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EDITORIAL

On account of the world-wide interest in the personal health and use of plant based drugs, the research activities in the traditional drugs have considerably increased. Over the years, a large number of traditional drugs, mainly herbal, have been subjected to clinical, pharmacological, phytochemical and pharmaceutical studies in an effort to validate them and prove their medical efficacy and safety. All these investigations have yielded extensive and valuable findings and insights, and there is a need for wide exchange of this information among scientists engaged in the development of new drugs of natural origin.

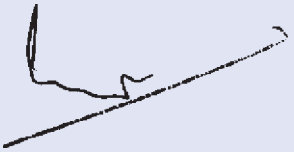
Unani system of Medicine, although originated in Greece, is one of the recognized systems of medicine of the country. Although, the Unani medicine have been in use for centuries and are known for their therapeutic efficacies, there is a need to scientifically establish their efficacy and safety in order to achieve global acceptance. Organized research work in this system was, therefore, a need of the hour. In post independent era, Central Council for Research in Unani Medicine, through its clinical, drug research, literary research, survey & cultivation of medicinal plants programme is contributing significantly for last three decades. *Vitiligo, Sinusitis, Filariasis, Eczema, Malaria, Infective Hepatitis, Asthma,* are some of the conditions where Unani therapies have earned recognition after scientific validation.

The Council has been publishing the *Hippocratic Journal of Unani Medicine* (HJUM), mainly to bring out fundamental and applied aspects of Unani Medicine. The journal also publishes recent advances in other related sciences and traditional medicines as well as different streams of medical sciences, which have bearing on validation and scientific interpretation of various concepts and strengths of Unani medicine.

In view of an overwhelming response, the journal earlier published twice a year, its periodicity has now been changed to quarterly w.e.f. January 2008 to accommodate more articles for quick dissemination of research data among scientific community. The journal has sufficient room for invited articles from luminaries of modern medicine and sciences as well as scholars of Unani medicine. The broad areas being covered include clinical research on single and compound Unani drugs, validation of regimental therapy, Clinical and experimental pharmacological studies, standardization of single and compound drugs, development of standard operating procedures, ethnobotanical studies, experimental studies on medicinal plants and development of agro-techniques thereof, and literary research on classics of Unani medicine. The journal is also open for studies on safety evaluation of Unani and other herbo-mineral drugs, nutraceuticals, cosmotherapeutics, aromatics, oral health, life style disorders, sports medicine etc. and such other newer areas which are the outcome of modern day living.

The current issue of this journal provides 16 original research and review papers in the areas of clinical research, drug standardization, pharmacology, ethnobotanical surveys and allied disciplines contributed by eminent scholars in their respective fields. It is hoped that data presented will contribute significantly in R&D sector of traditional drugs and prove to be an excellent exposition of current research efforts of scientists in this direction. Council acknowledges the authors for their contributions included in this issue and hope for their continued support in this endeavor. We wish to ensure the readers to bring out the future issues of the journal on time.

We at the CCRUM have been constantly striving to reach to higher standards and make HJUM the leading journal of Unani Medicine and related sciences. In this context, we thank our learned reviewers for their invaluable inputs in improving the manuscripts. We sincerely hope and trust that the mission can be accomplished with active partnership of quality-conscious individuals and institutions. Through these lines we seek your cooperation and support in materializing our dreams about the HJUM. In this regard, we request you for your as well as your colleagues' contributions for publication in and subscription to the journal. Further, we will appreciate if the journal is introduced far and wide. We would also welcome esteemed suggestions for achieving the highest standards of quality for the journal.



(Dr. Mohammad Khalid Siddiqui)
Editor-in-Chief

Efficacy of Post Beekhe Madar (*Calotropis gigantea*) Root Bark in Experimentally Induced Diarrhoea

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Abstract

The anti diarrhoeal activity of the aqueous extract of Post Beekhe Madar (PBM) (Root bark of *Calotropis gigantea*) was carried out in healthy adult Wistar rats of either sex weighing 150-200 gm. The effect of the test drug (in two doses of 73 mg and 125 mg/kg in A and B) was studied against 'castor oil induced diarrhoea' and 'castor oil induced enteropooling' in two different tests. Number of diarrhea in different groups and the purging index was calculated in the former while the volumes of intestinal content in the later test. The effect produced by the test drug was compared with control and the standard drug Lomotil (Diphenoxylate 2.5 mg + Atropine 0.025 mg).

The study revealed that the aqu. ext. of PMB has significant anti diarrhoeal effect as the mean number of defecation was reduced from 6.83 ± 0.95 in control group to 3.0 ± 0.63 and 3.0 ± 0.89 ($P< 0.05$) in test group A and B, respectively, in castor oil induced diarrhoea. Inhibitory activity against castor oil induced enteropooling was also found to be significant as the total intestinal volume was 1.55 ± 0.42 ml and 1.45 ± 0.19 ml ($P< 0.05$) in test group A and B, respectively, as compared to the control group where it amounted to 2.58 ± 0.20 ml. The experimental data was analyzed by using ANOVA one way with Dunnett multiple comparison test. The findings suggest that the aqu. extract of PBM possesses significant anti diarrhoeal activity against chemically induced diarrhoea.

Key Words: Diarrhoea, Unani Medicine, Castor oil, *Calotropis gigantea,* Diphenoxylate

Introduction

Diarrhoea is a major health problem especially for children under the age of 5 years, and up to 17 % of all deaths in the indoor paediatric patients have been estimated to be related to diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children, especially in developing countries. (Fauci *et al.,* 1998)

Medicinal plants are promising source of anti diarrhoeal drugs. (Makerie *et al.,* 19 89). Safety of the medicinal plants has been claimed to their main advantage as therapeutic agent in various ailments, besides being effective, economical and easily available. (Anonymous, 1979) It thus, becomes important to identify and evaluate the efficacy of commonly available plants and other natural products to develop safe, effective and low cost anti diarrhoeal agents.

Post Beekhe Madar (PBM) (Root bark of *Calotropis gigantea*) is an important drug of Unani system of medicine described to be *Habis* (Haemostatic) (Zakai, 2000; Multani, ynm; Ghani, ynm), *Qabiz* (Astringent) (Zakai, 2000; Multani, ynm; Ghani, ynm) and *Mujaffif* (Siccative) (Zakai, 2000; Multani, ynm; Ghani, ynm), effective in

almost all forms of diarrhoea. It is widely used for this purpose by Unani Physicians. (Zakai, 2000; Multani, ynm; Ghani, ynm) Although, its aerial part has been reported to possess anti diarrhoeal activity in experimental models (Havagiray, 2004) but PBM has not been studied scientifically for anti diarrhoeal effect.

In view of the described anti diarrhoeal effect of PBM in Unani literature and centuries old practice of Unani physicians to use it in patients of diarrhoea and also the scientific reports showing anti diarrhoeal effect in its aerial parts, the present study was envisaged to study the anti diarrhoeal activity of PBM on castor oil induced diarrhoea and enteropooling in animal models.

Materials & Methods

Animals

Wistar rats of either sex weighing 150-200 gm, 3-4 months of age, were used for the study. They were obtained from central animal house facility, National Institute of Mental Health and Neuroscience (NIMHANS), Bangalore.

Animals were housed in polypropylene cages at 25+ 2 ^{0}C with 12 h light / 12 h dark cycle. They were given free water and standard pellet diet (Hindustan Lever Ltd.) under strict hygienic conditions. All the animals were given sufficient time to acclimatize to laboratory conditions before the testing schedule. CPCSEA guide lines were followed for the experimentation and the Institutional animal ethics committee approved the experimental protocol.

Drugs and Dosage

Preparation of test drug

PBM was procured from the wild source at NIUM, Kottigepalya, Magadi Road, Bangalore-91. The authorized committee of NIUM (comprising of pharmacognosist, medicinal chemist and Unani expert) Bangalore, confirmed the identity of the drug. The drug was pulverized in a grinder to convert it into coarse powder. The aqueous extract of the powdered drug was prepared in Soxhlet's apparatus. The drug was subjected to 7 hours treatment at boiling temperature. The liquid extract was filtered and evaporated on a water bath till it dried. The extract yield was found to be 10 % w/w. The dose of the extract for Wistar rats was calculated by multiplying the Unani clinical dose by the conversion factor of 7 (Freireich, 1966) and found to be 73 mg/kg. A second dose was also used for this study, which was calculated by the method of Miller (1944), and was found to be 125 mg/kg. Drugs were administered by oral route with the help of gastric canula. The extract was suspended in distilled water before the administration.

Lomotil (Diphenoxylate 2.5 mg +Atropine 0.025 mg) manufactured by RPG Life Science Ltd. was used as the reference drug. It was administered in the dose of 0.7 mg/kg. The castor oil for the study was procured from Sigma Aldrich, USA.

Castor Oil Induced Diarrhoea

The effect of the test drug was studied on "castor oil induced diarrhoea" by the method of Awouters, *et al.,* 1978. Wistar rats of either sex, weighing 150- 200 gm were divided into 4 groups of 6 animals each. They were fasted for 18 hours prior to the experimentation, but had free access to water. Animals in Group I treated with distilled water orally in the dose of 2 ml/rats served as the control group. Animals in Group II were treated with the standard drug Lomotil (Diphenoxylate 2.5 mg + Atropine 0.025 mg) in the dose of 0.7 mg/kg and served as the standard group. The animals in Group III and IV were treated with the aqueous extract of test drug in the dose of 73 mg /kg and 125 mg/kg respectively, and served as test group A and test group B.

Thirty minutes after the treatment all the rats were administered 1 ml of castor oil, orally. Each animal was housed in separate cage over clean filter paper for 4 hours. The number of both wet and dry diarrhoeal droppings was counted at every hour over this period. Mean number of the stool passed by the test group was compared with that of the control group and the "Purgative Index" (PI) of each group was calculated by the following formula:

$$PI = \frac{\text{Percentage of respondents x Average no. of stool}}{\text{Average latent period}}$$

Castor Oil Induced Enteropooling

This test was carried out by the method of Robert *et al*. (1976). Overnight fasted rats of either sex, weighing 150-200 gm were divided into 4 groups of 6 animals each. Group I served as control and received distilled water 2 ml/rats, orally. Group II served as standard control and received 0.7-mg/kg of Lomotil (Diphenoxylate 2.5 mg + Atropine 0.025 mg). Group III & IV served as test group A & B and were treated with aqueous extract of the test drug in the dose of 73 mg/kg and 125 mg/kg, respectively.

Thirty minutes after the treatment each rat was administered 1 ml of castor oil orally. After 2 hours of the castor oil treatment rats were sacrificed under pentobarbitone anesthesia. The small intestine from pylorus to ileocaecal junction was removed after tying the ends with thread. The intestinal contents were collected by milking it into a graduated tubes and the volume was measured.

The results were statistically evaluated by using ANOVA one-way non repeated measure with post hoc pair comparison test followed by Dunnett Multiple comparison test, using Graphpad instat version 3. $P<0.05$ was considered significant.

Observation and Results

In "castor oil induced diarrhoea test", mean latent period was found to be 1.5 ± 0.22 h in control group, 1.67 ± 0.49 h, in standard group, 2.33 ± 0.49 h in test group A and 1.33 ± 0.42 h in test group B. The mean number of stool passed by the animals in 4 hours in control group was found to be 6.83 ± 0.95, which in standard group, was markedly reduced to 2.5 ± 0.80 ($P<0.01$). In test group A and B the mean number of stool passed during the same period was found to be significantly reduced to 3.0 ± 0.63 and 3.0 ± 0.89 ($P<0.05$), respectively. The purgative index of control group was calculated as 453, while it came down to 130.2 in standard group, 128 in test group A and 187 in test group B. The results indicated that the standard drug and both doses of the test drug caused very low purging indexes when compared to control group (Table 1).

In "castor oil induced enteropooling test", a significant decrease in the volume of intestinal fluid was observed in test group when compared with control group. Volume of intestinal fluid was found to be 2.58 ± 0.20 ml in control group, while in standard control it was reduced to 1.32 ± 0.14 ml ($P<0.01$). In test group A & B volume of intestinal fluid was decreased to 1.55 ± 0.42 ml and 1.45 ± 0.19 ml ($P<0.05$), respectively (Table 2).

Table-1. Effect of aqueous extract of Post beekhe madar on Castor Oil Induced Diarrhoea

Treatment	Mean latent period in hour ± SEM	Mean defecation in 4 hour±SEM	Purgative Index (PI)
Control group Distilled water 2 ml + Castor oil (1 ml /rat p.o.)	1.5±0.22	6.83±0.95	453
Standard Control Lomotil (0.7mg/kg p.o.) + Castor oil 1 ml/rat	1.67±0.49	2.5±0.80**	130.2
Test group A Aqu. ext. of PBM (73 mg /kg p.o.) + Castor oil 1ml/rat	2.33±0.49	3.0±0.63*	128
Test group B Aqu. ext. of PBM (125 mg/kg p.o.) + Castor oil 1 ml/rat	1.33±0.42	3.0±0.89*	187

* $P<0.05$, ** $P<0.01$

Table-2. Effect of aqueous extract of Post Beekhe Madar on Castor Oil Induced Enteropooling

Treatment	Volume of Intestinal fluid (ml) Mean ±SEM
Control group Distilled water 2 ml + Castor oil (1 ml/rat p.o.)	2.58±0.20
Standard Control Lomotil (0.7mg/kg p.o.) + Castor oil 1 ml/rat	1.32±0.14**
Test Group A Aqu. ext. of PBM (73 mg/kg p.o.) + Castor oil 1ml/rat	1.55±0.42*
Test Group B Aqu. ext. of PBM (125 mg/kg p.o.) + Castor oil 1 ml/rat	1.45±0.19*

*P<0.05, **P<0.01

Discussion

The findings of the two tests demonstrated that both the doses of Post Beekhe Madar (*Calotropis gigantea*) produced significant degree of anti diarrhoeal effect. In castor oil induced diarrhoea test, the administration of castor oil produced characteristic semi solid diarrhoeal droppings in 18-hour starved rats of the control group during the 4 hours of observation period. Both the doses of the test drug exhibited significant anti diarrhoeal activity (P<0.05) and the effect was almost equal to that of standard group.

The two doses of the test drug also scored very low purging index of 128 and 187 (test group A and B, respectively) as compared to 453 scored by the negative control. The purging index of test group A was even found to be lower than the purging index scored by standard drug. Thus, the above findings demonstrated that the two doses of the test drug possess striking anti diarrhoeal activity, both in terms of frequency of defecation and the purging index. The findings also suggested that the two doses of the test drug produced similar degree of effect in terms of frequency of stool, and the lower dose interestingly, scored a lower purging index than the higher dose. Thus, we can say that the therapeutic dose described in Unani literature and prescribed by the physicians is sufficient to produce anti diarrhoeal activity. The higher dose though did not apparently produce any adverse effect, but it was not found to produce dose dependent response either. The higher dose therefore need not be always recommended for therapeutic purposes.

In castor oil induced enteropooling test, it was noted that the two doses of the test drug significantly inhibited castor oil induced intestinal fluid accumulation as the volume of intestinal content was found to be significantly reduced. As the reduction

in intestinal volume itself is an indicator of anti diarrhoeal activity, therefore it can be inferred that the test drug possesses such an activity, because it decreased the intestinal volume significantly ($P<0.01$). It also indicated that probably anti secretory mechanism was involved in reducing the intestinal content and thereby improving the diarrhoeal condition both in terms of frequency and the volume of liquid. Since, castor oil induced diarrhoea allows the observation of measurable changes in the number of stools and the volume of enteropooling therefore, it has been considered as an appropriate model to study the anti diarrhoeal activity. (Havagiray, 2004) The test drug by reducing the number of stool and the volume of intestinal contents signified its usefulness as an anti diarrhoeal agent.

It has been shown that drugs affecting motility, frequency and consistency of diarrhoea also affect secretion most probably, because of the anti cholinergic effect. It is possible therefore that the test drug in our study may have affected the accumulation of fluid in the intestine through anti muscarinic or a_2 adrenoreceptor agonist activity. (Bolton, 1979)

Diarrhoea results from an imbalance between the absorption and secretory mechanism in the intestinal tract resulting in an excessive loss of fluid in the faeces. In some forms of diarrhoea, the secretory component predominates, while others are characterized by hyper motility. The inhibition of experimental diarrhoea and the reduction in faecal output by a drug substance are the basis of pharmacological evaluation of a potential anti diarrhoeal agent. The use of castor oil induced diarrhoea model in this study is logical because, the autacoids and prostaglandins have been described to be instrumental in the causation of diarrhoea in human being, so this model appears to have similarity with the diarrhoea in human being (Horton, 1968; Greenbargena, 1978) because, the liberation of ricinoleic acid from castor oil causes irritation and inflammation of the intestinal mucosa leading to release of prostaglandins, which in turn stimulate motility and secretion to cause increased frequency of defecation. (Pierce, 1971) The results in the present study showed that PBM produces a significant reduction in the severity and frequency of diarrhoea produced by castor oil. It is likely therefore, that the test drug possesses an activity, which either neutralized the effect of ricinoleic acid or decreased somehow the secretion of prostaglandin or produced an effect, which negotiated the effect of prostaglandin itself, causing cessation of diarrhoea. It can be explained by the view point of Unani medicine that the two properties of the test drug i.e. *qabiz* (astringent) and *mujaffif* (Siccative) (Zakai, 2000; Multani, ynm; Ghani, ynm) simultaneously acted to bring about normalcy.

On the basis of these findings we can conclude that the anti secretory and the pro absorptive effect in the test drug are more prominent and can be attained even at low dose. These findings are in consonance with the Unani description that suggests that *Quwate jazba* (Absorptive power) and *Quwate maska* (Retentive power) are improved after the administration of the test drug because of the two important effects i.e. *qabiz* and *mujaffif*, ascribed to the test drug .

Anti diarrhoeal activity of medicinal plants has been attributed mainly to the phytochemicals found in them such as tannins, alkaloids, saponins, sterols, terpene etc. Since the test drug apart from various active constituents, also possesses the above-mentioned constituents, it is likely therefore that they have contributory effect on the anti diarrhoeal activity. Sesquiterpene which is present in good quantity in the test drug has been reported to have anti inflammatory activity and the ability to relax smooth muscle and thereby relieve the gastrointestinal distress. This points towards the possible mechanism of action of the test drug. (Heinrich, 1998)

Further, sesquiterpenes, diterpenes, terpenes, flavonoids and terpenoid derivatives, which have been isolated from the test drug, are known for inhibiting the release of autacoids and prostaglandins, which in turn inhibit the secretion induced by castor oil. (Vimala, 1997; Veiga, 2001; Milanova, 1995; Nikiema, 2001) It is likely therefore that the test drug produced anti secretory effects also by inhibiting the prostaglandins.

On the basis of results and the findings it can be concluded that the test drug PBM possesses significant anti diarrhoeal effect.

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The authors are thankful to the Director, National Institute of Unani Medicine (NIUM), Bangalore, for providing necessary facilities in the department. The authors are also grateful to Dr. Suresh Chandra, Incharge of Central Animal Research Facility (CARF), National Institute of Mental Health Science, (NIMHANS), Bangalore for providing animals .

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Clinical Evaluation of Unani Compound Coded Drug (UNIM-353) on Zeequn Nafas (Bronchial Asthma): A Preliminary Clinical Study

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Abstract

The efficacy of Unani Compound Coded formulation (UNIM-353) was evaluated in one hundred sixty patients of Zeequn Nafas (Bronchial Asthma). The Patients were treated for a period of 180 days in the OPD, at RRIUM, Mumbai. The clinical assessment and Peak Expiratory Flow Rate (PEFR) was done before, during and after the treatment.

The clinical assessment was done in term of relief in sign and symptoms and reduction in number of asthmatic attack. The inclusion were presence of three or all the following signs and symptoms; Cough, Chest pain, Dyspnoea, Sneezing, Wheezing sound, Vesicular breathing, Bronchial breathing, Changes in Vocal fremitus, Rhonchi, Crepitations. The clinical results suggested that the drug (UNIM-353) is effective in treating mild, moderate and severe type of Bronchial Asthma.

Key Words: Zeequn Nafas, PEFR, UNIM-353, Bronchial Asthma, Unani Medicine.

Introduction

The word asthma is a Greek one meaning breathless or breath with open mouth (Seaton *et al.,* 1989). A condition in witch there is variable breathlessness due to widespread narrowing of intrapulmonary air-ways which varies in severity over short period of time, either spontaneous or with treatment, (.Batten, 1978). In the 1960s two primary features were incorporated into the definition of asthma: bronchial hyper-responsiveness to variety of stimuli and in response to such stimuli, a wide spread narrowing of the air-ways that was either partially or totally reversible, either spontaneously or in response to treatment (Barbee, 1997). Laitinen *et al.,* (1985) were first to describe the presence of disquantative eosinophilic inflammatory infiltrate in the bronchi of the patient with mild, stable asthma. Shortly there after a large number of reports confirmed by biopsy and bronchoalveolar lavage analysis, the presence of airway inflammation as constant feature, even in the mildest form of asthma (Bousqet, *et. al.,* 1990).

The definition of asthma in 1990 has been expended to include three primary features (1) chronic air ways inflammation, which in susceptible individuals, causes recurrent episodes of wheezing, breathlessness, chest tightness and cough. (2)Wide spread but variable airflow limitation that is at lest partially reversible, either spontaneously or with treatment. (3) Bronchial hyper-responsiveness to a variety of stimuli. (Anonymous, 1993). Ibn Sina (980-1037 CE) described that Warm-e-har (inflammation) occurs in orooq-e-khasna (bronchioles) as a result hypertonic condition develops in its internal surface. Subsequently phlegmatic material begins to exude from it and accumulates within the air passage. If this condition prolongs subsequently distention of air passage occurs this complication is called Ittasauttajaweef (bronchiactasis) Allama Samarqandi (d 1222 CE) also endorsed this condition.

Methodology

A clinical study was conducted to evaluate the efficacy of Unani Compound Coded formulation (UNIM-352) in 160 patients of Zeequn Nafas (Bronchial Asthma). The present study was carried out in the OPD of Regional Research Institute of Unani Medicine (CCRUM), Mumbai. Patients were treated for a period of 180 days. Clinical assessment and Peak Expiratory Flow Rate (PEFR) was also done before during and after the treatment.

The clinical assessment was done in term of relief in signs and symptoms and reduction in number of asthmatic attack. The inclusions were presence of three or all the following signs and symptoms. Cough, Chest pain, Dyspnoea, Sneezing, Wheezing sound, Vesicular breathing, Bronchial breathing, Changes in Vocal framitus, Rhonchi, Crepitations. All the signs and symptoms were assessed on each follow-up and scored accordingly.

The improvement criteria were; (a) Complete Relief: 71% - 100% relief in subjective symptoms and objectives signs with reduction in physical signs as assessed on clinical examination with no history of relapse.(b) Partial Relief: 29%-70% relief in subjective symptoms reported by the patients and objective signs along with reduction in physical signs in lungs as assessed by clinical examination. (c) No Relief: Indicates less than 0-29% subjective or objective improvement.

The following Investigations were carried out: ESR; Sputum Examination: its amount, character, test for acid fast bacilli and other bacterial; Dynamic pulmonary function test: Peak Expiratory Flow rate; Stool Examination: Microscopic for any cyst and ova to rule out any parasitic infection.

Drug Dose and Mode of Administration

Patients were given a coded Unani compound drug UNIM-353 in the form of Majoone (Paste) 10grams thrice daily orally after meal.

Pre and Post Treatment Observations

During treatment, on 15^{th} 30^{th} 60^{th} days and after completion of treatment, follow up was done once a month for two months.

Observations

One hundred sixty patients of either sex in the age group of 01 to 60 years were taken in the clinical study and the effect of Unani coded formulation UNIM 353 was assessed on the above mentioned parameters. Maximum number of patients registered in the age group of above sixty years (Table 1) Diet shows an important role in the Zeequn Nafas. Out of 160 patient 85.62% patients were non- vegetarian (Table 2) and 46.25% patients were belongs to middle class group.

Table 1. Showing classification age and sex wise

S.No.	Age Group	Male No. %age	Female No. (%age)	Total
1	1-10	4	2	10
2	11-20	23	3	52
3	21-30	12	4	28
4	31-40	26	7	57
5	41-50	28	9	77
6	51-60	31	11	95
	Total	124 (77.5%)	36. (22.5%)	160

Table 2. Showing dietary habit

S.No.	Dietary habit	No of cases	%age
1	Vegetarian	23	14.37 %
2	Non- Vegetarian	137	85.62%
	Total	160	

Table 3. Showing classification of Social status

S.No.	Social status	No of cases .	%age
1	Upper class	33	20.62%
2	Middle class	74	46.25%
3	Poor class	53	33.12%
	Total	160	

Table 4. Showing classification according to temperament

S.No.	Temperament	No. of cases	% age
1	Damvi		14 8.75 %
2	Balghami	121	75.62 %
3	Safravi	22	13.75 %
4	Saudavi	04	2.5 %
	Total	160	

Table 5. Showing Response of drug of Bronchial Asthma.

Coded Drug	Relieved 71-100%	Partially Relieved	No Relief Less than 29%	No. of Cassese
UNIM 353	18(12 %)	89(56%)	53(34 %)	160

Table 6. Showing Chronicity wise Response of Bronchial Asthma

Chronicity	Relieved (%) (71-100%)	Partially (%) Relieved (30-70%)	No Relief (%) Less than 29%	Total
0-1	02(22.2)	04(44.44)	3(33.33)	6
2-3 years	07(28)	10(40)	8(32)	9
4-5 years	05(25)	6(55)	9(45)	20
6-7 years	04(21.05)	6(46.03)	9(31.57)	19
8-9 years	06(24)	9(36)	11(44)	25
10-11 years	05(22.72)	9(40.30)	8(36.36)	22
12-13 years	1(16.66)	01(16.66)	04(66.66)	25
14-15 years	02(6.66)	11(36.66)	17(56.66)	30
More than 15 years	-	1(25)	03(75)	04
Total				160

Table 7. Response of cough to UNIM 353

Coded Drug	Before treatment (%)	After treatment (%)	No of cases relieved (%)
UNIM-353	142 (100)	27 (18%)	114 (82%)

Table 8. Response of dyspnoea to UNIM 353

Coded Drug	Before treatment No.(%)	After treatment No.(%)	No of cases relieved (%)
UNIM-353	58 (100)	32 (55)	26 (45)

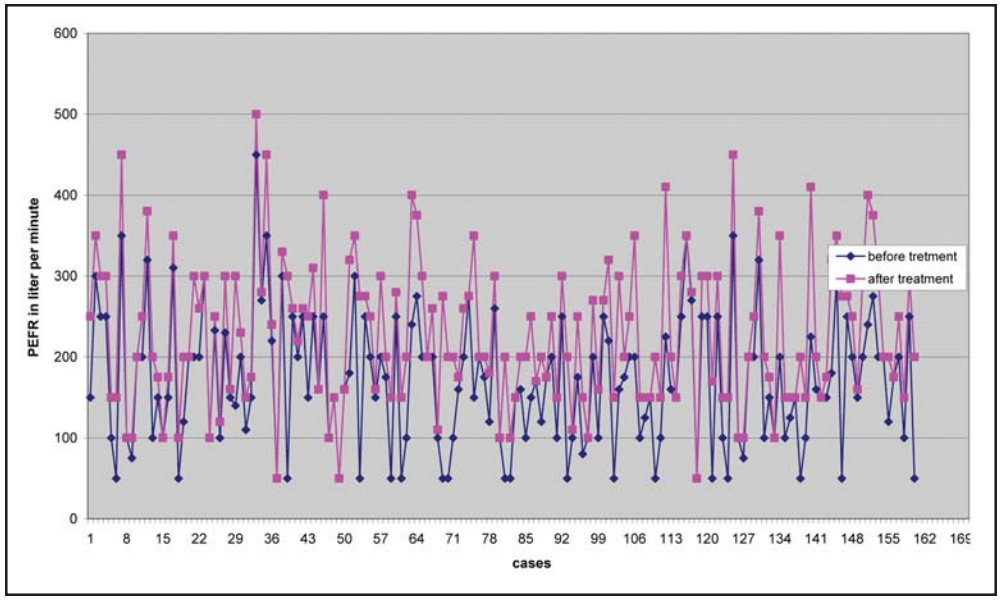
Table 9. Response of wheezing to UNIM 353

Coded Drug	Before treatment (%)	After treatment (%)	No of cases relieved (%)
UNIM-353	72 (100)	28 (38.88)	44 (61)

Table 10. Response of Rhonchi to UNIM 353

Coded Drug	Before treatment (%)	After treatment (%)	No of cases relieved (%)
UNIM-353	42 (100)	14 (34)	28 (67)

Graph showing PEFR value before and after treatment in cases treated with Unani coded drug UNIM 353



Results and Discussion

One hundred sixty patients suffering from Bronchial Asthma were treated with Unani compound coded drug UNIM -353 for a period of 180 days the response of the drug was assessed on the basis of clinical sing and symptoms and PEFR value. It was observed that out of 160 patients 75.62% patients were assessed Balghami temperament. UNIM -353 was found to be very effective. Out of 160 patients 12% patients were relived 56% partially relieved and only 34% patients were found not relieved. It has been observed that 28% patients were relived in 2-3 years of chronicity group and 55 % patients were partially relived in the group of 4-5 years

chronicity and maximum no of patients who were found not relieved were in the chronicity group of more than above 15 years. The response of drug was also observed on individual clinical sign and symptoms of bronchial asthma. It was observed that out of 142 patients having severe cough 82% were relived. Similarly Dysponea was relived in 45 % patients (Table 8). 61 % patients suffering from wheezing were relieved (Table 9). It has been observed that out of 67 patients having Rhonchi, 42 % were relived (Table 10).

It was observed that there is enhancement in the Peak Expiratory Flow Rate values after the treatment with coded drug (UNIM-353).

During the drug study no adverse effects of the Unani drugs were noticed. Thus, the study indicates that Unani compound coded drug (UNIM-353) is effective and safe in cases of Zeequn Nafas (Bronchial Asthma).

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Physico-chemical and Phytochemical Studies of Majoon-e-Baladur: A Herbal Formulation

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Abstract

Majoon-e-Baladur is a Unani Polyherbal formulation used mainly for the treatment of Nervine Disorders. The Physico-chemical standardization of Majoon-e-Baladur has not yet been reported. Thus the present work was undertaken in order to bridge the gap. The study includes the physico-chemical standardization of this drug under various parameters. Parameters undertaken are based on national and international pharmacopoeias. The findings can be utilized in controlling the quality of the formulation.

Key Words: Unani Medicine, Majoon-e-Baladur, Nervine disorder, Physico-chemical, Drug standardization.

Introduction

During the last century, the modern system of medicine which actually began with the findings of the Traditional System of Medicine has made tremendous progress both in term of process technology as well as development of desired product for a targeted disease. The system for assessing quality, efficacy and safety has been put in place with scientific data based generated through basic and applied research to take care of consumer' interest besides insuring regulatory compliance. The success of Modern system of medicine may be attributed to (a) scientific research and database (b) validated methods, procedures and (c) systems of quality control, quality assurance and safety.

A report was published that there are continuous banning of the synthetic medicine developed for various types of diseases on the one hand and the non availability of drugs for several chronic and systemic ailments on the other hand are the two main driving forces responsible for enhanced interest in Ayurvedic, Siddha & Unani (ASU) drugs. The world today is looking to traditional medicine to provide and answer to many of its health care needs. Given this scenario ASU drugs have the potential to capture the world market by existing as well as new drugs.

In Unani literature Majoon-e-Baladur is reported as Nervine and Brain Tonic. It is extensively used in tremors, paralysis, Bells palsy, and vertigo. (Aziz, 1244; Kareem, 1924; Israyilly, 1266 H; Ghani, 1928; Lutfi, 1924; Anonymous, 1981). So far no major effort has been done to standardize Majoon-e –Baladur. In the present study, the standardization of Majoon-e-Baladur was done under various parameters according to considerations mentioned above.

Materials and Methods

The crude material was procured from Dawakhana Tibbiya College, Aligarh Muslim University. The authenticity of these crude materials was confirmed from the pharmacognosy Section of the Department of Ilmul Advia, Ajmal Khan Tibbiya

College, AMU, Aligarh. The ingredients of Majoon-e- Baladur were taken as described in National Formulary of Unani Medicine, Part-1. The Majoon-e-Baladur was prepared from the crude material according to the method given in the same National Formulary of Unani Medicine (NFUM). The Physico-chemical studies were carried out based on pharmacopoeial parameters. NFUM (1987). The Successive Extraction was done in Soxhlet's apparatus in different solvents (B.P; 1986). Moisture contents (Jenkin *et. al.,* 1967), Loss on Drying and Extractive values (Hot and Cold Method) were also determined (Anonymous; 1987). Determination of Carbohydrate was made by the method of Peach & Tracey; 1995. Qualitative Phytochemical Analysis, PH Values, Bulk Density, Ash value, and sugar were also estimated (Afaq *et al.,* 1994). Thin Layer Chromatography (TLC) of the drug extract in different solvents was determined by using pre-coated silica gel 60 F254 aluminum plates (layer thickness 0.25mm, Merk).

Table-1. Physico-chemical Parameters of Majoon-e-Baladur

S.No.	Parameters	Results (%)	
		Mean *	S.E ±
I.	**Water Soluble Contents**		
	A] (Cold Method)	43.36	0.32
	B] (Hot Method)	45.96	0.39
	Alcohol Soluble Contents		
	A] (Cold Method)	29.97	0.26
	B] (Hot Method)	36.84	0.49
2.	**Ash Values**		
	i) Total Ash	2.13	0.01
	ii) Acid Insoluble Ash	1.18	0.01
	iii) Water Soluble Ash	0.80	0.05
3.	**Successive Extractive Value**		
	i) Petroleum Ether	16.84	0.02
	ii) Di-ethyl Ether	4.55	0.02
	iii) Chloroform	0.46	0.01
	iv) Benzene	0.45	0.01
	v) Alcohol	9.43	0.02
	vi) Aqueous	10.02	0.06
4.	**Percentage of Moisture Contents**	13.66	0.33
5.	**Loss of Weight on Drying on at 105°C**	14.57	0.22
6.	**Percentage of Carbohydrate**	68.16	0.44
7.	**Ph Value**		
	i) 1% solution	5.95	0.01
	ii) 10% solution	3.80	0.01
	Reducing Sugar	40.17	0.59
8.	**Non-Reducing Sugar**	31.31	0.43_
9.	**Bulk Density** (%)	1.14	0.01

*The values are mean of three experiments.

Observations and Results

The physico-chemical and phytochemical studies were carried out based on pharmacopoeial parameters. The results obtained are depicted in Table 1 to 3.

Discussion

It is very important to standardize herbal products before bringing it to market and clinical use. It is also necessary to ensure that all the forthcoming batches should be standardized according to appropriate “finger prints”. The standard should be laid down very cautiously keeping in view all the possibilities of variance and quality control. The standardization should be done according to the parameters assigned by WHO in order to ensure global comparability. Most of the qualitative test is colour tests which are specific for certain substances and can be used as a tool to distinguish between the drugs and adulterants having similar appearance. The extractive value is also a valuable test to check the quality of the drug and any variation in the chemical constituents will cause variation in the extractive values so it is a good tool to check the purity of a drug. The extractive value in different organic solvents viz. Pet. Ether, Di-ethyl Ether, Chloroform, Benzene, Alcohol and water were found to be 16.84%, 4.55%, 0.46%, 9.43% and 10.02% similarly, the percentage of water and alcohol soluble matter obtained from both cold and hot method were found to be different. The water soluble content (43.36%) is higher than alcohol soluble content (29.97%) in cold method, while in hot method water

Table-2. Qualitative Tests for Various Chemical Constituents in Majoon-e-Baladur

S.No.	Test	Results
1.	Alkaloid	+ve
2.	Amino acid	-ve
3.	Protein	+ve
4.	Glycoside	+ve
5.	Flavonoid	-ve
6.	Phenol	-ve
7.	Resin	+ve
8.	Sterol/Terpene	+ve
9.	Tannin	-ve
10.	Saponins	+ve
11.	Steroid	+ve

Table-3. Thin Layer Chromatogramic Evaluation of Majoon-e-Baladur after Spray Treatments

S. No.	Extract	Solvent System	Spray Treatment/ Detection	No. of Spots	Rf Value
1.	Pet. Ether	Petroleum Ether-. Di-ethyl Ether (8:2)	Vanillin Sulphuric acid	10	0.06 (purple), 0.16 (pink), 0.21 (light blue), 0.26 (purple), 0.29 (light brown), 0.34 (pink), 0.47 (light purple), 0.54 (pink), 0.06 (light blue), 0.96 (blue)
2.	Di-ethyl Ether	Petroleum Ether: Di-ethyl Ether (6:4)	Vanillin Sulphuric acid	12	0.05 (pink), 0.10 (pink), 0.14 (yellow), 0.17 (light green), 0.22 (light blue), 0.28 (pink), 0.36 (pink), 0.40 (dark blue), 0.43 (light blue), 0.54 (light blue), 0.71 (light blue), 0.94 (blue)
3.	Chloroform	Chloroform : Benzene (8:2)	Vanillin Sulphuric acid	05	0.06 (pink), 0.15 (purple), 0.18 (light green), 0.27 (purple), 0.57 (black)
4.	Benzene	Benzene: Methanol (7:3)	Vanillin Sulphuric acid	09	0.20 (pink), 0.21 (green), 0.23 (pink), 0.26 (brown), 0.38 (blue), 0.40 (blue), 0.43 (yellow), 0.46 (pink), 0.51 (green)

soluble content (45.96%) is higher than alcoholic soluble content (36.84%). Therefore, temperature related differences may also be considered as specific data in the identification and purity of present drug. The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy and earthy matters. The percentage of total ash, acid insoluble ash, and water soluble ash were 2.13%, 1.18%, and 0.80%, respectively. The other parameters like bulk density (1.14%), moisture content (13.66%), loss on drying (14.57%) and PH also indicate the quality of Majoon-e-Baladur sold in market. Chromatography can be used as an aid to identifying the authenticity of the drug. In the present study TLC has been conducted for the separation of different compounds and Rf value of developed spots in different solvent system is calculated.

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Ethnobotanical Survey of Khammam and Bhadrachalam Forests of Andhra Pradesh

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Abstract

Based on an ethnopharmacological survey of Khammam and Bhadrachalam forests of Andhra Pradesh conducted during February – March 2010, the paper presents some 43 contemporary folk recipes comprising 43 taxa of folk medicinal plants used by various tribes e.g. Koyas, Lambadas, Kondareddys and Naiks etc. for the treatment of various common ailments. Botanical name, family, local name, Unani name, field book number, part(s) used, name of the disease(s) against which used and, mode of administration are given for each recipe discussed. The information provided will help to discover new drugs of natural origin for many of the diseases and conditions, thus far, incurable in modern medicine.

Key Words: Ethnopharmacological survey, Tribal medicine, Khammam and Bhadrachalam Forests.

Introduction

In the course of an ethnopharmacological survey of Khammam and Bhadrachalam Forests of Khammam district of Andhra Pradesh undertaken in February – March 2010, first-hand information on folk medicinal uses of plants for treatment of various diseases and conditions were recorded. The area from which data were derived is situated in North Latitude 16º45' and 18º35' and between East longitudes of 79º47' and 81º37'. The areas explored included Khammam, Aswaraopet, Sattupally, Dammapet, karepally, Bhadrachalam, Charla, Chintoor, Motagudam, Sumbampeta and Vinayakpuram.

The study presents 45 folk medicinal species used by the tribal and other ethnic groups for treatment of various ailments among local population in the study area. The area has not been investigated exhaustively earlier in this direction except for some sporadic reports on medicinal uses of plants (Chetty & Rao, 1989; Balaji Rao *et. al.,* 1995, 1996; Gupta *et. al.,* 1997, 2005, 2007, 2008, 2009 & 2010, Reddy *et. al.,* 1988, 1989, 1991, 1996; Suryanarayana, 1996; Hemadri, 1981, 1991, 1992; Hemadri and Rao, 1983, 1984; Hemadri *et. al.,* 1987; Vedavathy, 1998; Vedavathy and Rao, 1992, 1995; Vedavathy and Mrudual, 1995 a, b; Vedavathy *et. al.,* 1991; Kapoor & Kapoor, 1973; Venkata Raju and Reddy, 1998, Vijay Kumar and Pullaiah, 1998a, 1998b.

Methodology

The data on folk medicinal uses of plants were collected from the well reputed herbalists (medicine men) through direct field interviews who also accompanied the survey team in the field to help identify the folk plants and also from the tribals who have long been prescribing the folk-medicines to locals for treatment of various

common and chronic diseases. Information about the degree of efficacy of the herbs was also recorded. Botanical specimens of all folk drugs were collected, identified and voucher specimens prepared and deposited in the Herbarium of Survey of Medicinal Plants Unit, Central Research Institute of Unani medicine, Hyderabad, for future reference and study. Ingredients and adjuvant drugs in a particular recipe were recorded by their local names in field and scientifically identified at the Institute.

Enumeration of Folk Medicinal Species

The medicinal plants used as folk medicine in the study area are arranged in alphabetical order. Each entry gives the information – plants' scientific name with family (in bracket), field Book No., Local Name (s), Unani name (wherever available), part(s) used, disease and condition against which used, and method of usage, in sequence.

- *Aegle marmelos* (Linn.) Correa; (Rutaceae); CRI 9303; Maredu; Bel; leaves; Infertility: (i) decoction of the leaves is to be taken 2-3 months, gives good response; (ii) bark powder is given with rice for the cure of loose motion; (iii) bark powder + Khash-2 + Misri is to be given as a aphrodisiac.
- *Andrographis paniculata* (Burm. f.) Wall. ex. Nees. (Acanthaceae); CRI 9472; Nelavemu; Kalmegh; Roots; Stomach pain: decoction of the root is used for stomach pain and also as anthelmintic.
- *Bambusa arundinacea* Willd. (Poaceae); CRI 9408; Bongu-Veduru; Bans; Leaves: Pneumonia; decoction of the leaves is boiled and mixed with honey and given to the patients.
- *Barleria prionitis* Linn. (Acanthaceae); CRI 9466; Mullu-goranta; Jhinti; whole plant; Liver tonic: powder of the whole plant is to be mixed with cow's urine, boiled and used as a liver tonic.
- *Bauhinia racemosa* Lam. (Caesalpiniaceae); CRI 9492; Ari; Kachnal; Leaves; Diarrhoea & Dysentery: decoction of the leaves with black pepper is given for diarrhoea and dysentery.
- *Bryophyllum pinnatum* (Lam.) Kurz. (Crassulaceae); CRI 9365; Sima-Jamudu; Zakhm-Haiyat; Leaves; Snake bite & Wounds: leaf-paste is applied to the affect part for snake bites and wounds.
- *Buchanania lanzen* Spreng. (Anacardiaceae); CRI 9305; Sara; Chironji; Seed oil; Joint Pains: seed oil is externally used for joint pains and swellings.
- *Calohpyllum inophyllum* Linn. (Guttiferae); CRI 9358; Pouna; Sultana Champa; Seeds; Rheumatism & Skin affections: the seed oil is used for rheumatism and skin affections.

- *Cassia fistula* Linn. (Caesalpiniaceae); CRI 9434; Rela-chattu; Amaltas; Leaves, Constipation: decoction of the leaves with tamarind or tomato is given two to three times for free motions.
- *Cassia occidentalis* Linn. (Caesalpiniaceae); CRI 9454; Kasinda; Kasondi; Whole Plant; Gonorrhoea: decoction of the whole plant is to be mixed with Raskapoor and is useful in gonorrhoea.
- *Cassytha filiformis* Linn. (Lauraceae); CRI 9484; Nulu-tega; Amarbeli; Whole plant; Skin diseases and diuretic: decoction of the whole plant with black pepper is given for skin diseases and also given as diuretic.
- *Chamaesyce hirta* (L.) Millsp. (Euphorbiaceae); CRI 9470; Bidarie; Dudhi; Whole Plant; Strangury: decoction of the whole plant is mixed with sugar and given to the patient 3-4 times for quick relief in cases of strangury.
- *Chloroxylon swietenia* DC. (Rutaceae); CRI 9420; Billydu; Bhirra; Leaves; Jaundice: decoction of the leaves is given as liver tonic and for treating Jaundice.
- *Cinnamomum camphora* (L.) Sieb. (Lauraceae); CRI 9480; Karpuram; Kapur; Leaves; Fever and measles: decoction of the leaves is given for fever and measles.
- *Cleome viscosa* Linn. (Capparaceae); CRI 9474; Kukhavominta; Hulhul; Whole plant; Joint pains: whole plant is to be crushed and mixed with mustard oil and used externally for joint pains.
- *Cocculus hirsutus* (Linn.) Diels. (Menispermaceae); CRI 9450; Dusaraitige; Jantiki bel: whole plant; irregular bleedings; Decoction of the whole plant with misri is given on empty stomach for irregular bleedings, for 3 to 10 days.
- *Crataeva nurvala* Buch-Ham. (Capparidaceae); CRI 9360; Varuna; Barna; Root bark; Bleeding piles: root bark powder is given for bleeding piles.
- *Datura innoxia* Mill. (Solanaceae); CRI 9461; Ummatta; Dhatura-sia; Seeds; Facial-paralysis: 25 gms seeds to be wet and then mixed with Kali mirchi and *Crocus sativa* Linn. (Zaffron) and tablet like mung dal is to be given to the patient 2-3 times, for facial paralysis.
- *Decalepis hamiltonii* Wt. & Arn. (Asclepiadaceae); CRI 9362; Mahali-kizhangu; Makaliberu; Roots; Leprosy & Healing wounds: root powder with black pepper is given for leprosy and healing wounds.
- *Diospyros melanoxylon* Roxb. (Ebenaceae); CRI 9309; Tumki, Tendu; Leaves; Carminative & Diuretic: decoction of the leaves with black pepper is given as carminative and diuretic
- *Emblica officinalis* Gaertn. (Euphorbiaceae); CRI 9451; Usirikai; Amla; Stem bark & Leaves; Constipation: decoction of the stem bark & Leaves with black pepper is given for free motions.

- *Ficus hispida* Linn.f. (Moraceae); CRI 9490; Bodamamidi; Jangli-Anjir; Stem bark; Joint pains & swellings: decoction of the stem bark with black pepper is given for joint pains and swellings.
- *Helicteres isora* Linn. (Sterculiaceae); CRI 9368; Kabanchi; Marorphali; Stem bark; Diabetes: stem bark powder is given for diabetes.
- *Jasminum auriculatum* Vahl. (Oleaceae); CRI 9370; Adavimolla; Juhi; Leaves; Asthma & Bronchitis: decoction of the leaves is used for treating asthma and bronchitis.
- *Lawsonia inermis* Linn. (Lythraceae); CRI 9456; Goranti; Mehndi; Leaves; Jaundice: decoction of leaves with black pepper is given orally for Jaundice, two times for 3 days.
- *Leucas aspera* Spreng. (Lamiaceae); CRI 9458; Tummachettu; Chota-Halkusa; Whole plant; Jaundice: decoction of the whole plant with black pepper is given orally for jaundice for 3 to 4 days.
- *Manihot esculenta* Crantz. (Euphorbiaceae); CRI 9372; Karrapen-dalamu; Marachini; Roots; Scorpion sting: root powder is given orally for scorpion-sting.
- *Morinda citrifolia* Linn. (Rubiaceae); CRI 9378; Maddi-Togaru; Nuna; Leaves; Gout & muscular swellings: juice of the leaves is applied for gout and also useful for muscular swellings.
- *Madhuca longifolia* (Koen.) Macbride (Sapotaceae); CRI 9400; Ippa; Mohwa; Seeds; Foot cracks; seed oil is used for foot cracks and also used in skin affections.
- *Mimosa pudica* Linn. (Mimosaceae); CRI 9468; Attapatti; Lajwanti; Whole plant; Mental Diseases (Mainia): decoction of the whole plant is mixed with black pepper and *Achyranthes aspera* Linn. (Chirchit) and given for 10-15 days to cure mental diseases.
- *Mesua ferrea* Linn. (Guttiferae); CRI 9392; Nagkesara; Nagkesar; Stem bark; Diuretic & Astringent: decoction of the stem bark is used as diuretic and astringent.
- *Nyctanthes arbor-tristis* Linn. (Oleaceae); CRI 9564); Parijatamu; Harshinghar; Leaves; Malaria: decoction of the whole plant is given with black pepper & honey to cure malaria.
- *Oroxylum indicum* Vent. (Bignoniaceae); CRI 9375; Araly; Pampini; Stem bark; Rheumatism & Swellings: stem bark is used for rheumatism and reduce swellings.
- *Pithecellobium dulce* Benth. (Mimosaceae); CRI 9479; Simachinta; Vilayati Babul; Seeds; Fevers and Antiseptic: seed powder is given to treat fevers and used as antiseptic to the wounds.

- *Plumbago indica* Linn. (Plumbaginaceae); CRI 9462; Errachitramulam; Rakat-chitra; Whole plant; Low BP: decoction of the whole plant is given with pure ghee and wheat chapatti for one week to cure low BP.
- *Premna herbacea* Roxb. (Verbenaceae); CRI 9380; Adavi-nalli; Bharangi; Roots; Fevers and cardiac tonic: root's powder is used for fevers and also as a cardiac tonic.
- *Pterocarpus marsupium* Roxb. (Fabaceae); CRI 9354; Yegi; Bijasar; Gum; Diarrhoea & Dysentery: gum is used for diarrhoea & Dysentery and wood is used to treat diabetes.
- *Punica granatum* Linn. (Punicaceae); CRI 9460; Danimma; Anar; Fruit rind; Ulcers: decoction of the fruit rind with 30% Ajwain is given for ulcers.
- *Salacia oblonga* Wall. (Hippocrataceae); CRI 9356; Ponkaranti; Chundan; Root Bark; Rheumatic-arthritis: root bark powder is used for rheumatic arthritis and also used for asthma.
- *Semecarpus anacardium* Linn.f. (Anacardiaceae); CRI 9332; Bhallataki, Bhilawa; Fruit's Kernel; Carminative & Anthelmintic: decoction of the kernels are used as carminative and anthelmintic.
- *Strychnos potatorum* Linn. (Loganiaceae); CRI 9328; Chilla-chettu; Nirmali; Seeds; Burning micturation: decoction of the seed powder is given two times a day for 2-3 days for quick relief.
- *Vitex negundo* Linn. (Verbenaceae); CRI 9452; Vaavili; Shambalu; Leaves; Joint pains: decoction of the leaves is boiled and mixed with honey and given to the patients.
- *Wrightia tinctoria* R.Br. (Apocynaceae); CRI 9477; Amkudu; Indrajao-Shireen; Stem bark; Aphrodisiac: stem bark powder with *Allium sativum* Linn. (Lasan); is externally applied to palm of both husband and wife. This acts as aphrodisiac.

Results and Discussion

The great potential of ethnobotanical knowledge as a key resource for developing new kinds of pharmaceuticals and other chemicals of industrial use has been increasingly realized. In the present study some traditional therapeutic methods employed by the natives of Khammam and Bhadrachalam forests of Khammam district have been discussed. Out of 95 taxa of medicinal plants collected and identified from the study area, 43 are used locally in folk medicines by the local tribals and other ethnic people viz. *Koyas, Lambadas, Kondareddys* and *Naiks* for the treatment of various common ailments; including fever, diarrhoea & dysentery, cough & cold; ulcers, rheumatic arthritis, cardiac troubles and skin diseases. The tribals normally prefer to do pod cultivation, which is of two types

(i) Chilakapodu and (ii) Kondapodu. It is a peculiar primitive system of co-operative farming.

The usual methods of application of folk medicines are as decoctions, paste, powder, juice and pills. These are taken internally or applied externally. Most of the folk medicines include only one plant species, however some preparations are the combination of several herbs. Moreover, the same plant is used for more than one diseases and the single disease may be treated by many species.

The data on folk-medicinal uses have been compared with recent available literature (Anonymous, 1948-1976, 1992; Hussain *et. al.,* 1992; Jain 1981, 1991; Rastogi and Mehrotra 1990-1998; Chetty & Rao, 1989; Hemadri, 1987, 1988, 1991; Vijay Kumar & Pullaiah; 1998; Nagaraju & Rao, 1989, 1990; Balaji Rao *et. al.,* 1995, 1996; Gupta, *et. al.,* 1997, 2005, 2007, 2008, 2009 & 2010; Surya Narayana, 1996; Imam *et. al.,* 1992; Hussain *et. al.,* 2002; Vedavathy, 1998 and found that most of the folk medicinal plants are duly reported in the literature, however, their mode of application, ingredients and parts used are different. Therefore, the present study represents contemporary folk uses of medicinal plants of the area investigated. It would therefore be worthwhile to subject all these folk drugs to scientific testing in the context of claims reported herein.

Further, the traditional knowledge has been eroding in the tribal society day by day. The crucial factors responsible for such erosion are the pressure of modernization and migration of youth from the tribal areas to semi urban or urban areas to take up employment. If such things continue to happen in these communities then knowledge related to ethnobotany will vanish from the region. Similar factors were believed to be the reason for the loss of traditional ethnobotanical knowledge in Iban community in Sarawak Malaysia (Yarvie and Perumal, 1994) and Rajitribal community of Central Himalaya, India (Negi *et. al.,* 2002). Through such investigations many more new plant drugs can be revealed from the unique folk-lores lying hidden among the traditional communities of other ethno-pharmacologically unexplored areas of India and elsewhere, which may be utilized to the well being of human health. However, experimental and clinical evidence are needed to demonstrate the effectiveness and safety of these folk drugs before they can be accepted by the modern health care system.

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Standardization of Habb-e-Man-e-Hamal – A Unani Contraceptive Pill

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Abstract

The interest in the use of herbal drugs for healthcare has increased tremendously during the past few decades. As a result several medium and large scale pharmaceutical industries have entered into this area. But, due to the lack of proper quality control measures, the Indian share in the world trade is quite insignificant. Lack of set parameters for standardization are a major set back for the Indian herbal industry. Today, there is an urgent need of developing properly standardized herbal drugs, both crude and in the form of formulations. In view of this, standardization of **Habb-e-Man-e-Hamal;** a unani contraceptive pill; have been carried out. Present communication reports proper authentication, taxonomic identification, organoleptic characters, ingredient identification, physico-chemical values and chromatographic profile; so as to set standards for quality assurance.

Key Words: Habb-e-Man-e-Hammal, Unani contraceptive, standardization

Introduction

Huboob (pills) are small, round and uniformly shaped medicinal preparations used in unani system of medicine (NFUM I). **Habb-e-Man-e-Hamal** is frequently prescribed by unani physicians to prevent conception. According to the formula composition, this drug contains five different plant ingredients (NFUM Part II). In order to lay down the standards for manufacturing the quality medicine, the drug was prepared in three different batches at laboratory scale. Present paper describes the salient features of preparation, micoscopical characters, physico-chemical and thin layer chromatography data of Habb-e-Man-e-Hamal.

Material and Methods

In order to develop the quality medicine with maximum therapeutic potential, all the ingredients were procured from the local raw drug dealers, New Delhi. After proper identification of each ingredient (by using pharmacognostical methods) Habb-e-Man-e-Hamal was prepared as per the formulation composition given in NFUM II (Anonymous, 2007)

Formulation Composition

S.No.	Ingredients	Botanical Name	Part used	Quantity
1.	Kalijiri	*Vernonia anthelmentica* Willd	Fruit	500g
2.	Tukhm-e-Halela Kabli	*Terminalia chebula* Retz	Seed	500g
3.	Nagkesar	*Mesua ferrea* Linn.	Floral bud	500g
4.	Narkachoor	*Zingiber zerumbet* Rosc.ex Smith	Rhizome	500g
5.	Shoneez (Kalonji)	*Nigella sativa* Linn.	Seed	500g
6.	Kaifal	*Myrica nagi* Thunb	Stem bark	500g

Preparation Method

All the ingredients taken were of pharmacopoeial quality. Cleaned, dried, powdered separately and sieved through a mesh no. 100. All the ingredients were then mixed together and kneaded with water to make the dough. The sticks of dough were made and rolled between the fingers to make the pills of approx. 500mg. size. Packed in tightly closed containers to protect from light and moisture. The formulation was prepared in three batches separately by the same method.

Microscopy

Few pills were broken into fine powder and mounted in different reagents to examine microscopically the various cells/ tissues/ cell contents. The representative photographs were taken from the computer with microscopic attachment (Johansen, 1940).

Chemical Analysis

All the prepared batch samples were subjected to chemical analysis. Physico-chemical studies like total ash, acid insoluble ash, solubility in alcohol and water, loss on drying at 105ºC were carried out (Anonymous, 1998).

Thin Lalyer Chromatography

Preparation of Extract for TLC

5g. powdered drug was extracted in 60 ml. of absolute alcohol under reflux of water bath for 10 minutes and filtered. Further, the filterate was concentrated upto 4 ml. and used for thin layer chromatography (Wagner *et. al.,* 1984).

Results and Discussion

Habb-e-Man-e-Hamal is brown coloured, tasteless, solid pills with pungent odour. The drug did not show any change or fungal growth when kept in a petri dish.

Microscopic Observation

Kalijiri: Non glandular trichomes, fragments of cotyledon filled with aleurone grains and oil globules, prismatic crystals of calcium oxalate and crystals bearing cellulosic fibers (Fig. 15-18)

Tukhm-e-Halela Kabli: Sclereids in groups, two types of sclereids one with highly thickened walls with numerous pits and striations, lumen small and other type of

sclereids with moderately thickened walls, numerous pits and wide lumen, fragments of endosperm with rosette shape calcium oxalate crystals having diameter 20.6µ - 47.8µ (Fig. 7-9)

Nagkesar: Numerous pollen grains which are spherical, smooth, thick walled, exine and intine distinct, some having 1-3 protuberances having diameter 65µ-82µ (Fig. 10)

Narkachoor: Trichomes simple, unbranched, thick walled, unseptate, parenchyma cells either single or in groups filled with starch granules (Fig. 12-14)

Kalonji: Fragments of endosperm cells filled with oil globules (Fig. 11)

Kaifal: Phloem fibers, crystal fibers, prismatic crystals of calcium oxalate measuring 32.5µ- 40µ (Fig. 1-6)

Fig. 1. Crystal fiber of *Myrica nagi* Thunb.x40

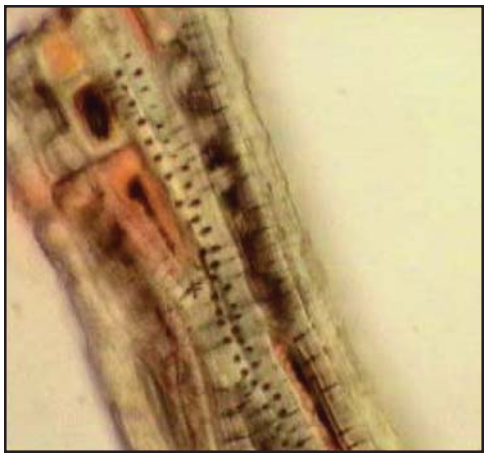

Fig. 2. Pitted fibre of *Myrica nagi* Thunb.x40

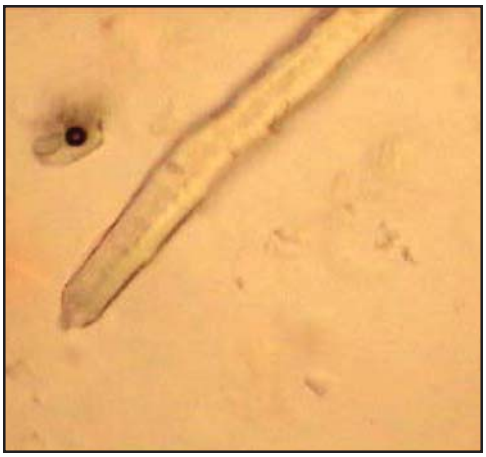

Fig. 3. Phloem fibre of *Myrica nagi* Thunb.x40

Fig. 4. Sclereids of *Myrica nagi* Thunb.x40

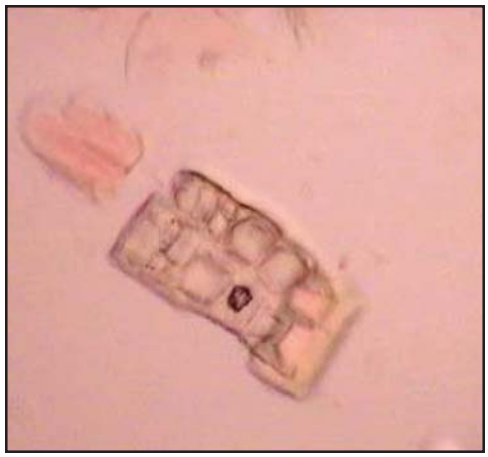

Fig. 5. Prismatic crystal of *Myrica nagi* Thunb.x40

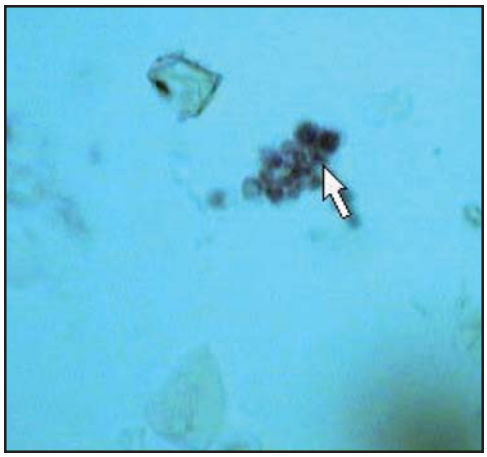

Fig. 6. Starch grains of *Myrica nagi* Thunb.x40

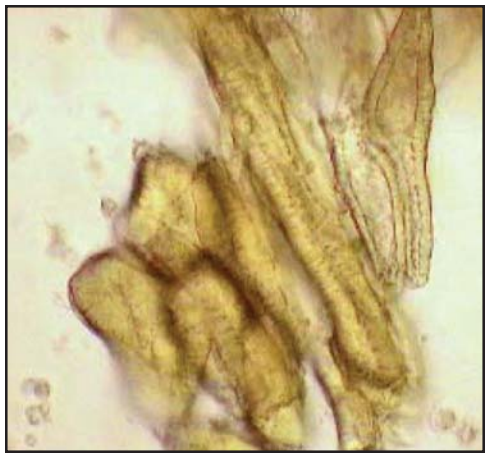

Fig. 7. Sclereids of *Terminalia chebula* Retz.x40

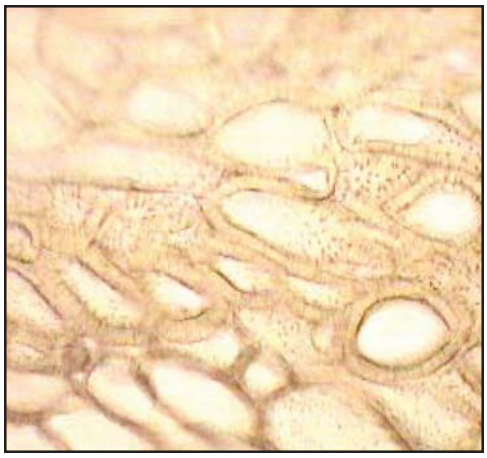

Fig. 8. Sclereids of *Terminalia chebula* Retz.x40

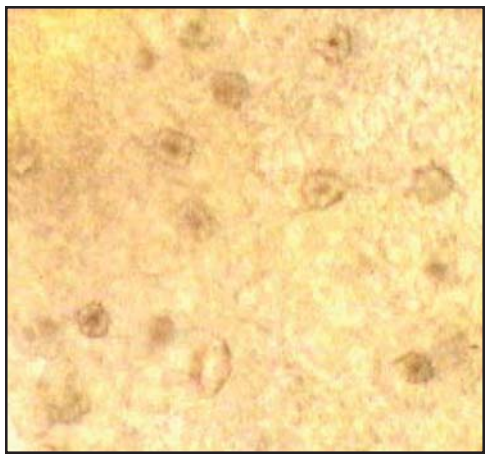

Fig. 9. Fragment of endosperm showing crystals in *T. chebula* Retz.x40

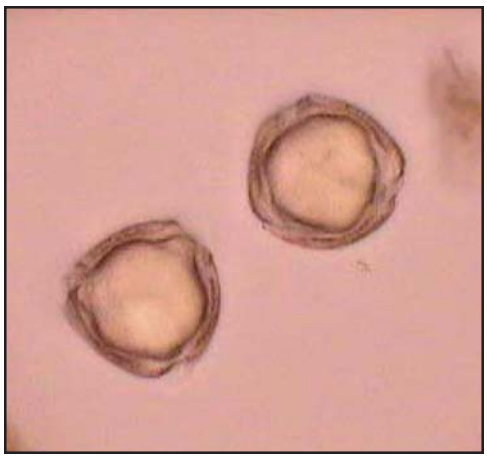

Fig. 10. Pollen grain of *Mesua ferrea* Linn.x100

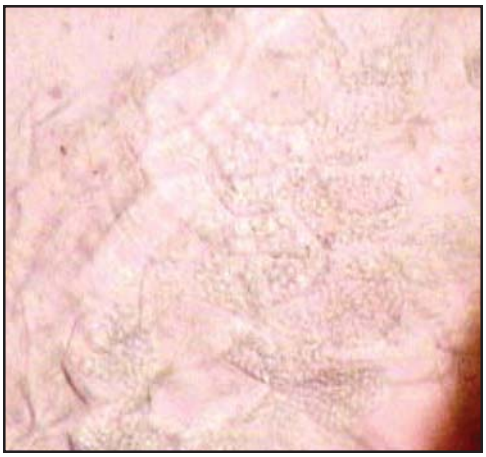

Fig. 11. Endosperm cells with aleurone grains and oil globules in *Nigella sativa* x40

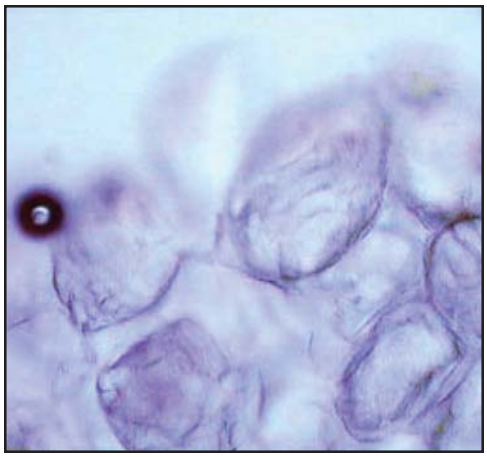

Fig. 12. Parenchyma cells with starch grains of *Zingiber zerumbet* Rosc. ex. Smith x40

Fig. 13. Trichome of *Zingiber zerumbet* Rosc. ex. Smith x40

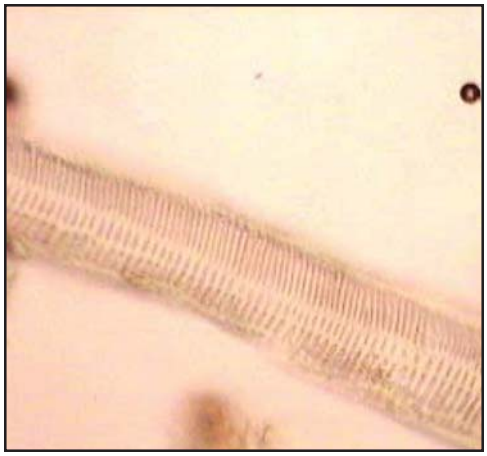

Fig. 14. Reticulate vessel of *Zingiber zerumbet* Rosc. ex. Smith x40

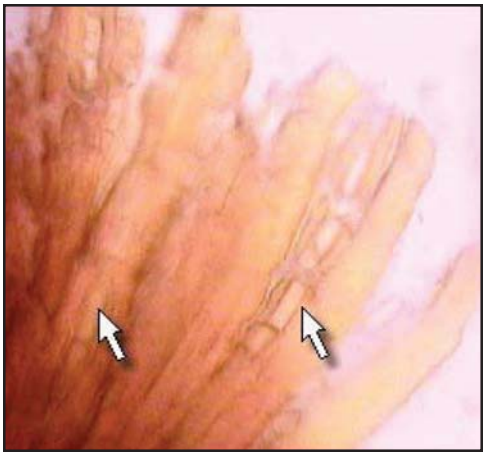

Fig. 15. Crystal fibre of *Vernonia anthelmentica* Willd. x100

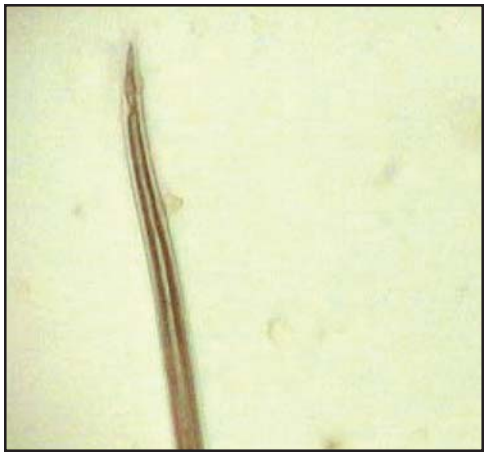

Fig. 16. Trichome of *Vernonia anthelmentica* Willd. x100

Chemical Analysis

Physico- chemical data of the drug are shown in Table-1.

Thin Layer Chromatography Analysis

The extract was applied on a pre- coated silica gel plate and the solvent system Toluene: Ethyl Acetate (70 : 30) was used in developing chamber to develop it. The plate was dried and sprayed with vanillin in sulphuric acid reagent and again dried

Table 1.

S.No.	Parameters	Batch No.		Batch No.		Batch No.	
		I	Mean Value	II	Mean Value	III	Mean Value
Extractives							
1.	Alcohol soluble matter	9.60 11.20 12.80	11.20	10.00 11.20 9.60	10.27	10.40 11.00 12.00	11.13
2.	Water soluble matter	17.60 17.60 18.00	17.73	18.00 19.60 20.40	19.33	19.60 23.00 18.00	20.20
Ash Values							
1.	Total ash	6.75 6.95 7.00	6.90	6.40 6.55 6.50	6.48	6.75 6.85 6.75	6.78
2.	Acid insoluble ash	2.90 3.00 3.00	2.97	2.90 2.75 2.95	2.87	2.90 3.00 3.00	2.97
3.	Loss in wt. on drying	8.60 7.77 9.10	8.49	9.25 8.80 9.00	9.02	8.65 9.05 9.25	8.98
pH Values							
1.	1% aq. Sol.	4.87 4.90 4.92	4.90	4.97 5.00 4.95	4.97	4.94 4.95 4.90	4.93
2.	10% aq. Sol.	4.38 4.42 4.41	4.40	4.32 4.38 4.30	4.33	4.34 4.34 4.38	4.35

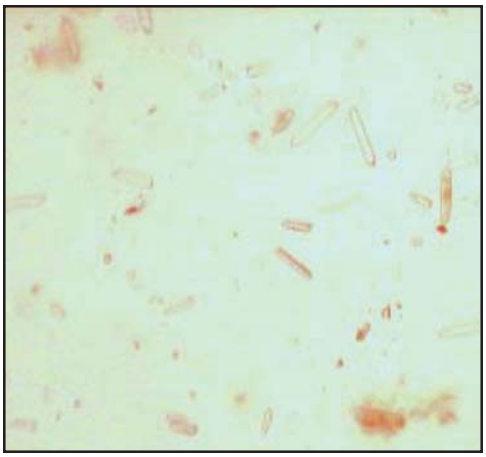

Fig. 17. Crystals of *Vernonia anthelmentica* Willd. x40

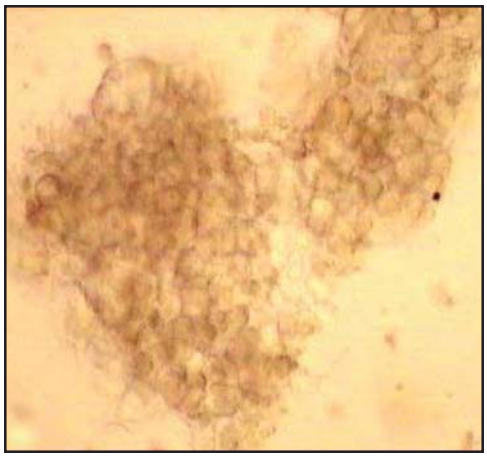

Fig. 18. Parenchyma cells with aleurone grains & oil globules of *Vernonia anthelmentica* Willd. x40

and kept in an oven for heating at 105ºC for 10 minutes. Rf. Values of the spots are 0.04; 0.23; 0.34; 0.38; 0.43; 0.47; 0.55; 0.72; 0.97.

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Ethnopharmacological Study of the Champawat Forests of Kumaon Region, Uttarakhand

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Abstract

Thirty-three plant species, used as folk medicine by indigenous people of Champawat district, are listed. For each plant, the family name, correct botanical and prevalent local names, voucher specimen, uses and therapeutic activity in folk medicine and mode of administration are given. Many uses have not been, hitherto, described.

Key Words: Ethnopharmacology, medicinal plants, Kumaon, Champawat, Uttarakhand.

Introduction

In various parts of Kumaon region, medicinal plants are still widely used as an alternative to pharmaceutical products which play an invaluable role in the maintenance and treatment of diseases among indigenous societies. This is the reason why a large number of information on folklore practices has been documented from this region of Uttarakhand (Agnihotri *et al.,* 2003; Ali *et al.,* 2008; Arya and Prakash, 1999; Arya *et al.,* 1999; Aswal, 1992; Bhatt and Gaur, 1992; Datt and Lal, 1993; Garbyal *et al.,* 2005; Gupta, 1960; Joshi *et al.,* 1993; Kalakoti and Pangtey, 1988; Pandey and Pande, 1990; Pandey *et al.,* 1995; Pant and Pandey, 1998; Rawat and Pangtey, 1988; Shah and Gupta, 1976; Shah and Jain, 1988; Shah and Joshi, 1971; Shah, 1982; Singh and Ali, 1998; Singh and Maheshwari, 1990, 1993, 1994; Singh *et al.,* 1980, 1987, 1997; Upreeti *et al.,* 2009). A perusal of this literature however reveals that no ethnobotanical exploration of Champawat district had previously been reported. Hence, the present report communicates some most commonly used herbal preparations, as a result of an ethnopharmacological survey carried out in this area of Uttarakhand.

Study Area

Champawat – a border district of Uttarakhand is situated on Indo-Nepal border and lying between 29º02' - 24º38'N latitude and 79º44'-80º25'E longitude in the outer Himalayan ranges (Fig. 1). It consists of seven forest ranges viz. Lohaghat, Kalikumaon, Champawat, Bhingara, Devidhura, Boom and Dugari with reserve forest covering an area of 6975.57 hectare. The entire district is a hilly terrain. It is inhabited by various indigenous castes and cultural groups (*Banrawat, Bhora, Chand, Bhat, Samant, Negi*). Agriculture and horticulture are the main occupations. Phytotherapy is a part of the cultural and social heritage of this area.

Methodology

The present investigation was carried out in November 2005, by interviewing traditional healers of good reputation and other knowledgeable village elders. Data

Fig. 1. Map of Champawat district showing the areas surveyed.

on common name of the plant or the crude drug, other ingredients added (if any), method of preparation and mode of administration were recorded for each species. Plant specimens along with relevant field information were collected. Plants were later identified by the senior author with the help of related floras (Gupta, 1968; Hooker, 1872-1897; Osmaston, 1972; Rau, 1975). Voucher specimens were prepared and deposited in the Herbarium of Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Aligarh (UP), India.

Results

In the course of this survey, some 187 taxa of medicinal plants were collected and identified. Out of these, 32 are best known in folk medicine and reported herein. Data on ethnomedicinal uses obtained during the fieldwork are summarized in Table-1. Species are arranged under their respective families which are listed alphabetically. For each plant are given the correct scientific and prevalent local names, voucher specimen number, plant part utilized, popular use(s) and mode of administration.

Table-1. Medicinal plants used by the natives of Champawat in Kumaon region.

Family, botanical and local names, voucher specimen no.	Part used	Use(s)	Mode of administration
AMARANTHACEAE *Achyranthes aspera* L., 'Apamarga', SMPA7343	Root	Asthma	Decoction is given orally.
ANACARDIACEAE *Lannea coromandelica* (Houtt.) Merrill, 'Gobarbhalya', SMPA7479	Leaf	Boil	Past is applied locally.
APIACEAE *Centella asiatica* (L.) Urban,'Barmi', SMPA7302	Leaves	As cicatrizant	Mash and applied externally.
APOCYNACEAE *Alstonia scholaris* (L.) R.Br., 'Chhatiyon', SMPA7453	Latex	Wounds	Fresh latex is obtained from the plant and applied locally.
ASTERACEAE *Artemisia nilagirica* (Clarke) Pamp.,'Kurja', SMPA7379 *Eupatorium adenophorum* Spr., 'Akali', SMPA7473	Leaves Leaves	Dandruff Sharp cut	Decoction is used to wash the hairs. Fresh paste is applied locally to check the bleeding.
BIGNONIACEAE *Oroxylum indicum* (L.) Vent., 'Pharee', SMPA7459	Seeds	Antidiarrheal (veterinary use)	Powder is given orally with fodder.
CORIARIACEAE *Coriaria nepalensis* Lam., 'Gingharu', SMPA7481	Fruits	Boils	Paste is applied locally for suppuration.

Family, botanical and local names, voucher specimen no.	Part used	Use(s)	Mode of administration
DIOSCOREACEAE *Dioscorea bulbifera* L., 'Gethi', SMPA7481	Bulbils	Anorexia	Potato like bulbils are cut into pieces, cooked and eaten.
ERICACEAE *Rhododendron arboreum* Sm., 'Burans', SMPA7448	Flowers	As cardiac tonic, refreshing and for gastric trouble	Sherbet is taken orally.
FABACEAE *Glycine max* (L.) Merr., 'Kala Bhatt', SMPA7327	Seeds	Jaundice	Socked seeds are cooked and taken.
FAGACEAE *Quercus lanuginosa* D. Don, 'Riyanj', SMPA7342	Leaves	For deficient lactation in buffaloes and cows	Fresh leaves are fed as fodder.
HYPOXIDACEAE *Hypoxis aurea* Lour., 'Lehsanjari', SMPA7439	Root	Joint pain	Paste is applied externally.
LILIACEAE *Asparagus curillus* Buch.-Ham. ex Roxb., 'Alora', SMPA7341	Root	As galactagogue	Paste is taken orally.
MORACEAE *Ficus auriculata* Lour., 'Timla', SMPA 7473 *Ficus subincisa* Buch.-Ham., ex J. E. Smith, 'Dudhila', SMPA7474	Fruits Leaves	Dysentery Galacta-gogue	Paste of boiled fruits is mixed with curd and eaten. Fresh leaves are fed to cows.
MYRICACEAE *Myrica esculenta* Ham., 'Kaiphal', SMPA7340	Fruits	As laxative	Paste of ripe fruits is taken.
ORCHIDACEAE *Dactylorhiza hatagirea* (D. Don) Soo, 'Salam misri', SMPA7351 *Vanda tessellata* (Roxb.) D. Don, 'Hadjojan', SMPA7427	Root Aerial parts	For general weakness Bone fracture	Powder is taken orally. Paste is used as plaster around the fractured limb.

Family, botanical and local names, voucher specimen no.	Part used	Use(s)	Mode of administration
PINACEAE *Pinus roxburghii* Sarg., 'Chir', SMPA7345	Oleo-resin	Scabies	Fresh material is applied to affected area.
RANUNCULACEAE *Thalictrum foliolosum* DC., 'Pilijari', SMPA7303	Root	Redness of eye	Fresh paste alone or with 'Padam' (fruit of *Prunus cerasoides* D. Don) is applied in the affected eye.
ROSACEAE *Rubus ellipticus* Sm., 'Hinsalu', SMPA7450	Root	Abdominal pain	Decoction is taken.
RUBIACEAE *Rubia manjith* Roxb. ex Flem., 'Jatkura', SMPA7301	Root	Common cold of children	Root is rubbed on metallic plate and paste is given orally.
RUTACEAE *Zanthoxylum armatum* DC., 'Temur', SMPA7330	Stem	For oral hygiene	Tender twig is used as toothbrush.
SAPOTACEAE *Diploknema butyracea* (Roxb.) Lam.,'Chieura', SMPA7368	Seed oil	For cracked skin	Oil is lightly messaged on affected area.
SCROPHULARIACEAE *Verbascum thapsus* L., 'Ekalbeer', SMPA7307	Leaf	Common cold and cough of children	Lukewarm paste is applied on chest.
SOLANACEAE *Solanum melongena* L. var. *incanum* (L.) O. Kuntze, 'Bhatt', SMPA7364	Leaf	Jaundice	Fresh juice is taken orally.
URTICACEAE *Girardia diversifolia* (Link) Fries, 'Al', and 'Chhina', SMPA7457	Aerial parts	Galacta-gogue for cows	Fresh material is cooked and given with fodder.

Family, botanical and local names, voucher specimen no.	Part used	Use(s)	Mode of administration
VERBENACEAE *Callicarpa macrophylla* Vahl, 'Dayya', SMPA7451	Fruits	Stomatitis	Ripe fruits are chewed.
Pygmaeopremna herbacea (Roxb.) Moldenke, 'Raktpatiya', SMPA7353	Leaves	For worm infestation in children	Juice is given orally.
ZINGIBERACEAE *Hydichium spicatum* Buch.-Ham. ex Sm., 'Bansupari', Kapoor Kachri, SMPA7331	Rhizome	Renal calculus	Fresh material is chewed and taken.
Zingiber roseum (Roxb.) Roscoe, 'Banhaldi', SMPA7332	Rhizome	Cough	Fresh paste is given orally.

Discussion

The reported plant species belong to 32 genera of 27 different families of angiosperms. The analysis of data obtained has revealed that knowledge of medicinal plants and their curing properties is usually limited only to a few elderly people who have long been using these plants in healthcare of humans and cattle. These adults generally teach the youngers about the importance and uses of medicinal plants. In spite of this, ancestral knowledge is decreasing from one generation to another. On the other hand, there is a threat to some of the wild medicinal taxa due to continued exploitation, unsustainable harvesting practices and habitat destruction. In this situation, this traditional knowledge is in danger of being lost. It was, therefore, considered worthwhile to document this information before some of the medicinal plants become extinct or their uses forgotten as a result of acculturation of indigenous people.

Data on medicinal uses were compared with the available literature (Ambasta, 1986; Anonymous, 2001; Chopra *et al.,* 1956; Jain, 1991; Kirtikar and Basu, 1935; Nadkarni, 1954). It was found that most of these uses are, hitherto, unreported. It is, therefore, desirable to investigate these plants scientifically, in the context of reported claims. The objectives of ethnopharmacology are to rescue and document the important cultural heritages before these are lost and to investigate as well as evaluate the agents employed. Thus, it plays an immense role in the evaluation of natural products and more particularly the herbal drugs from traditional and folklore resources (Cordell and Colvard, 2005). Many potent drugs have origin in traditional

Fig. 2. Bhatt (*Solanum melongina* L. var. *incanum* (L.) O. Kuntze)

Fig. 3. Burans (*Rhododendron arboreum* Sm.)

Fig. 4. Dayya (*Callicarpa macrophylla* Vahl)

Fig. 5. Gethi (*Dioscorea bulbifera* L.)

Fig. 6. Hadjojan (*Vanda tessellata* (Roxb.) D. Don)

medicine and ethnopharmacology. Such knowledge can still serve as an innovative and powerful discovery engine for newer and affordable medicines (Patwardhan, 2005). The aim of present study is to report the information on most commonly used medicinal plants from the Champawat district. With it we hope to contribute to the rich herbal heritage of Kumaon region of Uttarakhand as well as to provide access to the researchers in development and search of new pharmaceuticals of natural origin.

Acknowledgements

We thank Dr. Mohammad Khalid Siddiqui, Director General, Central Council for Research in Unani Medicine, New Delhi for necessary facilities and funds for the present survey and to Mr. Rajmany Pandey, Divisional Forest Officer, Champawat

Fig. 7. Jatkura (*Rubia manjith* Roxb. ex Flem.).

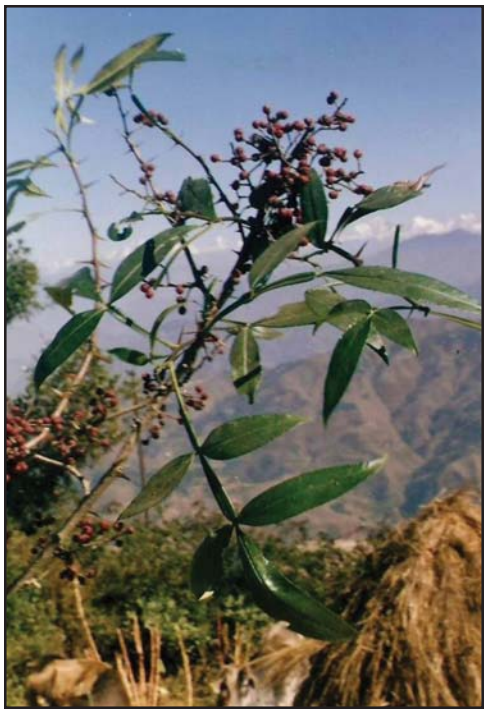

Fig. 8. Temur (*Zanthoxylum armatum* DC.)

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Kala-Azar (Leishmaniasis) and its Management in Unani Medicine

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Abstarct

Kala azar is a chronic and potentially fatal parasitic disease of viscera (the internal organs particularly liver, spleen) bone marrow and lymph nodes. It is caused by the bite of sand flies. In unani system of medicine it is described under the heading of *Humma-e-Aswad* (Black fever) and *Yarqaan-e-Aswad* (Black jaundice). Unani physicians has described its etiology, pathology and other related symptoms and also the management of this disease.

Key Words: Kala Azar, Leishmaniasis, Humma-e-Aswad, Yarqaan-e-Aswad etc.

Introduction

The term "*Kala-azar*" is a combination of words of two different languages (Hindi & Persian). Kala is a Hindi word means black and Azar is a Persian word means trouble/disease. It is also known as Black Fever (Boyd 1990). Kala azar is a chronic and potentially fatal parasitic disease of viscera (the internal organs particularly liver, spleen) bone marrow and lymph nodes. In Unani system of medicine it has been described by many terms i.e. *Humma-e-Aswad* (*Humma* means Fever, *Aswad* means Black), *Humma-e-Ruba'* (*Ruba'* means four). (A type of remitant fever in which fever relapses on fourth day), and *Yarqaan-e-Aswad* (Yarqan means Jaundice). This is a fever due to the predominance of *Khilt-e-Sauda* (black bile) characterized by the blackish discoloration of skin. (Khan, 1290H, Ibn Rushd, (1126-1198 AD) 1987, Tabri, (810-895 AD) 1417, Razi, (841-926 AD) 2000, Farahi, 1999, Ahmad, undated), Chandpuri, 1984, Kabiruddin, 1959, Ajmali, 1949, Ahmad, 1942, Jurjani, (11th century AD) 1903, Arzani, 1870, Ibne Sina, (980-1037 AD) (1317H).

History of Kala Azar

Description of cutaneous leishmaniasis has been discovered on tablets from King Ashurbanipal from the 7th century BC. (Cox 1996). Cox has quoted in his book "*The welcome trust illustrated history of tropical disease*" that Muslim physician of 10th century, Ibn-e-Sina described this disease as Qurooh-e-Balkhiya (Ghaznavi 1993). Although cutaneous leishmaniasis can be traced back many hundreds of years, one of the first and most important clinical descriptions was made in 1756 by Alexander Russell following an examination of a Turkish patient.

In the old World, Indian physicians applied the Sanskrit term Kala azar (meaning "***black fever***)" to an ancient disease later defined as visceral leishmaniasis (Anonymous, 2007).

Who first discover this organism is somewhat dispute. Surgeon major Cunnigham of British Army saw it first in 1885 with out being able to relate it to the disease (Cunnighma 1885, Cox 2002). In 1901, Leishman identified certain organism in

smears taken from the spleen of a patient who had died from **"*Dum dum fever*".** At the time "Dum-dum", a town not far from Calcutta, was considered to be particularly unhealthy. The disease was characterized by general debility, irregular and repetitive bouts of fever, severe anemia, muscular atrophy and excessive swelling of the spleen. Initially, these organisms were considered to be trypanosomes, but in 1903 Captain Donovan described them as being new. The link between these organisms and Kala azar was eventually discovered by Major Ross, who named them *Leishmania donovani* (Bora, 1999).

Etiology in Unani Medicine

According to the Unani classical literature *Humma-e-Aswad* is caused due to the putrefaction of humours especially *Khilt e Sauda* (Black bile). (Tabri, (810-895 AD) 1417, Ibn-e-Sina (980-1037 AD) 1926, Jurjani (11th century AD) 1903, Ajmali 1949, Razi 2000, Jeelani, undated). (In Unani medicine, this disease is included under *Amraz-e-Tarsili* (Communicable diseases) (Ibn Rushd, (1126-1198 AD) 1987). *Ismail Jurjani* (11 century AD) has written clearly in his book *Zakherah-e-Khawarizam Shahi* that *Yarqan-e-Aswad* can be developed some time due to the bite of *Hasharaat* (insect) (Jurjani 1903). He has clearly indicated the involvement of the spleen in this disease. He has written that due to the change of *Safra* (bile) into *Sauda* (black bile) in spleen there is *Azm-e-Tihal* (splenomegaly) (Jurjani 1903).

According to the author of *Makhzan-ul-Jawahar*, this disease is usually caused by a specific organism and spread by the bite of specific fly. He has not described about the blackish discoloration of the skin (Jeelani, Undated).

Rabban Tabri (810-895 AD) has written in his book *Firdaus Al Hikmat* "this type of fever occurs when the black bile becomes putrefied and enters into the vascular system". It can be caused by (1) Disturbance in the metabolism and conversion of humours into black bile. (2) Temperament of the individual that is cold and dry and hot and dry. (3) Fatigue. (4) Emotional disturbances. (5) Excessive use of things that produce black bile (Sauda) e.g. diets which are cold and dry and hot and dry in temperament. (6) Prolonged illness or fever of any other kind (Tabri 1417).

Unani physician *Ibne-Zohr* (1091-1162 AD) mentioned in his book *Kitab-al-Taisir-fil Mudawat-wat-Tadbir* that *Humma-e-Saudavi* generally develops in persons with *Saudavi* temperament i.e. predominance of *Khilt-e-Sauda* which can be congenital, or due to the use of foods which help in excess production of *Khilt-e-sauda* (Black Bile). i.e. paneer, red meat, meat of camel, donkey, water animals and fish of stagnant water, and dried meat of animals (Ibne Zohr 1986).

According to *Buqrat* (460 BC) this disease commonly occurs in autumn season because in this season there is more dryness, which in turn favours the production of *Sauda* (black bile) (Ibn Rushd 1987).

Signs and symptoms in Unani medicine

Unani Scholars described the signs and symptoms of *Humma-e-Aswad* similar to that of visceral leishmaniasis (Kala Azar). e.g. (*Azm-e-Tihal, Warm-e-Tihal*) Splenomegaly, (*Azm-e-kabid, warm-e-Kabid*) Hepatomegaly, *Humma-e-Nobati* (remittent fever), Loss of appetite (*Zof-e-Ishtiha*), Blackish skin (*Taghayyur-e-Jild*), bleeding from nose and gum (*Ruaaf* and *Nafsud Dam*), Sore (*Qurooh*), etc. In *Al Akseer* Hakim Azam Khan has mentioned about the sign and symptoms of *Yarqaan-e-Aswad* similar to that of *Kala Azar* (Khan 1290H). *Zakaria Razi* wrote in his book *Al-Hawi Fit Tib* with reference to Jalinoos (95 AD) (Galen) that when both spleen and liver are involved then only *Yarqan-e-Aswad* (*Black Jaundice*) occurs. (Razi (841-93-26 AD) 2000, Khan 1290 H).

According to *Firduas-Al-Hikmat* when the putrefied black bile exits outside the vascular system the fever will be of remittent variety. He also mentioned sign and symptoms similar that of Kala Azar e.g. urine is colourless initially, but it become blackish in later stage, splenomegaly, blackish discoloration of skin, rate of pulse decreases due to the predominance of sauda (black bile) (Tabri 1417 AD). According to the author of *Makhzan-ul-Jawahar* this type of fever is usually remittent, low grade, and it characterized by splenomegaly and hepatomegaly. (Jeelani (YNM)).

Geographical distribution

Currently the leishmaniasis (Kala Azar) is prevalent in 88 countries, 72 of which are developing countries, 90% of all visceral leishmaniasis (Kala Azar) cases occur in Bangladesh, Brazil, India, Nepal and Sudan. 90% of mucocutaneous leishmaniasis (Kala Azar) occurs in Bolivia, Brazil and Peru. 90% of cutaneous leishmaniasis (Kala Azar) cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria. In India it occurs most in the states of Bihar and West Bengal (Bora, 1999).

The annual estimate for the incidence and prevalence of *Kala-Azar* cases worldwide is 0.5 million and 2.5 million, respectively. Of these, 90% of the confirmed cases occur in India, Nepal, Bangladesh and Sudan. In India, it is a serious problem in Bihar, West Bengal and eastern Uttar Pradesh. In these region women and children 0-9 years of age are generally affected by this disease (Anonymous, 2007).

Incubation period

The incubation period in man is quite variable, generally 1 to 4 months; a range is 10 days to 2 years (Park, 2002).

Types of leishmaniasis (Kala Azar)

1. Visceral leishmaniasis (Kala Azar): This is the most serious form of *Kala Azar* and potentially fatal if untreated. More than 90% of the world's cases of visceral

leishmaniasis (Kala Azar) occur in Bangladesh, northeastern India (particularly Bihar State), Nepal, Sudan, and northeastern Brazil (Heslett, *et. al.* 2002). The organisms can be transmitted not only by sand flies but also congenitally and parenterally (e.g. through blood transfusions or needle sharing). Infection begins in macrophages at the inoculation site (e.g. in dermal macrophages at the site of a sand fly bite) and disseminates throughout the reticuloendothelial system. Whereas the general term visceral leishmaniasis (Kala Azar) covers a broad spectrum of severity and manifestations. These are Cachexia, Fever, Splenomegaly, Hepatomegaly, Peripheral lymphadenopathy (Herwaldt, 1999, Braunwald, *et. al.* 2001). These symptoms of Kala azar are in accordance with the saying of Buqrat quoted in *Kitab-ul Kulliyat* by *Ibn Rushd* that when ever there is splenomegaly it weakens the body system leading to Cachexia. (Ibn Rushd, 1987).

2. Cutaneous leishmaniasis (Kala Azar): The most common manifestation of this form is a sore at the site of bite, which heals in a few months to a year, leaving an unpleasant looking scar (Goldman & Bennett, 2001; Braunwald, *et. al.,* 2001). This form can progress to any of the other three forms. In classical unani literature, it is also known as Qurooh-e-Balkhiya, Lahori Phora, Dilli ka Phora, Qandhari Phora, and Baghdadi Phora etc. This condition occurs after fever. (Ibne Sina, 1317H; Park, 2002; Herwaldt, 1999; Goldman & Bennett, 2001; Cotron, 2000; Braunwald, *et. al.,* 2001; Ghaznavi, 1993).

3. Diffuse cutaneous leishmaniasis (Kala Azar): This form produces widespread skin lesions which resemble leprosy and is particularly difficult to treat (Herwaldt, 1999; Goldman & Bennett, 2001; Cotron, 2000; Braunwald, *et. al.,* 2001).

4. Mucocutaneous leishmaniasis (Kala Azar): This form commences with skin ulcers which spread causing tissue damage to (particularly) nose and mouth (Park, 2002; Herwaldt, 1999; Goldman & Bennett, 2001; Cotron, 2000; Braunwald, *et. al.* 2001).

Laboratory examination

Laboratorial advances and investigational processes may help to diagnose this disease easily.

- Demonstration of parasites in a biopsy specimen obtained from bone marrow, Lymph node or preferably spleen.
- Culture of biopsied materials to confirm the presence and absence of Leishmania. Animal inoculation is done if culture facilities are unavailable.
- Serology for anti-Leishmania antibodies. This includes IFA (Indirect fluorescent antibody) and ELISA (Enzymes linked immunosorbent Assay).
- Aldehyde Test.

- Hematological finding: These include progressive leucopenia, anemia and reverse albumin-globulin ratio with greatly increased IgG. The WBC:RBC ratio is 1:1500 or even 1:2000, ESR is increased (Lawrence, *et. al.* 2005; Park, 2002).

Prevention and control

The transmission of Leishmania species is typically focal, because of the limited flight range of sand flies. These insects usually remain within a few hundred meters of their breeding site. They rest in dark, moist places, in habitats ranging from deserts to rain forests; peridomestic sand flies rest in debris, so vector control may be useful to a great extent. (Braunwald, 2001).

Personal protective measures include avoiding outdoor activities when sand flies are most active (dusk to dawn); using mechanical barriers such as screens and bed-nets that keep out sand flies, which typically are about one-third the size of mosquitoes; wearing protective clothing; and applying insect repellent to exposed skin. Impregnating clothing, bed-nets, and screens with pyrethrum may also be useful, as may spraying dwellings with insecticide, if transmission of infection is intra or per domiciliary. (Braunwald, 2001).

Management

India is one of the world's largest foci of *Kala Azar* (Visceral Leishmaniasis), accounting for 50% of the total burden of this disease. In March 2002, the country's regulatory authorities registered the first oral drug, milefosine, for the treatment of *Kala Azar* (Visceral Leishmaniasis), marking a true breakthrough for India's National Leishmaniasis (*Kala Azar*) Control Programme. The drug is relatively safe and highly effective, achieving cure rates of up to 98% (More, *et. al.* 2003). However, the drug does have some gastrointestinal side-effects, mainly vomiting and diarrhoea, and due to its potential tetrogenicity, it should not be given to pregnant women. It does require refrigeration for storage and has been used successfully to treat cases resistant to conventional antimony therapy. Use of *Kushta-e-Asmad* (Calx of Antimony) is well documented in Unani literature (Ajmali, 1949). It was practiced for more than 50 years. India has now launched programmes to eliminate visceral leishmaniasis (*Kala Azar*) from the country by 2010. In this regard Unani system of medicine also provides some very effective treatment for this disease which is well documented in its classical text. These treatments include regimental therapy such as the evacuation of dominant putrefied black bile from the body. Some of the formulations are used orally and some locally for the treatment of this disease. Since dietotherapy is one of the most important parts of the treatment in Unani medicine, dietary recommendation and restrictions are also there.

Ilaj Bid Tadbir (Regimental Therapy)

Regimental therapies which are recommended in Kala Azar patients are:

Fasad (Venesection) is avoided in these patients and patients are advised to take *Hammam* (Turkish bath).

Massage with Roughan-e-Gul-e-Khairi (*Lavandula officinalis* Chaix)

Enema if necessary is given with the oil of Walnut (Akhroot) (*Juglans regia* Linn.) Sweet Almond (Badam) (*Prunus amygdalus* Batsch).

Emesis is recommended in the initial days of month or the last days, because in these two periods waste materials get accumulated in the body. (Tabri 1417)

Ilaj Bid Dawa (Pharmacotherapy)

Some words used in the formulation are (Baikh=Root, Shagufa=Bud, Gul=Flower, Barg=Leaf, Tukhm=Seeds, Post=Peel, Usara=Extract, Samar=Fruit, Maghz=Pulp,)

Following compound formulations are mentioned in Unani classical and other Unani texts. Which are may be beneficial for this disease.

1 Formulations

Dawa-e-Mushil (Jurjani 1903, Khan, 1290)

Halela	*Terminalia chebula* Retz.	54 gm
Halela Kabli	*Terminalia chebula* Retz.	24 gm
Beekh-e-Ajmood	*Apium graveolens* Linn.	24 gm
Badiyaan	*Foeniculum vulgare* Mill.	24 gm
Khurbaq Siyah	*Helleborus niger* Linn.	7 gm
Bisfaij	*Polypodium vulgare* Linn.	14 gm
Shagufa-e-Kibr	*Capparis spinosa* Linn.	21 gm
Usquloqandaryon		21 gm
Maveez Munaqqa	*Vitis vinifera* Linn.	72 gm
Aaolo Bukhara	*Prunus domestica* Linn.	20 nos
Tamar Hindi	*Tamarindus indica* Linn.	36 gm
Aftimoon	*Cuscuta epithymum*	17.5 gm
Decoction of it in 252 gm adjuvant of		
Iyarij		3.5 gm
Gariqoon	*Agaricus alba*	1.75 gm
Nasoot	*Operculina turpethum* Linn.	3.5 gm

Nuskha-e-Zimaad (Jurjani, 1903)

Shagufa-e-Izkhar	*Cymbopogon citratus* (DC. ex Nees) Stapf	36 gm
Shagufa-e-Haasha	*Thymus serpyllum* Linn.	36 gm
Afsanteen	*Artemisia absinthium* Linn.	36 gm
Qirdmaana	*Carum carvi* Linn.	36 gm
Qanturiyoon	*Centauria centaurium*	36 gm
Baikh-e-Kibr	*Capparis spinosa* Linn.	36 gm
Gul-e-Surkh	*Rosa damascena* Mill.	54 gm
Guggul	*Commiphora wightii*, Hook. ex Stocks	17.5 gm
Ushaq	*Dorema ammoniacum* D. Don.	24 gm
Vinegar	QS	

Except Ushaq (*Dorema ammoniacum* D. Don.) and Guggul (*Commiphora wightii*, Hook. ex Stocks) all above drugs are grounded and sieved thereafter Guggul (*Commiphora wightii,* Hook. ex Stocks) and Ushaq (*Dorema ammoniacum* D. Don.) are dissolved in vinegar, the grounded powder are mixed with it and prepared as zimaad. Before applying this ointment the affected part should be washed with the decoction of Sowa (*Anethum sowa* Roxb. ex Flem.), Sudab (*Ruta graveolens* Linn.), Pudina (*Mentha arvensis* Linn.) and vinegar.

Sikanjabeen (Ibn-e-Sina, 1926)

Baikh-e-Kasni	*Cichorium intybus* Linn.	1 part
Baikh-e-Ajmood	*Apium graveolans* linn.	1 part
Kalonji	*Nigella sativa* Linn.	1 part
Badiyan Roomi	*Foeniculum vulgare* Mill.	1 part
Baikh-e-Badiyan	*Foeniculum vulgare* Mill.	1 part
Vinegar		

All the above ingredient are taken in equal part and boiled in Vinegar and prepared as Sikanjabeen

2 Formulation (Azmi, 1997)

In the initial stage of disease following treatment is recommended

Gul-e-Banafsha	*Viola odorata* Linn.	6 gm
Barg-e-Jhaoo	*Tamarix articulata* Vahl.	6 gm
Barg-e-Shahtra	*Fumaria officinalis* Linn.	6 gm
Tukhm-e-Kasni	*Cichorium intybus* Linn.	6 gm

Tukhme Khatmi	*Althaea officinalis* Linn.	6 gm
Mako Khushk	*Solanum nigram* Linn.	6 gm

All of these ingredients are soaked over night in hot water and strained in the morning. Thereafter it is mixed in 25 ml of Sharbat Buzoori and taken orally.

If patient is mildly febrile then add

Tukhme-Sambhaloo	*Vitex negundo* Linn.	3 gm
Afsanteen Roomi	*Artemisia absinthium* Linn.	3 gm
Anjeer Zard	*Vitis vinifera* Linn.	3 nos

In place of Tukhme Khatmi (*Althaea officinalis* Linn.) and Gul-e-Banafsha (*Viola odorata* Linn.)

If there is diarrhea and any symptom of Ascitis accompanies with fever then the treatment will be (Azmi, 1997)

Shikai	*Onopordum nervosum*	3.5 gm
Tukhm-e-Kasni	*Cichorium intybus* Linn.	5 gm
Baikh-e-Kasni	*Cichorium intybus* Linn.	5 gm
Tukhme Kharpaza	*Cucumis melo* Linn.	5 gm
Khar-e-Khask Khurd	*Tribulus terrestris* Linn.	5 gm
Tukhme Kasoos	*Cuscuta reflexa* Roxb.	3 gm (tied in a cloth pouch)
Gul-e-Ghafis	*Gentiana dahurica* Fisch.	3 gm

These all are soaked in water treated with iron over night it is then taken orally in the morning after straining and sprinkling 6 gm Bartang (*Plantago lanceolata* Linn.) over it.

Since this disease is due to the predominance of Khilt Sauda (black bile) then it is necessary to include Munzij Mushily therapy (MMT) in its treatment.

Munjiz-e-Sauda is: (Azmi, 1997)

Shahtra	*Fumaria officinalis* Linn.	7 gm
Baadranjboya	*Nepeta hindostana* Haines	7 gm
Badiyaan	*Foeniculum vulgare* Mill.	7 gm
Gaozubaan	*Onosma bracteatum*	5 gm
Asl-us-soos Muqashahar	*Glycyrrhiza glabra* Linn.	5 gm
Unnab	*Zizyphus jujuba* Linn.	5 Nos.
Sapistan	*Cordia myxa* Linn.	9 Nos.

These drugs are soaked in hot water over night and taken with 25 gm of Gulqand in morning. It is followed by Mushil-e-Sauda which is: (Azmi, 1997)

Iyarij-e-Faiqra	31 gm	
Lajward Maghsool	*Lapis lazuli*	4 gm
Ghariqoon	*Agaricus Alba*	4 gm
Mash Safaid (Urad)		4 gm
Gugul	*Commiphora wightii* Bhan.	4 gm
Turbud Safaid Mudabbar	*Operculina turpethum* Linn.	4 gm
Kateera	*Astragalus gummifer*	4 gm
Post Halela Zard	*Terminalia chebula* Retz.	4 gm

All the above drugs are ground and after sieving, fried in 75 ml of Roughan-e-Badam Shireen (*Prunus dulcis* Mill.) and made as pills of 3-7 Gms

Then these are wrapped in silver foils. In this form it is taken in midnight in the dose of 3 pills with warm water.

In the morning the following formulation is given

Sheer-e-Khisht	*Fraxinus ornus* Linn.	40 gm
Gulqand Aftabi	*Rosa damascena* Mill.	40 gm
Magz Faloos Khayarshamber	*Cassia fistula* Linn.	60 gm

These are mixed in Aab-e-Barg-e-Kasni Sabz Murawwaq (*Cichorium intybus* Linn.), Aab-e-Badiyan Sabz Murawwaq (*Foeniculum vulgare* Mill.) 200 ml each and Roughan-e-badam Shireen (*Prunus amygdalus* Mill.) 4 ml and taken orally.

3 Formulation (Azmi, 1997)

Arq-e-Kasni	*Cichorium intybus* Linn.	50 ml
Arq-e-mako	*Solanum nigram* Linn.	50 ml
Arq-e-shahtra	*Fumaria officinalis* Linn.	50 ml
Sharbat Buzoori		25 ml

These are mixed and taken orally

4 Formulation (Azmi, 1997)

Aab-e-Barg-e-Karanjwa	*Caesalpinia bonducella* Flem.	50 ml
Aab-e-Mako	*Solanum nigram* Linn.	50 ml
Aab-e-Kasni	*Cichorium intybus* Linn.	50 ml

All are Sabz-e-Murawwaq mixed and taken orally in morning

In case of Aazm-e-Kabid following formulation is used: (Azmi 1997)

Badiyan	*Foeniculum vulgare* Mill.	6.5 gm
Afsanteen	*Artemisia absinthium* Linn.	3 gm
Bisfaij Fisatqi	*Polyjpodium vulgare* Linn.	5 gm
Tukhm-e-Kasoos	*Cuscuta reflexa* Roxb.	5 gm (tied in a cloth pouch)
Gul-e-Ghafis	*Gentiana dahurica* Fisch.	5 gm
Mako Khuskh	*Solanum nigram* Linn.	6 gm
Tukhm-e-Kasni	*Cichorium intybus* Linn.	6 gm
Barg-e-Gaozabaan	*Onosma bracteatum*	4 gm
Maveez Munaqqa	*Vitis vinifera* Linn.	9 nos

All these drugs are soaked in water over night and boiled in morning. After straining it is taken orally with 25 ml Sharbat-e-Dinaar.

5 Formulation (Ajmali, 1949, Jeelani, undated)

Qurs-e-Shifa

Gul-e-Surkh	*Rosa damascena* Mill.	25 gm
Zarishk	*Berberis aristata* DC.	12 gm
Tukhm-e-Khyarain	*Cucumis melo* Linn.	7 gm
Tukhm-e-Khurfa	*Portulaca oleracea* Linn.	7 gm
Kafoor Qaisoori	*Cinnamomum camphora* Linn	3 gm
Usara-e-Afsanteen	*Artemisia absinthium* Linn.	3 gm
Zafraan	*Crocus sativus* Linn.	3 gm
Irsa	*Iris ensata* Thunb.	3 gm
Sumbulutteeb	*Nardostachys Jatamansi* D.C.	3 gm
Revand Chini	*Rheum officinale* Baill.	3 gm
Lac Maghsool	Lac	3 gm
Tabasheer	*Bambusa arundinacea* Retz.	3 gm
Samara-ut-Turfa		3 gm
Usara-e-Ghafis	*Gentiana dahurica* Fisch.	2 gm

All are ground and made into pills with the help of Luaab-e-Behidana (*Cydonia oblonga* Mill.).

It is taken orally in the dose of 5 gm adjuvant of Aab-e-Kasni Sabz Murawwaq (*Cichorium intybus* Linn.) Aab-e-Shahtra Sabz Murawwaq (*Fumaria officinalis* Linn.) Sikanjabeen-e-Bazoori 60 and 25 ml respectively.

6 Formulation (Ajmali, 1949)

Habb-e-Sammul Faar

Sammulfar	Arsenic oxide	1 gm
Aahak	Lime	12 gm
Kaat Safaid	*Acacia leucophloea*	12 gm

All these are made in the form of pills (similar that of Bajra) with the help of Arq-e-Lemo (*Citrus limo* (Linn.) Burm.f.)

It is taken 1 pill with water (before onset of fever)

7 Formulation (Azmi, 1997)

For Aazm-e-Tihaal

Barg-r-Jhaoo	*Tamarix articulata* Vahl.	12 gm
Maghz-e-Kadu Sheerin	*Cucurbita moschata*.	12 gm
Tukhm-e-Khurfa	*Portulaca oleracea* Linn.	12 gm
Tukhm-e-Kasni	*Cichorium intybus* Linn.	12 gm
Tukhm-e-Kharpaza	*Cucumis melo* Linn.	12 gm
Tukhme-Qurtum	*Carthamus tinctorius* Linn.	12 gm
Badiyan	*Foeniculum vulgare* Mill.	12 gm
Tukhm-e-Sambhaloo	*Vitex negundo* Linn.	12 gm
Sumbul ut Teeb	*Nardostachys Jatamansi* D.C.	7 gm
Gul Banafsha	*Viola odorata* Linn.	7 gm
Aslas Soos Muqashshar	*Glycyrrhiza glabra* Linn.	7 gm
Tukhme Khatmi	*Althaea officinalis* Linn.	7 gm
Afsanteen Roomi	*Artemisia absinthium* Linn.	7 gm
Gul-e-Ghafis	*Gentiana dahurica* Fisch.	7 gm
Post Beekh-e-Kasni	*Cichorium intybus* Linn.	7 gm
Kazmazaj	*Tamarix orientalis* Vahl	7 gm
Maveez Munaqqa	*Vitis vinifera* Linn.	25 gm

These are soaked over night in water and boiled in morning and mixed with Qand Siyah 50 gm Sirka Jamun 175 ml Aab-e-Mako (*Solanum nigrum* Linn.) 180 ml Aab-e-Kasni (*Cichorium intybus* Linn.) 60 ml (Murawwaqain) this is taken orally twice a day.

8 Formulation (Jeelani, undated)

Yarqan-e-Aswad

Concoction of the following drugs mixed with Sharbat-e-Buzoori (48 gm) Aab-e-Mako Sabz Murawwaq (*Solanum nigrum* Linn.) (48 gm) Aab-e-Turb Sabz Murawwaq (48 gm) is given orally.

Tukhm-e-Kasni	*Cichorium intybus* Linn.	6 gm
Gul Banafsha	*Viola odorata* Linn.	6 gm
Maveez Munaqqa	*Vitis vinifera* Linn.	9 Nos
Badiyan	*Foeniculum vulgare* Mill.	6 gm
Barg-e-Shahtra	*Fumaria officinalis* Linn.	6 gm
Mundi	*Sphaeranthus indicus* Linn.	6 gm
Unnab	*Zizyphus jujuba* Linn.	5 Nos
Anjeer zard	*Vitis vinifera* Linn.	3 Nos

In case of Constipation Gulqand may replace with Sharbat Buzoori

For Tanqia (Evacuation) this formulation is added with Barg-e-Sana Makki (*Cassia Angustifolia* Vahl) (108 gm) Magh-e-Faloos Khayarshamber (*Cassia fistula* Linn.) (48 gm) Khameera Banafsha (*Viola odorata* Linn.) (48 gm) Sharbat Dinaar (48 gm) Sheera Maghz-e-Badaam (*Prunus dulcis* (Mill.) (5 Nos).

Thereafter formulation of Tabreed is given

9 Formulation (Jeelani, undated)

Nuskha-e-Tabreed

Dawaul Misk Moutadil 5 gm is given first then sheera unnab (*Zizyphus jujuba* Linn.) (5 Nos) Arq-eShahtra(*Fumaria indica*) (72gm.) Arq-e-Mundi (*Sphaeranthus indicus* Linn.) (72 gm) Sharbat unnab (*Zizyphus jujuba* Linn.) (24gm) Tukhm-e-Rihaan (*Ocimum basilicum* Linn) (5gm) sprinkled over it and taken orally.

Thereafter following formulation is given for a few days

Aab-e-Turb Sabz (*Raphanus indicus*) /Aab-e-Pudina Sabz (*Mentha arvensis* Linn.) (84 gm), Sikanjabeen Bazoori (48gm).

10 Formulation (Jeelani, undated)

Qurs-Tabasheer Qabiz

Tabasheer Safaid	*Bambusa arundinacea* Retz.	6 gm
Gul-e-Surkh	*Rosa damascena* Mill.	6 gm

Tukhme Kahu	*Lactuca sativa* Linn.	6 gm
Tukhme Kasni	*Cichorium intybus* Linn.	6 gm
Tukhme Khurfa	*Portulaca oleracea* Linn.	6 gm
Sumaq	*Rhus coriaria* Linn.	6 gm
Gulnar	*Punica granatum* Linn.	3 gm
Sandal Safaid	*Santalum album* Linn.	3 gm
Tukhme Hamaz	*Rumex vesicarius* Linn	3 gm
Afiyun	*Papaver somniferum* Linn.	0.1 gm

These are grounded and made in the form of tablet with the help of Arq-e-Gulab (*Rosa damascena* Mill) and it is taken orally in the dose of 3 gm with the adjuvant of arq-e-Gaozabaan (*Onosma bracteatum*) (12 tola) Sharbat Habbul Aas (*Myrtus communis* Linn.) (1 tola).

11 Formulation (Jeelani, undated)

Sikanjabeen

Tukhme-Kasni	*Cichorium intybus* Linn.	24 gm
Badiyaan	*Foeniculum vulgare* Mill.	24 gm
Tukhm-e-Karafs	*Apium graveolens* Linn.	24 gm
Water		1.5 L
Sirka		120 gm
Qand Safaid		1 kg

This is prepared in the form of Sikanjabeen

This is mixed with Arq-e-Gaozabaan (*Onosma bracteatum*) 120 gm and taken orally taken in the dose of 36-48 gm.

12 Formulation (Jeelani, undated)

Arq-e-Murakkab

Gul-e-Nilofar	*Nymphaea alba* Linn.	120 gm
Gul banafsha	*Viola odorata* Linn.	60 gm
Gul-e-Surkh	*Rosa damascena* Mill.	84 gm
Gul-e-Gaozabaan	*Onosma bracteatum*	48 gm
Gul-e-Swevti	*Rosa alba* Linn.	36 gm
Tukhme Kasni	*Cichorium intybus* Linn.	60 gm

Tukhme kahu	*Lactuca sativa* Linn.	60 gm
Tukhme Khurfa	*Portulaca oleracea* Linn.	60 gm
Tukhme Shahtra	*Fumaria officinalis* Linn.	60 gm
Tukhme Palak	*Spinacia oleracea* Linn.	60 gm
Chiraita	*Swertia chirayita* (Roxb. ex Flem.)	60 gm
Shahtra	*Fumaria officinalis* Linn.	48 gm
Gilo	*Tinospora cordifolia* Miers	84 gm
Mundi	*Sphaeranthus indicus* Linn.	60 gm
Barg-e-Tulsi	*Ocimum basilicum* Linn.	36 gm
Barg-e Hina	*Lawsonia inermis* Linn.	24 gm
Buraada Sandal Safaid	*Santalum album* Linn	36 gm
Buraada Sandal Surkh	*Pterocarpus santalinus* Linn. f.	24 gm
Khas	*Vetiveria zizanioides* (Linn.) Nash	36 gm
Kishneez	*Coriandrum sativum* Linn.	60 gm
Badranjaboya	*Nepeta hindostana* Haines	36 gm
Unnab	*Zizyphus jujuba* Linn.	101 nos
Aalo Bukhra	*Prunus domestica* Linn.	101 nos
Tamar hindi	*Tamarindus indica* Linn.	180 gm

All these drugs are soaked in Arq-e-Kasni (*Cichorium intybus* Linn.) (3 L) Arq-e-Nilofar (*Nymphaea alba* Linn) (3 L) Arq-e-Bed Sada (*Salix caprea* Linn.) (3 L) Arq-e-Mako (*Solanum nigram* Linn.) (3 L) Arq-e-Keora (*Pandanus tectorius* Soland. ex Park.) (2 L) Arq-e-Gulab (*Rosa damascena* Mill) (2 L) or water (15 L) for 24 hours thereafter prepared as Arq.

Dose 84 gm twice in a day either alone or with adjuvant of Sharbat-e-Anar (*Punica granatum* Linn.) (24 gm)/ Sharbat Buzoori (24 gm)/ Sharbat Nilofar (*Nymphaea alba* Linn) (24 gm).

13 Formulation (Ajmali, 1949)

Kushta Asmad

Surma Siyah is kept in the fruit of Colocynth thereafter it is made into calyx after burning then it is grounded in the form of fine powder. This powder of calyx (15 mgm) is mixed with the powder of Revand Chini (*Rheum officinale* Baill.) (125 mgm), Naushadar (Sal Ammoniac/Ammonium chloride) (125 mgm), Shora Qalmi (125 mgm), this formulation is then taken in the dose of 15 mgm with Butter.

14 Formulation (Ahmad, 1942)

Zimaad-1

Barg-e-Sudab	*Ruta graveolens* Linn.	10 gm
Ushaq	*Dorema ammoniacum* D. Don	7 gm
Pudina Khushk	*Mentha arvensis* Linn.	7 gm
Boora Armani	Armenian Earth/Aluminum silicate	3 gm

These are grounded in vinegar and prepared as Zimaad. Used locally on the site of spleen.

15 Formulation (Ahmad, 1942**)**

Zimaad-2

Sibr*Aloe barbadensis* Mill. 6 gm

Zafran	*Crocus sativus* Linn.	2 gm
Ash of Chobe Angoor	*Vitis vinifera* Linn.	24 gm

These are grounded in the extract of Barg-e-Jhaoo Sabz (*Tamarix articulata* Vahl.) and mixed with Sirka (vinegar) and applied locally on the site of spleen in the form of Zimaad.

16 Formulation (Ahmad, 1942)

Zimaad-3

Anzaroot	*Astragalus sarcocola*	6 gm
Kateera	*Astragalus gummifer*	12 gm
Ushaq	*Dorema ammoniacum* D. Don.	24 gm
Zarawand Madharaj	*Aristolochia rotunda*	12 gm

These are all grounded in Vinegar (old) and applied at the site of spleen as Latooq (medicine smeared on cloth for local application)

17 Formulation (Ahmad, 1942)

Joshanda

Unnab	*Zizyphus jujuba* Linn.	35 gm
Sapistan	*Cordia myxa* Linn.	35 gm
Tamar hindi	*Tamarindus indica* Linn.	35 gm
Aaloo Bukhara	*Prunus domestica* Linn.	35 gm

Sana-e-makki	*Cassia Angustifolia* Vahl	17 gm
Bisfaij Fistaqui	*Polypodium vulgare* Linn.	17 gm
Badawarad	*Cirsium tuberosum* (L.) All.	17 gm
Tukhm-e-Rihaan	*Ocimum basilicum* Linn	17 gm
Shahtra	*Fumaria officinalis* Linn.	17 gm
Haleela Siyah	*Terminalia chebula* Retz.	17 gm
Haleela Kabli	*Terminalia chebula* Retz.	17 gm
Gul banafsha	*Viola odorata* Linn.	17 gm
Barg-e-Gaozabaan	*Onosma bracteatum*	17 gm
Tukhm-e-Khayaar	*Cucumis sativus* Linn.	10 gm
Tukhm-e-Kasni	*Cichorium intybus* Linn.	10 gm
Zarishk	*Berberis aristata* DC.	10 gm
Aftimoon	*Cuscuta epithymum*	10 gm

All the above ingredient are boiled with water and strain and mixed with Sheera-e-Amaltas (*Cassis fistula*) (268 mg) and Roughan-e-Badam Shirin (*Prunus amygdalus*) and taken orally.

Recent Researches

Recent researches have proved some claim by Unani physician scientifically that herbal compounds have been found effective against this disease. Hakim Najmul Ghani says in his book *Khazainul Advia* that Aspand (*Peganum harmala* Linn.) is useful in persons with Saudavi temperament and it is use for cleaning of spleen from viscous humours. (Ghani, 1911). Vasicine (Peganine) found in the plant *Peganum harmala* (Aspand Sokhtani/Hurmul/Sheersa/ Astarmaatoos) has been tested *in-vitro* against the *promastogote* stage of *Leismania donovani*, the causative agent of visceral leishmaniasis. It was shown that this compound induces apoptosis in *Leishmania prostogotes*. Peganine hydrochloride dehydrate, beside being safe, was found to induce apoptosis in both the stages of *L. donovani* via loss of mitochondrial transmembrane potential (Misra, *et. al.* 2008).

An other alkaloid harmine found in the same plant found appreciable efficacy in destroying intracellular parasites as well as non hepatotoxic and non nephrotoxic nature, harmine in the vesicular forms may be considered for clinical application in humans (Lala, *et. al.* 2004).

Dietary recommendations

Lateef and Zood hazam Ghiza (light and easily digestible diets) has been recommended (Ibn Rushd, (1126-1198 AD) 1987). Half boiled egg, grapes (*Vitis*

vinifera Linn.), dried figs (*Ficus carica* Linn) and Maweez (*Vitis vinifera* Linn.) etc. In the initial stage of disease *Maa-us-Shaeer* (Barley water) thereafter Chicken soup, Mutton Soup, water strained from Chana (*Cicer arietinum* Linn.) soaked over night, green vegetables, mint (*Mentha aquatica* Linn.), radish (*Raphanus sativus* Linn.), figs (*Ficus carica* Linn.), and Kharpaza (*Cucumis melo* Linn.) is recommended. Curry is prepared by cooking meat of lamb/chick/Murgabi in beet root (*Beta vulgaris* Linn.), Halyun (*Asparagus officinalis* Linn.) and Jirjeer (*Brucea sativa* Mill.). This is a diet which is recommended by Unani physician in the month of Autumn/Khareef to avoid disease of the season including *Humma-e-Aswad* (Tabri 1917).

Dietary restrictions

Saqeel and Naffaq diets (heavy and flatus producing diets) are restricted e.g. Potato (*Solanum tuberosum* Linn.), arvi (coco yam), Kachaloo, Dal mash (black gram), lady finger (*Abelmoschus esculentus* (Linn.) Moench), Milk, Butter, ghee (clarified butter) (Tabri 1917).

Conclusion

For a long time, little was known about the transmission cycles of the disease, but over the last few years, field research and the application of molecular biology have enabled substantial progress to be made in understanding the different links in the transmission chain. Moreover, simple new diagnostic tests have recently been developed which are practical, reliable and inexpensive. These techniques are available to concerned countries for the early detection and rapid treatment of the disease. Until a safe and effective vaccine is developed, a combination of sand fly control, detection and treatment of patients and prevention of drug resistance and the use of age old formulations and treatment according to Unani system of medicine is the best approach for controlling Kala-Azar. In this regard Central Council for Research in Unani Medicine (CCRUM) is playing an important role for the prevention of this disease. Several Clinical trials and research programme are being conducted in the Regional Research Institute of Unani Medicine (RRIUM) under the Dept. of AYUSH in Bihar, Kolkata, Assam, and Orissa (Anonymous, 2010).

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Role of Chromatography in the Identification and Quality Control of Herbal Drugs 1. HPTLC Finger Prints of "Qurs-e-Kundur": a Unani Compound Formulation

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Abstract

The impending world is reliant on traditional medicine, and its recognition escalating at present as essentials. In regulating the therapeutic efficacy of herbal drugs, standardization and quality control are the key factors. Organoleptic parameters are not much consistent in establishing the standards of herbal drugs, for which instrumental analysis of the drugs provides a more accurate picture regarding the qualitative and quantitative aspects of bioactive molecules, which are held liable for therapeutic action and is widely accepted in the quality assessment of herbal drugs. However, such work is lacking or at infantile stage. In this study, a comparative account of the HPTLC finger prints of the ingredients of compound Unani formulation, **Qurs-e-Kundur** in methanolic extract as well as petroleum ether extracts are given and discussed in detail.

Key Words: Qurs-e-Kundur, HPTLC Fingerprints, Quality control, Safety evaluation.

Introduction

Qurs-e-Kundur is yellowish brown, acrid Unani tablet with odour like that of camphor and bitter taste. It is made up of Barg-e-Sudab (Leaves of *Ruta graveolens* Linn.), Kundur (Gum-resin of *Boswellia serrata* Roxb.), Nankhwah (Fruitlets of *Trachyspermum roxburghianum* DC.), Pudina Khusk (Shoot of *Mentha arvensis* Linn.), Rasan (Stem of *Innula racemosa* Hook.f.) and Satar Farsi (Leaves of *Zataria multiflora* Boiss.). Therapeutic uses of the formulation are Fuwaq and Burudat-e-Meda. The drug has many medicinal uses in the ailment of skin diseases, and Haematemesis-wounds healing, cough and Bronchial asthma, Amnesia, Incontinence of urine, Diseases of eye, sexual debility. (Anonymous, 2007; Imam *et al.,* 2009). Curative efficacies of compound herbal medicines is reliant on the quality and the quantity of the constituent single drugs as they contain specific bio-active marker species with specific pharmacological actions. Though, it is very difficult to identify the ingredients after the formulation is prepared and the organoleptic parameters like taste, odour, colour etc. will not establish the standard quality of the medicine. Therefore, there should be some analytical method to ensure the presence of all the ingredients in the formulation. Chromatographic finger printing of both the compound formulation and it's constitute single drugs will definitely ascertain the presence of the ingredients, and quality of the preparation. But such studies in Indian Systems of Medicines are lacking. Therefore, experiments have been designed to analyze the ingredient single drugs and the compound formulation, Qurs-e-Kundur by HPTLC studies and the results are presented.

Materials and Methods

Preparation of the formulation

Qurs-e-Kundur was prepared according to the composition of the formulation (Anonymous, 2007) and three batches are shown in figure 6.

Processing of raw materials

Barg-e-sudab, kundur, Nankhwah, Pudina Khushk, Rasan and Satar Farsi, are the ingredients of the preparation, after identification by botanist and ascertaining the quality, were cleaned by the removal of foreign matter, if present and by washing two to three times with sterile distilled water, if needed. The drugs were air dried in shade under aseptic conditions. Later, all the drugs were powdered separately by pulveriser, sieved through a mesh with a pore size of 150µ.

Preparation of the Tablets

The tablets were prepared as per the procedure described by Mohammad Azam Khan.1315 AD[3]. Required quantities of the powders were mixed thoroughly and moistened with sterile distilled water. Samagh-e-Arabi, ten percent of the total weight of the powders of the ingredients, was added to the powdered drugs to get a semisolid paste and subjected for granulation, using mechanical granulator. The granules thus formed were dried in drier at low temperature or in the sun light. The granules were later subjected for making of tablets of desire weight using Rotary tablet punching machine (Cadmach).

Determination of Physico-Chemical Standards

Physico-Chemical Standards of the prepared compound formulation, **Qurs-e-Kundur** were developed as per the methods described in The Unani Pharmacopoeia of India, (Anonymous, 2007).

Preparation of Extract of the drug sample for HPTLC

Five grams powder of Qurs-e-Kundur was dissolved in each of 100 ml of methanol for polar extract and 100ml of petroleum ether for non-polar extract in a stoppered conical flask and was kept for 2 hours shaking in regular intervals. Later the contents were filtered through Whattmann No. 41 filter paper and evaporate the solution to 20 ml. The solution obtained was used as sample for the determination of components. (Imam, 2009)

Development and determination of the solvent system

Sample applied	:	Sample drug solution of about 10µl.
Solvent system	:	Toluene: Ethyl acetate: Methanol (7: 2: 1)
Scanning wavelength	:	366nm

The sample applied as a band of 8mm with the help of Automatic TLC applicator system of the DESAGA Sarstedt Gruppe on Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck). After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the Twin through chamber of TLC to the maximum height of the plate so that it can be able to separate the components on the polar phase of silica gel and that of mobile phase of solvent system. (Pozharitskaya Olga *et al*., 2006; Shah *et al*., 2007; Kumar, 2003).

Development of HPTLC technique

After developing, TLC plates were dried completely and detected with the suitable detection system like UV Cabinet system in order to examine number of spots at 366nm and 254nm as shown in figures 1-4. Further it was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366nm appearing a maximum number of components. A corresponding densitograms was obtained as shown in the figures (7.a-7.g), in which peaks are appeared for the corresponding spots being detected in the densitometer while scanning and the peaks area under the curve corresponds to the concentration of the component in the sample for the concentration we applied on the TLC plate.

Results and Discussion

Analytical Profile

Organoleptic Character

A yellowish brown color, acrid Unani tablet with odour like that of camphor and bitter taste.

Identification

Microscopy

Anomocytic and anisocytic stomata, cellular residue with schizogenou cavities filled with oils and vessels with wide lumen and spiral thickenings (**Barg-e-Sudab**), thick walled rectangular cells containing brownish pigment, cuboid stone cell containing prismatic calcium oxalate crystals and tannin (**Nankhwa**), uniseriate nonglandular

hairs, glandular trichomes, diacytic stomata, and brick shaped cels with wavy walls (**Pudina**), brick shaped stratified cork cells, cells containing solitary, prismatic calcium oxalate crystals, vessel fragments with pitted thickenings (**Rasan**) Anomocytic and anisocytic stomata, cellular residue with schizogenou cavities filled with oils and vessels with wide lumen and spiral thickenings (**Satar Farsi**).

Physico-Chemical Standards

Parameters	Batch-I	Batch-II	Batch-III
1. Ash Values			
(i) Total Ash:	14.00-16.53	14.20-16.80	14.40-16.93
(ii) Water soluble (% w/w):	03.15-04.02	03.45-04.42	03.25-04.22
(iii) Acid insoluble (% w/w):	04.90-05.63	04.92-05.68	04.80-05.60
2. Alcohol sol. matter (% w/w):	20.26-23.57	21.26-24.50	20.80-23.80
3. Water sol. matter (% w/w):	39.65-41.59	40.00-41.90	39.00-41.00
4. P^H of			
5. A. 1% Aqueous Solution:	06.23-06.51	06.20-06.40	06.26-06.52
B. 10% Aqueous Solution:	05.66-05.87	05.60-05.80	05.62-05.82
6. Disintegration Time in min.:	10.00-10.50	10.20-10.30	10.30-10.40
7. Total moisture content: (Loss of weight on drying at 105°C)	07.40-07.60	07.20-07.50	07.80-07.90

Safety Evaluation of drug

1. Microbial Contamination

Total *Bacterial* Load	:	4 x 10^4 (Not more than 10^5/g)
Salmonella Spp.	:	Nil
Escherichia Coli	:	Nil
Total *Fungal* count	:	3x10^2 (Not more than 10^3/g)

2. Aflatoxin Contamination

B1	:	Nil (Not more than 0.50 ppm)
B2	:	Nil (Not more than 0.10 ppm)
G1	:	Nil (Not more than 0.50 ppm)
G2	:	Nil. (Not more than 0.10 ppm)

3. Heavy Metal Analysis

Arsenic	:	Nil (Not more than 3.0 ppm)
Cadmium	:	Nil (Not more than 0.3 ppm)
Lead	:	Nil (Not more than 10.0 ppm)
Mercury	:	Nil (Not more than 1.0 ppm)

4. Pesticide residue

Chlorpyriphos	:	Nil (Not more than 0.20mg/Kg)
DDT	:	Nil (Not more than 1.00mg/Kg)
Endosulfan	:	Nil (Not more than 3.00mg/Kg)
Malathion	:	Nil (Not more than 1.00mg/Kg)
Parathion	:	Nil (Not more than 0.50mg/Kg)

HPTLC *Analysis*: A comparative account of the Chromatograms of the methanolic extract and petroleum ether (60^0C-80^0C) extract of Qurs-e-Kundur and it's single drugs extracts. Developed chromatograms at UV 366nm wavelength and at 254 nm for methanolic extracts of drug and its ingredients are given in figures 1, 2 and 3, 4 respectively. The Densitogram of formulation and its ingredients of methanolic extract at 366nm wave length is given in figure-5. The individual densitograms of compound formulation Qurs-e-Kundur and its ingredients of methanolic extracts are given in figures (7.a-7.g) respectively. Similarly the data represented in tabular forms for petroleum ether extract of compound formulation Qurs-e-Kundur and its ingredients. A comparative account of the Rf Values of the formulation Qurs-e-Kundur and its ingredients for methanolic extracts at UV 366nm and at UV 254 nm are shown in table 1 and 2; where as for petroleum ether extracts shown in table 3 and 4. The individual Rf values of Qurs-e-Kundur and its ingredients Barg-e-sudab, Kundur, Nankhwah, Pudina khusk, Rasan and Satar farsi for methanolic extracts are given in tables from 5-11 respectively. It is evident from figures 1, 2,

Table 1. Rf Values of methanolic extract of Qurs-e-Kundur (formulation) corresponding to the specific Rf values of the ingredients at UV 366nm.

Peaks	Q. Kundur	Barg-e-Sudab	Kundur	Nankhwah	Pudina Khusk	Rasan	Satar Farsi
1.	0.02	–	0.02	–	–	–	–
2.	0.06	–	–	–	–	–	–
3.	0.19	0.18	–	–	0.18	–	–
4.	0.28	–	–	–	–	–	–
5.	0.33	–	–	–	–	–	0.33
6.	0.40	0.41	–	–	–	–	–
7.	0.53	–	–	–	–	–	–
8.	0.64	0.63	–	–	–	–	–
9.	0.77	0.77	–	–	–	–	–
10.	0.91	–	0.92	–	–	–	–

Table 2. Rf values of methanolic extract of Qurs-e-Kundur (formulation) corresponding to the Rf values of the ingredients at UV 254nm.

Peaks	Q. Kundur	Barg-e-Sudab	Kundur	Nankhwah	Pudina Khusk	Rasan	Satar Farsi
1.	0.02	–	0.02	0.02	0.02	0.02	–
2.	0.45	–	–	–	–	–	0.44
3.	0.64	0.65	–	–	–	–	–
4.	0.89	0.89	–	–	–	–	–

Table 3. Rf Values of Petroleum ether extract of Qurs-e-Kundur (formulation) corresponding to the specific Rf values of the ingredients at UV 366nm.

Peaks	Q. Kundur	Barg-e-Sudab	Kundur	Nankhwah	Pudina Khusk	Rasan	Satar Farsi
1.	0.04	–	0.04	–	–	–	–
2.	0.11	–	–	–	–	–	–
3.	0.18	–	0.18	–	–	–	–
4.	0.23	–	0.22	–	–	–	–
5.	0.28	0.27	–	–	–	–	–
6.	0.46	0.46	–	–	–	–	–
7.	0.57	0.57	–	–	–	–	–
8.	0.70	–	–	–	0.70	–	–
9.	0.78	–	–	–	–	–	–
10.	0.85	–	–	0.84	–	–	–

3 and 4 that the number of spots at UV 366nm wavelength is more than at 254 nm and also number of spots in methanolic extract are less when compared to that of the petroleum ether extract at both wavelength. It has been observed from methanolic extract chromatogram at UV 366nm as in table 5 that there are ten spots in the chromatogram of Qurs-e-Kundur at Rf values 0.02,0.06, 0.19,0.28, 0.33,0.40, 0.53, 0.64, 0.77, and 0.91; from table 6 that there are nine spots in the chromatogram of Barg-e-sudab at Rf values 0.01, 0.18, 0.22, 0.26, 0.36, 0.41, 0.63, 0.77, and 0.88 ; from table 7 that there are five spots in the chromatogram of Kundur at Rf values

Table 4. Rf values of Petroleum ether extract of Qurs-e-Kundur (formulation) corresponding to the Rf values of the ingredients at UV 254nm.

Peaks	Q. Kundur	Barg-e-Sudab	Kundur	Nankhwah	Pudina Khusk	Rasan	Satar Farsi
1.	0.01	0.01	–	0.01	0.01	–	0.01
2.	0.15	0.15	–	–	–	0.15	–
3.	0.40	0.41	–	–	–	–	–
4.	0.59	–	0.60	–	–	–	–
5.	0.65	0.66	–	–	–	–	–
6.	0.73	–	0.72	–	–	–	0.72
7.	0.80	0.81	–	–	–	–	–

Table 5. Peak list and Rf values of various spots of methanolic extract of Qurs-e-Kundur

Methanolic extract of Qurs-e-Kundur, HPTLC parameters					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	14.3	2535.93	56.0	753.23	0.02
2	17.8	1094.82	24.2	218.30	0.06
3	29.1	71.27	1.6	29.69	0.19
4	36.1	4.51	0.1	3.34	0.28
5	40.4	45.77	1.0	15.21	0.33
6	46.0	47.48	1.0	16.00	0.40
7	56.8	7.10	0.2	4.00	0.53
8	66.2	301.27	6.6	79.61	0.64
9	77.1	54.59	1.2	15.89	0.77
10	88.4	368.97	8.1	53.20	0.91

0.02, 0.31, 0.43, 0.80, and 0.92; from table 8 that there are six spots in the chromatogram of Nankhwah at Rf values 0.02, 0.10, 0.35, 0.46, 0.82, and 0.90; from table 9 that there are seven spots in the chromatogram of Pudina khusk at Rf values 0.02, 0.18, 0.25, 0.35, 0.48, 0.57 and 0.73; from table 10 that there is only

Table 6. Peak list and Rf values of various spots of methanolic extract of Barg-e-sudab.

Methanolic extract of Barg-e-sudab, HPTLC parameters					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	14.2	2088.01	53.5	555.91	0.01
2	28.2	144.18	3.7	59.02	0.18
3	31.6	4.48	0.1	3.41	0.22
4	34.8	36.54	0.9	19.00	0.26
5	43.1	206.80	5.3	79.16	0.36
6	47.3	19.04	0.5	10.31	0.41
7	65.3	848.15	21.7	190.39	0.63
8	77.3	213.53	5.5	54.64	0.77
9	85.8	339.01	8.7	62.76	0.88

Table 7. Peak list and Rf values of various spots of methanolic extract of Kundur.

Methanolic extract of Kundur, HPTLC analysis					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	14.5	63.21	34.8	16.17	0.02
2	39.1	9.70	5.3	6.39	0.31
3	48.7	25.11	13.8	11.60	0.43
4	79.4	12.51	6.9	8.24	0.80
5	89.1	71.34	39.2	18.03	0.92

one spots in the chromatogram of Rasan at Rf values 0.02; from table 11 that there are seven spots in the chromatogram of satar farsi at Rf values 0.03, 0.33, 0.38, 0.45. 0.60, 0.82 and 0.93;

It is of utmost significance to know whether all the required single drugs are mixed in the compound formulation, because each and every single drug has its own bioactive molecules responsible for a particular therapeutic activity. It is very difficult to identify the single drugs once they are powdered and mixed for preparing compound formulation. A comparative account of the finger print TLC of any

Table 8. Peak list and Rf values of various spots of methanolic extract of Nankhwah.

Methanolic extract of Nankhwah, HPTLC analysis					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	14.4	1690.77	84.5	583.37	0.02
2	21.1	147.59	7.4	50.91	0.10
3	42.0	6.43	0.3	3.40	0.35
4	51.3	5.32	0.3	3.00	0.46
5	81.2	91.93	4.6	17.46	0.82
6	88.0	59.48	3.0	19.91	0.90

Table 9. Peak list and Rf values of various spots of methanolic extract of Pudina Khusk.

Methanolic extract of Pudina Khusk, HPTLC analysis					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	14.4	2702.48	86.8	763.81	0.02
2	27.6	103.61	3.3	45.69	0.18
3	33.8	57.34	1.8	19.70	0.25
4	42.2	163.31	5.2	53.64	0.35
5	52.7	27.10	0.9	9.45	0.48
6	60.1	39.48	1.3	9.08	0.57
7	73.8	21.17	0.7	5.24	0.73

Table 10. Peak list and Rf values of various spots of methanolic extract of Rasan.

Methanolic extract of Rasan, HPTLC analysis					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	14.9	848.82	100	255.31	0.02

Table 11. Peak list and Rf values of various spots of methanolic extract of Satar Farsi.

Methanolic extract of Satar Farsi, HPTLC analysis					
Peak no.	y-pos[mm]	Area	Area (%)	Height	Rf
1	15.3	2865.45	87.1	562.66	0.03
2	40.4	15.43	0.5	5.32	0.33
3	44.6	11.31	0.3	5.55	0.38
4	50.1	23.46	0.7	9.12	0.45
5	63.1	142.08	4.3	21.27	0.60
6	80.9	41.30	1.3	9.67	0.82
7	90.2	189.62	5.8	39.78	0.93

Table 12 Rf Values of Petroleum ether extract of Qurs-e-Kundur (formulation), and all its ingredients at UV 366nm.

Peaks	Q. Kundur	Barg-e-Sudab	Kundur	Nankhwah	Pudina Khusk	Rasan	Satar Farsi
1.	0.04	0.05	0.04	0.05	0.05	0.05	0.05
2.	0.11	0.08	0.18	0.19	0.08	0.82	0.17
3.	0.18	0.15	0.22	0.48	0.17	0.88	0.25
4.	0.23	0.19	0.32	0.62	0.21		0.31
5.	0.28	0.27	0.45	0.74	0.33		0.75
6.	0.46	0.46		0.84	0.51		0.89
7.	0.57	0.57			0.64		
8.	0.70	0.65			0.70		
9.	0.78	0.71			0.86		
10.	0.85	0.83					

compound formulation along with it's constituent ingredients will help in determining whether the genuine single drugs are mixed or not. Table 1, 2, 3 and 4 shows the Rf Values of the TLC spots of the formulation, Qurs-e-Kundur, corresponding to the specific Rf values of the spots of the individual ingredients for methanolic extract and petroleum ether extract at both wavelength. It is evident from table 1 that there

Table 13. Rf Values of Petroleum ether extract of Qurs-e-Kundur (formulation), and all its ingredients at UV 254nm.

Peaks	Q. Kundur	Barg-e-Sudab	Kundur	Nankhwah	Pudina Khusk	Rasan	Satar Farsi
1.	0.01	0.01	0.03	0.01	0.01	0.02	0.01
2.	0.15	0.05	0.12	0.17	0.13	0.15	0.13
3.	0.40	0.11	0.24	0.57	0.17	0.76	0.27
4.	0.59	0.15	0.39	0.70	0.29	0.84	0.31
5.	0.65	0.25	0.53	0.82	0.58		0.72
6.	0.73	0.41	0.60		0.83		0.85
7.	0.80	0.52	0.67				
8.		0.66	0.72				
9.		0.81					

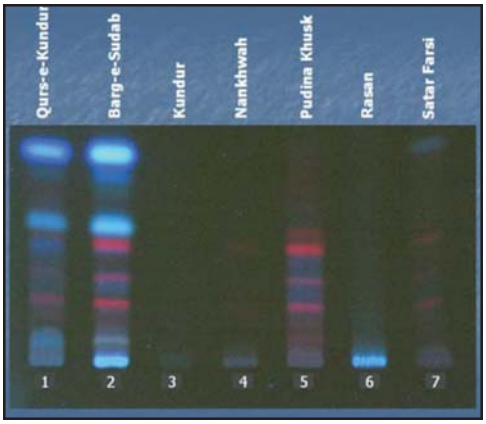


Fig. 1. Chromatograms of the methanolic extracts of Qurs-e-Kundur and it's single drugs extracts at UV 366nm wavelength

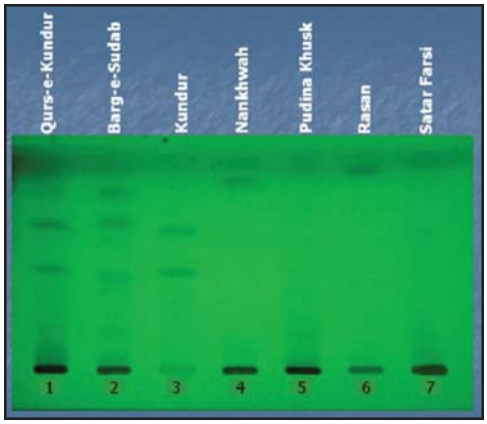


Fig. 2. Chromatograms of the methanolic extracts of Qurs-e-Kundur and it's single drugs extracts at UV 254nm wavelength

are ten spots in the compound formulation with Rf values at 0.02, 0.06, 0.19,0.28, 0.33,0.40, 0.53, 0.64, 0.77, and 0.91 which correspond to the Rf values of various ingredients. Spot of compound formulation with Rf value at 0.02 corresponds to the spot specific to Kundur; spot of compound formulation with Rf values at 0.19 correspond to the spots specific respectively to Barg-e-sudab and Pudina Khusk; Spot of compound formulation with Rf value at 0.33 corresponds to the spot specific to Satar Farsi; Spots of compound formulation with Rf values at 0.40, 0.64 and 0.77 corresponds to the spots specific to Barg-e-sudab; Spot of compound formulation

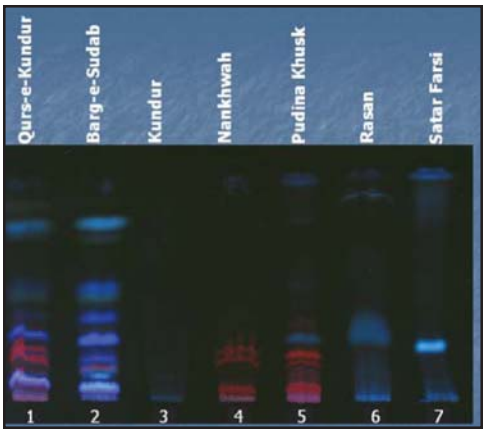


Fig. 3. Chromatograms of the Petroleum ether extracts of Qurs-e-Kundur and it's single drugs extracts at UV 366nm wavelength

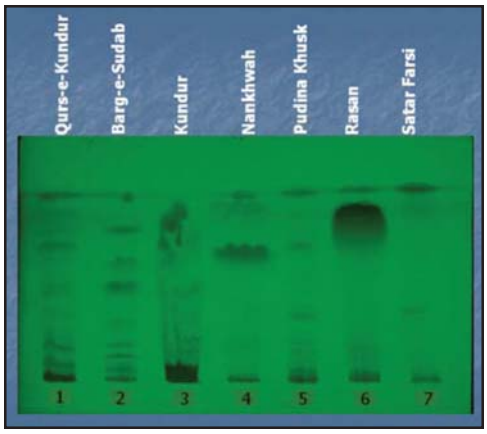


Fig. 4. Chromatograms of the petroleum ether extracts of Qurs-e-Kundur and it's single drugs extracts at UV 254nm wavelength

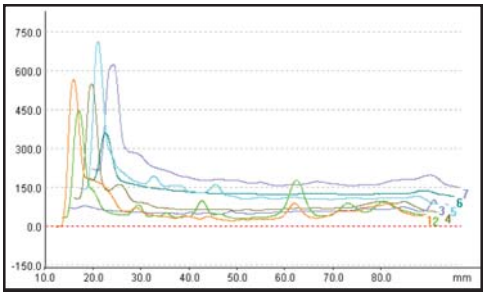


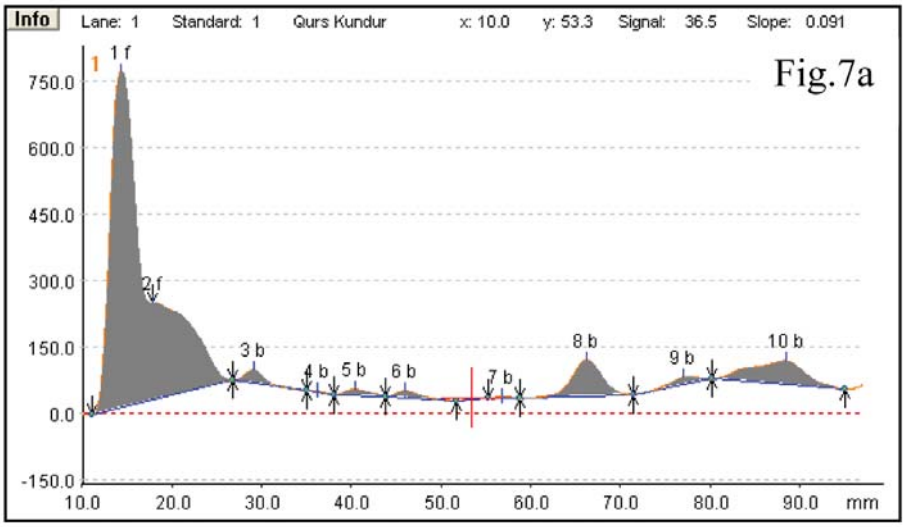
Fig. 5. Densitograms of the methanolic extracts of Qurs-e-Kundur and it's single drugs extracts at UV 366nm wavelength

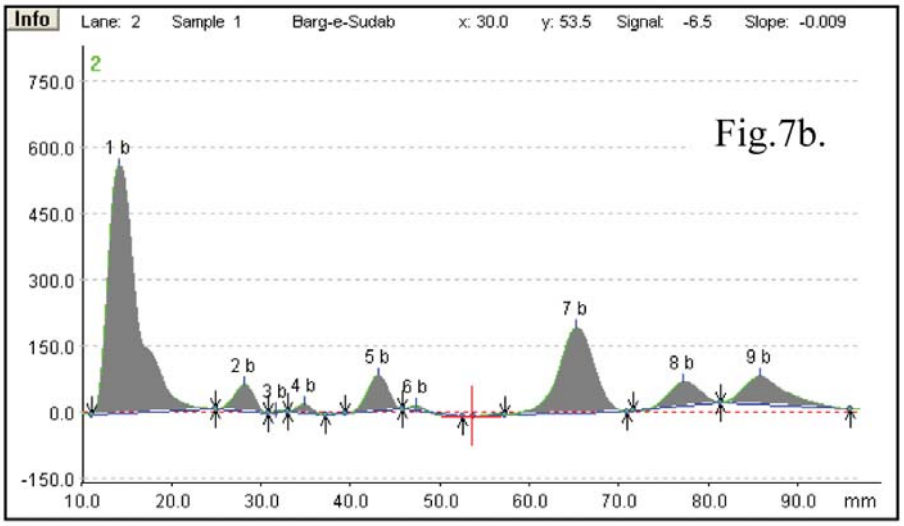
Fig. 6. Qurs-e-Kundur formulation in three batches.

with Rf value at 0.91 corresponds to the spot specific to Kundur, all these indicating the presence of ingredients in the compound formulation.

Similarly methanolic extract of Qurs-e-Kundur and all its ingredients at UV 254nm shows related information as shown in the table 2. Spot of compound formulation with Rf value at 0.02 corresponds to the spots specific to Kundur, Nankhwah, Pudina khusk and Rasan; Spots of compound formulation with Rf value at 0.45, and 0.64 corresponds to spot specific to Satar farsi and Barg-e-Sudab respectively. Spot of formulation with Rf value at 0.89 corresponds to the spot of Barg-e-sudab indicating the presence of these ingredients in the compound formulation.

Analogous to methanolic extract results of Qurs-e-kundur similar information can be acquire for the petroleum ether extracts at UV 366nm and at UV 254 nm which guide to a comparative account of Rf values presented in the table 3 and 4. At UV 366nm in petroleum ether extract spots of compound formulation with Rf value at 0.02, 0.18, 0.23 corresponds to the spots specific to Kundur; spots of compound formulation with Rf value at 0.28, 0.46, 0.57 corresponds to the spots specific to





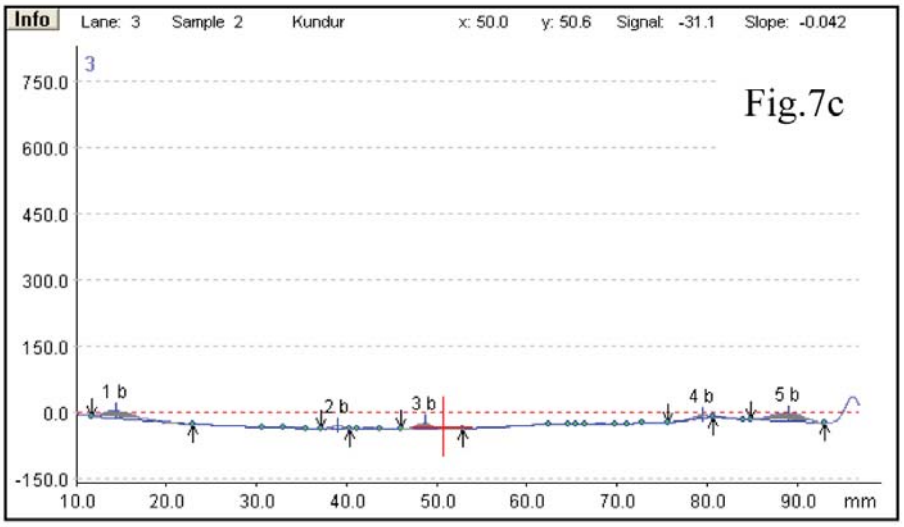
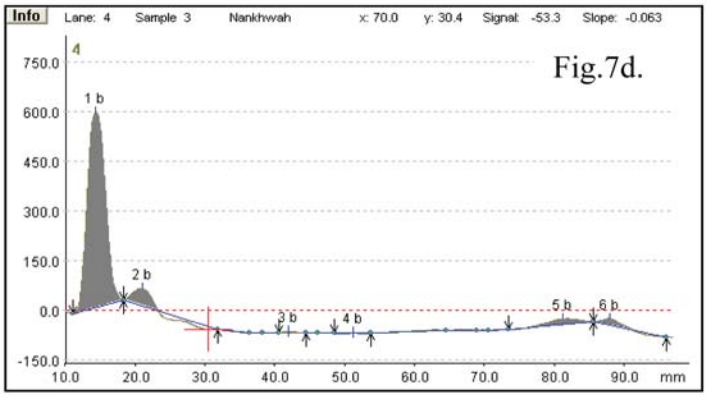
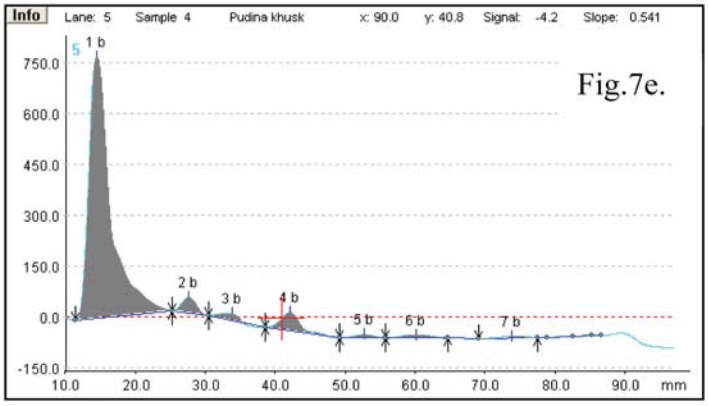
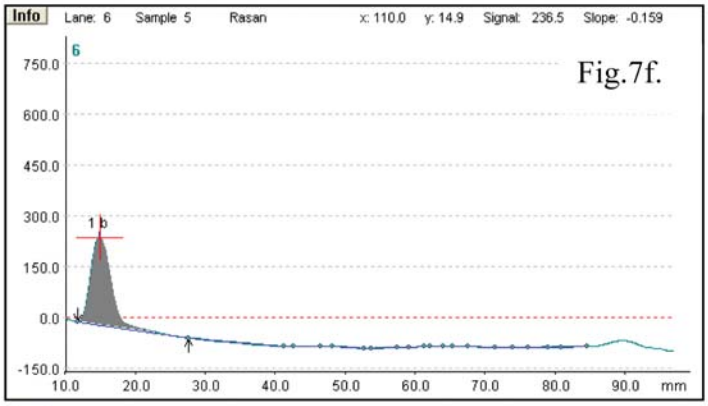


Fig. 7(a, b, c) Densitograms of the methanolic extract of Qurs-e-Kundur, Barg-e-sudab, Kundur







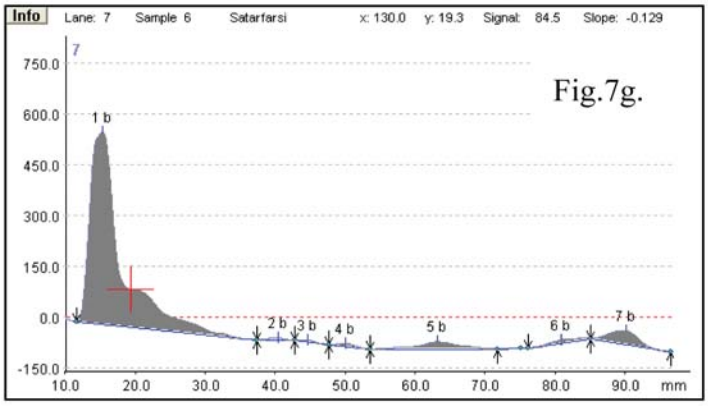


Fig. 7(d, e, f, g) Densitograms of the methanolic extract of Nankhwah, Pudina Khusk, Rasan, Satar Farsi.

Barg-e-sudab; Spot of compound formulation with Rf value at 0.70 corresponds to the spot specific to Pudina Khusk; Spot of compound formulation with Rf value at 0.85 corresponds to the spot specific to Nankhwah approximately. Where as at UV 254nm of petroleum ether extract shows that in table 4 i.e., spots of compound formulation with Rf values at 0.01 correspond to the spots specific to Barg-e-sudab, Nankhwah, Pudina Khusk, Satar Farsi; spots of compound formulation with Rf values at 0.15 correspond to the spots specific to Barg-e-sudab, Rasan; spots of compound formulation with Rf values at 0.40, 0.65, 0.80 correspond to the spots nearby to Barg-e-sudab. spot of compound formulation with Rf values at 0.59 correspond to the spot nearby to Kundur; spot of compound formulation with Rf values at 0.73 correspond to the spots nearby to Kundur and Satar Farsi. Hence ensuring the polar and non-polar compounds in the compound Unani fomulation to that of the mixed ingredients are present or not and also provides a suitable technique in determing the quality standard and method for analysis along with the safety evalution of formulation and the results as mentioned.

Conclusion

It is very difficult to identify the single drugs once they are powdered and mixed for preparing compound formulation. Organoleptic parameters are not much reliable in establishing the standards of herbal drugs. A comparative account of the finger print HPTLC of compound formulation along with its constituent ingredients in polar and non-polar extracts will help in determining whether the genuine single drugs are mixed or not. Such studies will definitely ensure the quality of a medicine and ensure the action for which it is used.

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A Study of Anti-salmonella Activity of Neem (*Azadirachta indica*) Stem Bark using different extracts

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Abstract

Anti-salmonella activity of Neem (*Azadirachta indica*) stem bark was tested against pathogenic *Salmonella paratyphi* and *Salmonella typhi* using various solvent extracts. The in vitro anti-salmonella activity was performed by agar well diffusion method and the results were expressed as the average diameter of zone of inhibition of bacterial growth around the well. The Ethanol and methanol extracts showed better anti-salmonella activity with zone of inhibition (20-25mm) when compared with other tested extracts and standard antibiotic Erythromycin (15 mcg) with zone of inhibition (13-14mm). Using Fisher's exact test significance difference was found between two salmonella strains sensitivity pattern against tested extracts (P</=0.035).

Key Words: Anti-salmonella activity, *Azadirachta indica*, *S. paratyphi, S.typhi*.

Introduction

Salmonella is a primary cause of food poisoning worldwide. The Centre for Disease Control and Prevention estimated that approximately 1.4 million cases of salmonellosis were annually reported in the United States (Mead *et al.,* 1999). Certain pathogenic Salmonella serotypes adapted to humans, such as *S. typhi* and *S. Para typhi* usually cause severe diseases in humans, such as enteric fever. Enteric fever (Typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate (John *et al.,* 2003). In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics, and a rapid rise in multidrug resistance. Resistance to the first line drugs, chloramphenicol, ciprofloxacin, and amoxicillin, in the course of salmonellosis management has been reported (Zulfigar *et al*., 1994; Benoit *et al*., 2003.). Resistance to antimicrobial agents such as antibiotics is emerging in a wide variety of organisms and multi drug resistant organisms pose serious threat to the treatment of infectious diseases. Hence, plant derived antimicrobials have received considerable attention in recent years.

Neem (*Azadirachta indica)* is a versatile tree of family Meliaceae, popularly known as 'Yavan Priya' meaning the beloved of Muslims. Neem bark has for long been used in the traditional Unani system of medicine for its beneficial properties. The aqueous extract of stem bark used as tonic, stimulant and as a remedy against various skin ailments (Dhawan and Ratnaik, 1993). It is a multi functional as well as multi utility natural product and without any side effects. The bark contains 3.43% protein, 0.68% alkaloids and 4.16% minerals. Studies made with Neem showed anti-pyretic, anti-inflammatory (OKpanyi Ezeukwa, 1981) diuretic (Binde *et al.,* 1958) antiseptic, antibacterial and anti-tumour and interferon inducing activities (Fuziwara *et al.,* 1982).

Methodology

Collection of Plant material

Bark of *Azadirachta indica A. Juss* (Maliaceae) were collected from local area (Hyderabad, Andhra Pradesh, India). The Bark was thoroughly washed with distilled water to remove dirt. It was shade dried and ground into fine powder. The botanical identification of leaves and flowers was done by Dr. V.C Gupta (Section of Botany, CRIUM, Hyderabad, Andhra Pradesh, India).

Preparation of extracts

The material was extracted with six solvents, independently viz. Methanol, Ethanol, Acetone, Chloroform, Benzene and Aqueous extracts. Briefly, 100g of the powder was soaked into respective solvent for three days and followed by filtration of the solvent using Whatman's filter paper under aseptic condition. A stock solution of the extracts was prepared at the concentration of 200mg/ml and stored at 2°C till further use.

Bacterial strains

Two pathogenic Salmonella species – *Salmonella typhi* (MTCC 734) and *Salmonella paratyphi (*MTCC 734), were obtained from Institute of Microbial Technolog (IMTECH), Chandigarh, India. They were sub cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4°C. Fresh inoculums were taken for the test.

Evaluation of anti-Salmonella activity

Anti-Salmonella activity of the extract was determined by agar diffusion assay (Reeves, 1989). Salmonella strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 4 h at 37 ºC and after the incubation period 0.1ml of the test organisms inoculum was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. The seeded plates were allowed to dry in the incubator at 37ºC, Wells were made using sterile 6mm cork borer in the inoculated MHA plate. The wells were filled with 200µl of the extracts (re-suspended in respective solvents) and negative controls (1:1[solvent: water]).The concentration of stock extracts was 200 mg/ml. The inoculated plates were incubated at 37ºC for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zone of inhibition was measured and the anti-salmonella activity was expressed in term of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics, Gentamycin (10 mcg), Ciprofloxacin (30mg) and Chloramphenicol (30mcg). The photograph was taken in U.V-Visible documentation system.

Statistical Analysis

Statistical analysis was done using SAS 9.0 version. Salmonella strains which were sensitive to different extracts and various standard antibiotics were compared using Fisher's Exact Test with P</ = 0.035 being considered as significant. Difference in sensitivity pattern of tested extracts was found individually on two Salmonella strains performing t-test [*Salmonella paratyphi* P</=0.0004, *Salmonella typhi* P</=0.0014, Table 1] which was considered as significant.

Results and Discussion

Neem (*Azadirachta indica*) Ethanol and Methanol extracts showed better zone of inhibition (20-25mm) when compared with the other tested extracts and standard antibiotic Erythromycin (15 mcg) which showed lesser zone of inhibition (13-14mm).

Salmonella typhi showed better sensitivity against Methanol extract with zone of inhibition (25mm) compared with the standard antibiotics Gentamycin and Erythromycin with zone of inhibition (21 and 13mm), Benzene and Chloroform extracts showed no zone of inhibition. There exist significant difference among the various tested extracts anti-salmonella activity (t-test: P</=0.0014, P</=0.0004, Table 1).

Among the two Salmonella strains, all six different tested extracts showed better activity against *Salmonella paratyphi* when compared with *Salmonella typhi* which was not sensitive to Benzene and Chloroform extracts, there found to be significant difference between two Salmonella strains sensitivity pattern against tested extracts (Fisher's exact test: P</=0.035, Table 1).

Salmonellosis and enteric fever are always a public health concern in most developing countries, which are mostly low or middle-income countries with inadequate sanitation and hygiene, particularly regarding food, water and disposal of human excreta.

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to or less sensitive to current antibiotics. Many plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A large number of this agents appear to have structures and modes of action that are distinct from those of the antibiotic in current use. In our study Ethanol and Methanol extracts of the Unani drug Neem were found to show better sensitivity compared with standard antibiotic Erythromycin.

The results obtained in this study gives some scientific support to the Unani use of Neem (*Azadirachta indica*) stem bark for the treatment of Typhoid. Further efforts should be directed at investigating the principle in this extract which has this anti-salmonella activity against Typhoid disease.

Table-1. Antibacterial activity of different extracts of Neem (Azadirachta indica) stem bark against Salmonella strains.

Extracts	Diameter of Inhibition zone (mm)	
	S. Paratyphi A	S.Typhi
Aqueous	10 ± 0.2	17 ± 0.6
Methanol	12 ± 0.4	25 ± 0.3
Acetone	10 ± 0.5	24 ± 0.2
Ethanol	20 ± 0.6	23 ± 0.4
Benzene	14 ± 0.3	0
Chloroform	14 ± 0.5	0
Ciprofloxacin (30 mcg)	31 ± 0.2	30 ± 0.3
Gentamycin (10 mcg)	25 ± 0.1	21 ± 0.2
Chloramphenicol (30 mcg)	30 ± 0.5	27 ± 0.4
Erythromycin (15 mcg)	14 ± 0.3	13 ± 0.5
Negative control:		
Methanol: water (1:1)	0	0
Ethanol: water (1:1)	0	0
Acetone: water (1:1)	0	0
t- Test	P< 0.0004	P<0.0014
Fisher's exact test	P< 0.035	

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A Chemical Standardization of a Unani Single Drug – 1. Ood-e-Saleeb (*Paeonia emodi* Wall.) and Evaluation of its Antimicrobial Activity Against Bacterial Strains

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Abstract

The dried rhizome of *Paeonia emodi* Wall. known as 'Ood-e-Saleeb' in Unani System belonging to Ranuculaceae family plays a foremost role in Unani system of medicine. It is used in the preparation of different Unani compound formulations and for the treatment of many diseases such as epilepsy, uterine disorders, blood purifier, haemoptysis & remedy for bruises, sprains, etc. Due to its diverse medicinal importance there is a need to standardize the drug according to WHO guidelines. Organo-leptic parameters are not much reliable in establishing the standards of herbal drugs for which instrumental analysis of herbal drug was carried out, which gives a more concrete picture regarding the qualitative and quantitative aspects which are widely accepted in the quality assessment of herbal drugs like HPTLC and Atomic Absorption Spectrophotometer. Further the antibacterial activity studies were studied on aqueous and methanolic extracts by agar well diffusion method and results were shown as average zone of inhibition of bacterial growth. The microscopic studies of dried root were carried out to lay down the standard for the genuine drug. Other parameters studied include fluorescence behavior and U.V. spectrum.

Key Words: Ood-e-Saleeb, Macroscopic and Microscopic properties, Physico-chemical analysis, HPTLC Studies, Heavy metals, Antimicrobial activity.

Introduction

A genus of ornamental herbs and undershrubs, paeonia is distributed in the north temperate zone, especially in the Mediterranean region and Asia. One species occurs in India. *Paeonia emodi* Wall. belonging to the familly of Ranunculacea, its dried shizones are used widely in the Unani Systems of Medicine and known differently in varied regions by different names such as Ood-e-Saleeb (Unani); Chandra (Sanskrit); Paeony rose (English); Ud-salap (Hindi); Momokh, Mamekh (Punjabi & kashmir); Ud-salam (Bombay), Bhuma madiya and yet ghas (Bhutan); (Nadkarni, 1976).

A herbaceous or a shrubby perennial with a cluster of fleshy roots found in west temperate Himalayas from Kumaon to Hazara, in the upper Tons Valley & kashmir at altitudes of 2000-3000 m. The plant often occurs in gregarious patches and is reported to be abundant in Liddar Valley near Pahlgam (Kashmir).

Herbaceous peonies are preferred for ornamental purposes in gardens on the hills. In delicacy of tint and fragrance they resemble the rose, the double flowered one being more popular. They grow well in the cool climate of the hills, thriving in a deep, rich, rather moisty loamy soil and propagation by division of the fleshy roots (Anonymous, 1985).

A glabrous, 1-2 feet high, erect, stout, leafy, perennial herb. Leaves alternate, 6-12cm long leaflets-3 lobed or segments lanceolate, pointed, entire. Flowers showy,

3-4 cm. across, long stalked, usually solitary axillary. Sepals-5, orbicular, green, persistent, the outer one ending in leaf like point. Petals 5-10, broadly ovate, concave, red or white. Stamens many ovaries 1-3, densely hairy. Follicle ovoid, 2-3cm. Seeds few large (Anonymous, 2008). The seeds are emetic and cathartic (Kirtikar and Basu, 1998).

Tuberous roots of *paeonia* are stored food, swell and form tuber like structure. The tubers are of two kinds with sweetish acrid taste; acts as an appetizer and are highly esteemed remedy for uterine disorders & bladder troubles, colic (Qulanj), bilious obstructions leading to dropsy, epilepsy(Sara), head ache, convulsions (Ikhtenaq-ur-Rahem) and hysteria; given to children with milk as a blood purifier; useful in diuretic and haemoptysis.

Many formulations reported in Unani system of Medicine containing Ood-e-saleeb as one of the major ingredient for its valuable actions such as Roughan-e-Jund for Daf-e-Tashannuj (Antispasmodic); Habb-e-kuchla, Majoon-e-Maddat-ul-Hayat-Jadwari and Dawa-e-Salasul Baul used for Muqawwi-e-Asab (Nervine tonic), Itrifal-e-Zabeeb for Mufatteh sudad (Deobstruent), Khamira-Gawzaban-Ambari-Jadwar-Ood-Saleeb-wala for Muqawwi-e-Aza-e-Raeesa (Tonic for vital organs), Majoon-Hamal-Ambari-Alvi-Khani for Muqawwi-e-Reham, Sharbat Faryad Ras; for Munaffis-e-Balgam (Expectorant), Habb-e-Fawania Mushil for Munaqqi-e-Asab, Sufoof-e-Binai for Habis (Retentive), Qabiz (Constipation) and Mohallil-e-Warm-e-Meda (Anti inflammatory); (Farah *et al.,* 2005).

The tender shoots are cooked and eaten as vegetable. The fleshy roots are used in uterine diseases, and nervous affections. Excessive doses cause headache, giddiness, confused vision and vomting. An infusion of the dried flowers is given to control diarrhoea (Chopra *et al.,* 1986). Root combined with other drugs such as bruised leaves of *Melia is* a fovourite remedy for bruises, sprains, etc. Root given to cattle to render them prolific. Hot aqueous extract of plant had direct depressant effect on frog myocardium and direct spasmodic effect on guinea pig and rabbit ileum (Rastogi and Mehrotra, 1990).

Constituents: This genus contain a toxic acrid juice; roots of *P. emodi* are reported to contain an essential oil, with salicylaldehyde as the chief component; fixed oil, Starch (9.5) sucrose (5.4) Malic acid (0.47) oxalic acid (0.36) tartaric acid (0.34%) and benzoic acid present in roots (Rastogi and Mehrotra, 1991). Root oil contained lipids (Rastogi and Mehrotra, 1993).

Materials and Methods

Collection of material

Ood-e-saleeb was procured from the Pharmacy of Central Research Institute of Unani Medicine, Hyderabad, and was identified with the help of a botanist. The

present investigation includes parameters such as morphological, physico-chemical analysis and HPTLC fingerprint and antimicrobial activity. Routine procedures were followed for external morphological and anatomical studies. Physico-chemical parameters such as ash and extractive values were determined according to the methods described in Anonymous (2009). Fluorescence analysis was carried out as per the method described by Trease and Evans (1972).

Preparation of Extract of the sample drug

Five grams of powdered drug was dissolved in 100 ml of methanol in a stoppered conical flask and was kept for 2 hours while shaking at regular intervals. Later the contents were filtered through whattmann No. 41 paper and evaporate the solution to 20 ml. The solution thus obtained was used as sample for the determination of components.

Development and determination of the solvent system

Sample Application	:	methanolic extract of drug about 10µl.
Solvent system	:	Chloroform: Methanol: Formic acid (6: 3: 1)
Migration distance	:	91mm
Scanning wavelength	:	380nm

The sample was spotted as 4mm band with the help of Automatic TLC applicator system of the DESAGA Sarstedt Gruppe on Precoated Aluminium Sheets of Silica Gel 60 F_{254} (Merck). After trying with various solvent system with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the Twin through TLC chamber to the maximum height of the plate so that components are separated on the polar phase of silica gel and mobile phase of solvent system.

Development of HPTLC technique

After developing, the TLC plate was dried completely and detected by spraying 5% methanolic Sulphuric acid on the plate heated at 105^0C for 5 minutes and observed in the UV Cabinet system for detection of spots and photographed at 366nm as shown in figure 4. Multiwavelength scan was carried out and found that the wave length having better resolution and intense peak at 380nm in all the components of the methonlic drug extract as shown in figure 5**.** Further the plate was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 380nm. A densitogram was obtained as shown in figure 6. in which peaks are appeared corresponding to spots, being detected in the densitometer while scanning. The peak area under the curves corresponds to the concentration of the component in the sample to the amount of solution applied on the plate.

Fig. 1. Macroscopical feature of drug (Dried root of *Paeonia emodi* Wall.)

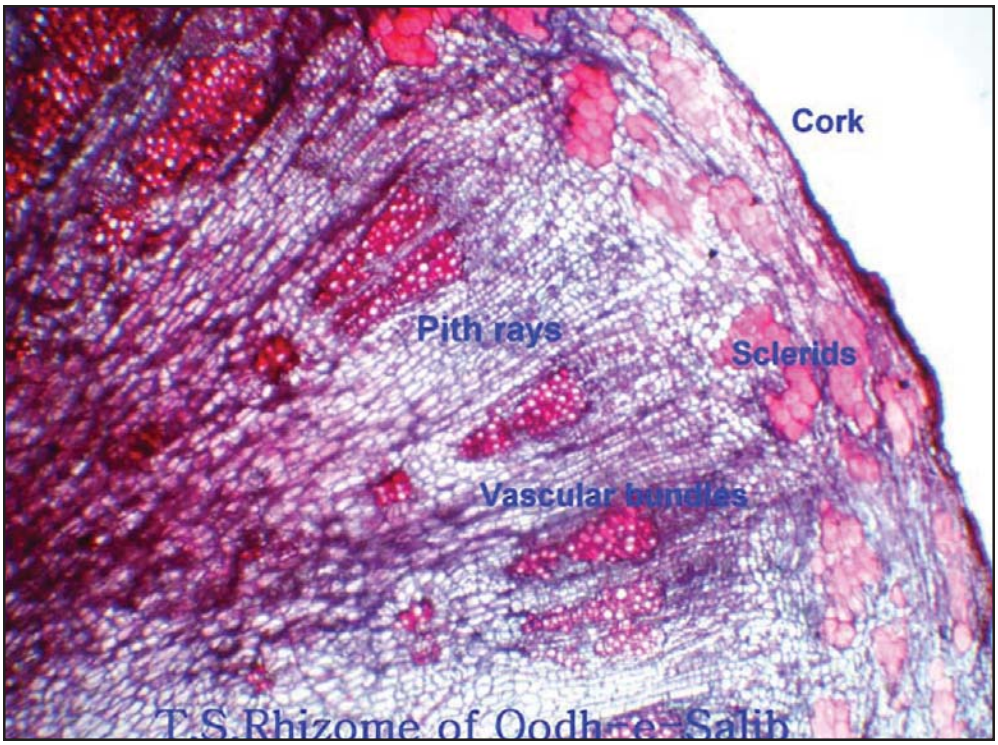

Fig. 2. Photo micrograph of transection of drug (Dried root of *Paeonia emodi* Wall.)

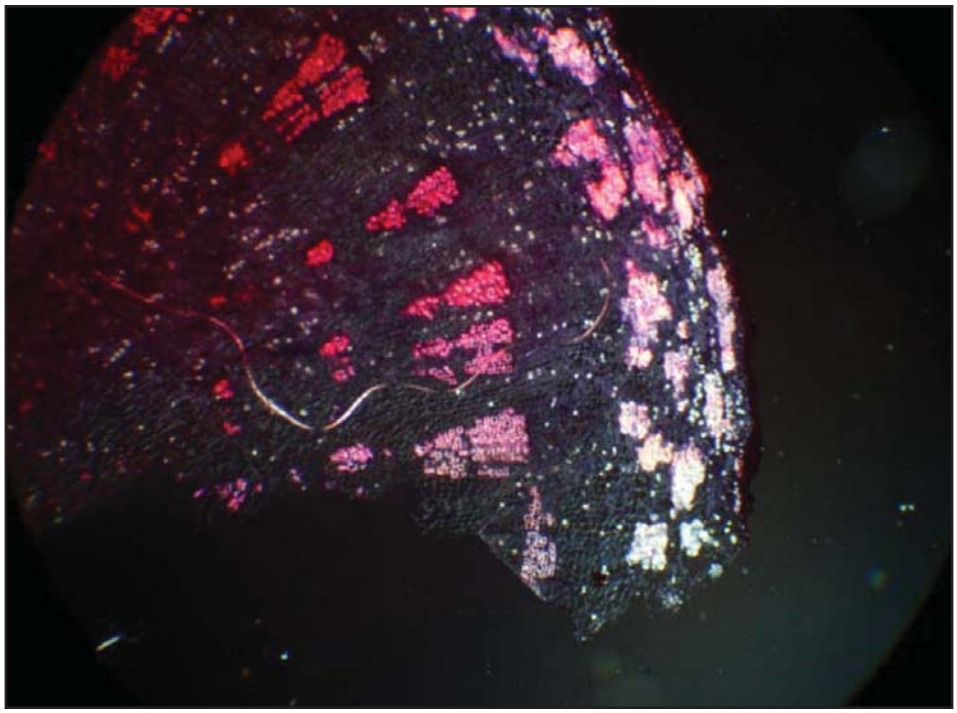

Fig. 3. Photo micrograph of drug (Dried root of *Paeonia emodi* Wall.)

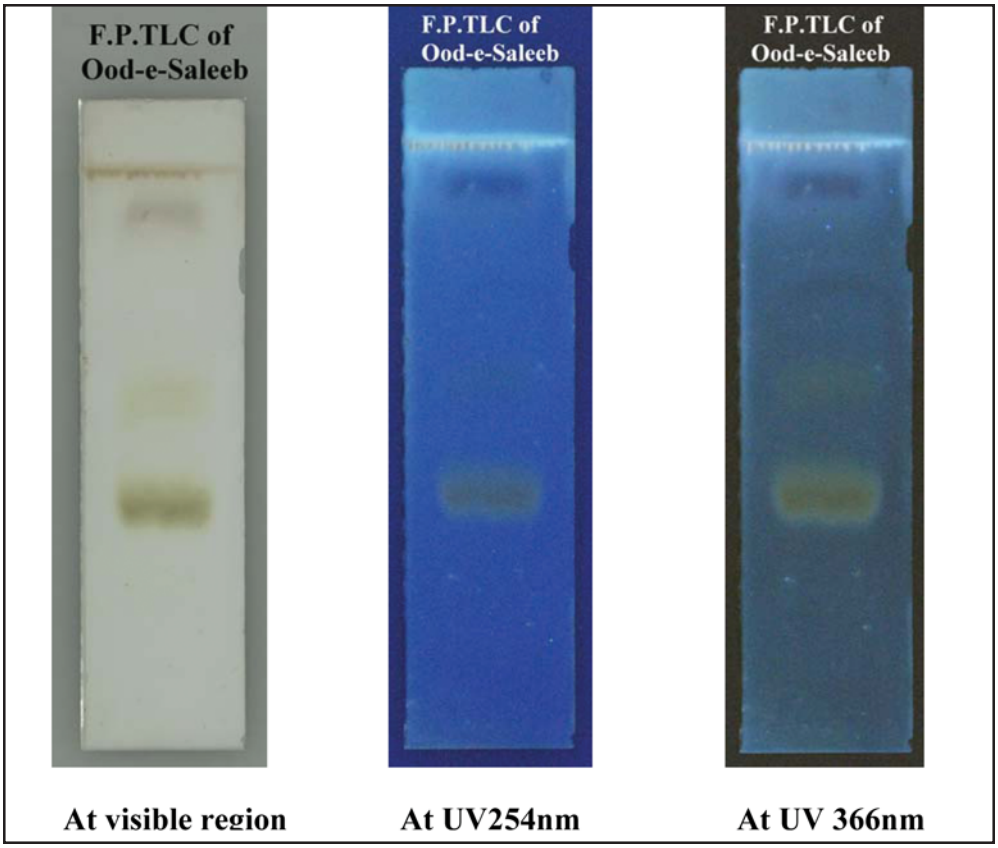

Fig. 4. Chromatograms of the methanolic extract of Ood-e-saleeb photographed at different ranges as mentioned

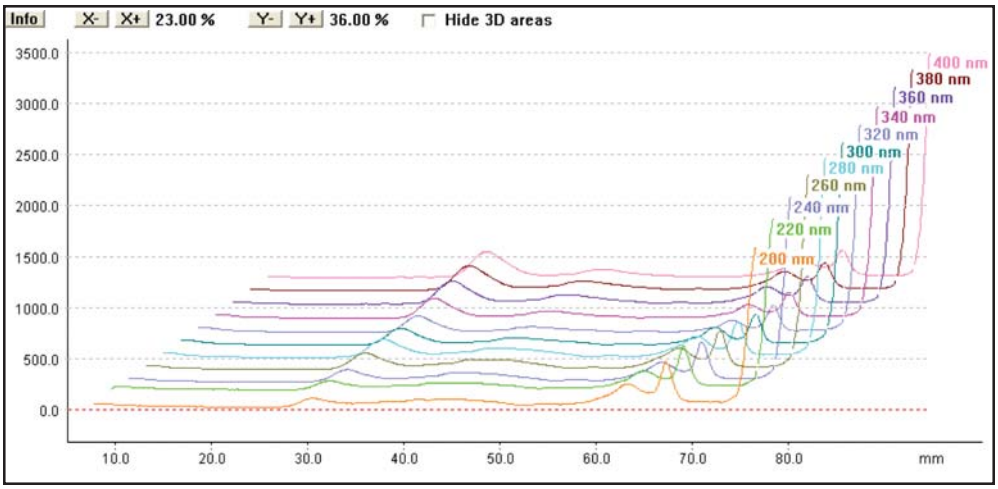

Fig. 5. Multiwavelength scan has carried out and found the wave length having better resolution and intense peak at 380nm in all the components of the sample.

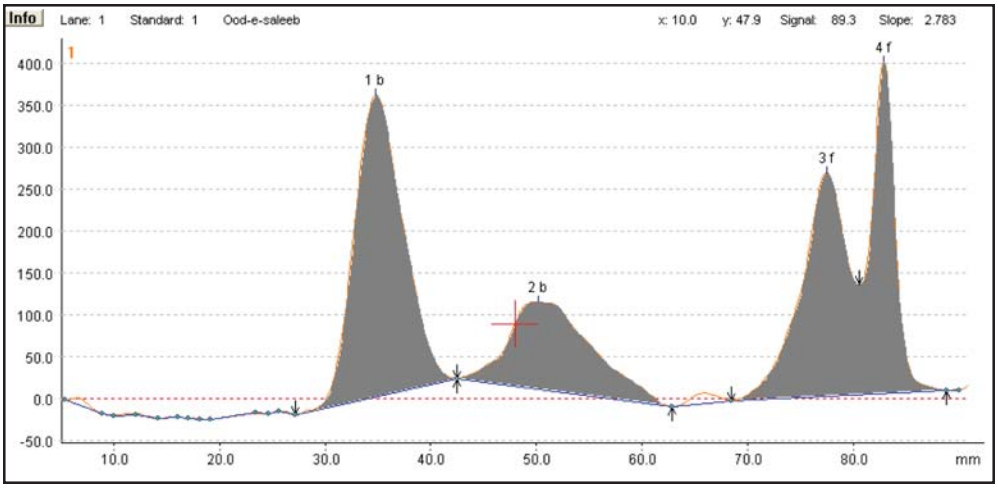

Fig. 6. Densitogram of the methanolic extract of Ood-e-saleeb

UV spectrum

With the help of HPTLC software system i.e., Proquant 1.6 version, the UV Spectrum was taken out for the peaks obtained in the densitogram with respect to their components positions and as shown in the figures (7a-7d). The UV spectrum was carried out under the UV range to get the absorption bands in the spectrum.

Heavy metals analysis

GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used in the analysis of toxic metals or heavy metals. Heavy metal analysis was carried out as per the WHO guidelines (Anonymous, 1998).The operating parameters of the

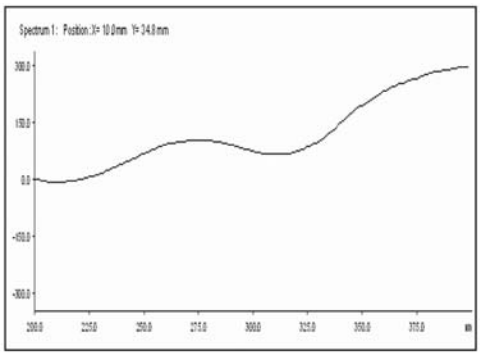

Fig.7a. UV spectrum of the component-1 positioned at 34.8mm

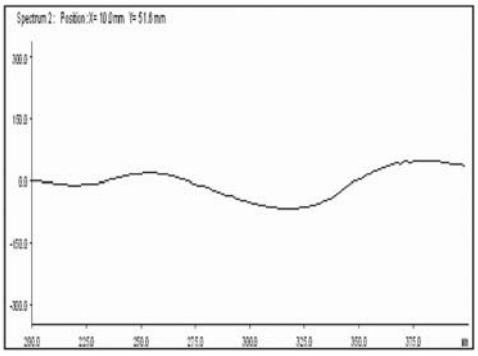

Fig.7b. UV spectrum of the component-2 positioned at 51.6mm

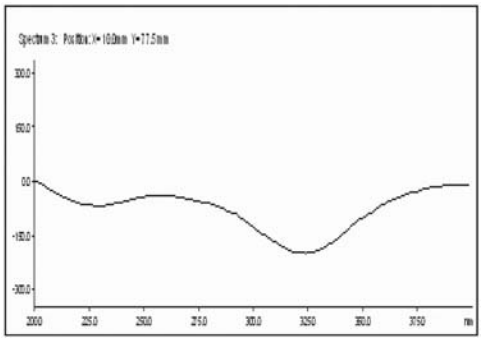

Fig.7c. UV spectrum of the component-3 positioned at 77.5mm

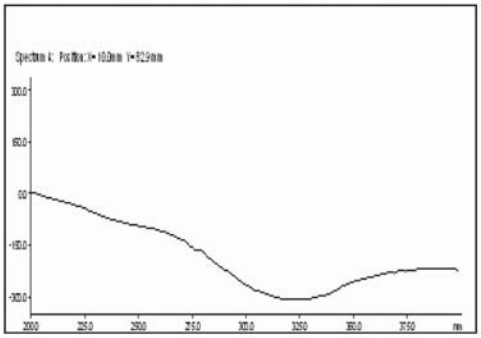

Fig.7d. UV spectrum of the component-4 positioned at 82.9mm

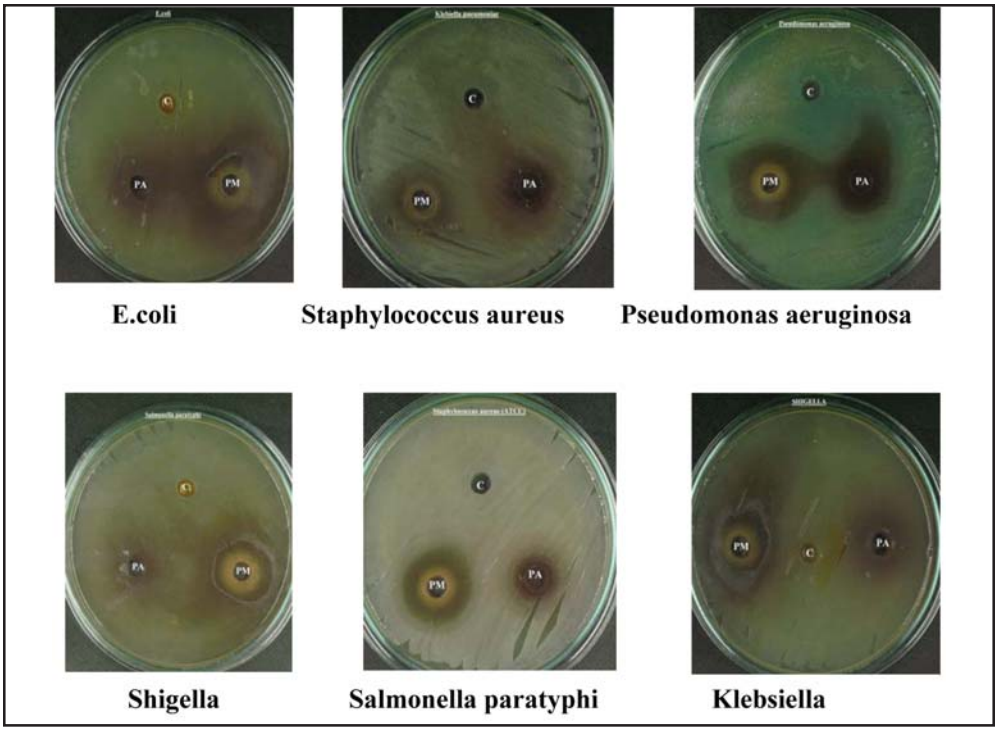

Fig. 8. Antimicrobial activity shown in the drug extract with specific pathogenic organism.

instrument were slit width: 0.5 mm, current: 3.0 mA, carrier gas: Air-acetylene, flow rate: 2ml/min. Stock solutions of Standard of the heavy metals were procured from Sisco Research Laboratory, Mumbai and their aliquots were made for the calibration of the equipment prior to the estimation of a particular element. Air-Acetylene gas was used for ignition of flame and Hollow cathode lamp were used for Cd, Hg and Pb elemental analysis, in order to determine their concentration in ppm levels with greater sensitivity.

Antibacterial activity

The methanolic and aqueous drug extracts were studied for antibacterial activity against pathogenic organisms such as *E.coli, Klebsiella, Pseudomonas aeruginosa, Salmonella paratyphi, Staphylococcus aureus, Shigella.*

Screening for antimicrobial activity

Antibacterial activity of the extract was determined by agar diffusion assay**.** Bacterial strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 4h at 37^0C and after the over night incubation period, 1ml of culture were spread on Mueller Hinton agar (MHA). The concentrations of the extracts used were 200 mg/ml. The wells were made using a sterile 6mm cork borer in the inoculated MHA plate and 200ìl of the extracts and along with that a control (Methanol : Water = 1 : 1) were loaded in the wells and the plates were incubated for 24h at 37^0C. Average “Zone of inhibition” was measured around the wells. The photographs of the plates were taken in Cab UV-VIS imaging system of Desaga Sarstedt Gruppe as shown in the figure 8 (Anonymous, 1998).

Results and Discussion

Analytical Profile

Organoleptic Characters

A glabrous erect, stout, leafy, perennial herb with tubers, sweetish acrid in taste.

Identification

Macroscopy

Rhizome pieces broadly spindle shaped, surface dark brown, rough with scars of persistent leaf bases 2.0-8.5 cm. long and 1.5-3.5 cm. wide; fracture brittle, surface rough; odour characteristically aromatic; taste indistinct. Macroscopic photograph as shown in figure 1.

Microscopy

T.S. of Rhizome as shown in figure 2 & 3 shows cork, cortex, vascular bundles, pith and pith rays. Cork 5-7 seriate, cells brick shaped, striated, 30-45 µ long and 18-20 ì wide, cell walls suberised. Cortex multiseriate, parenchymatous, cells polygonal, compactly arranged, 20-25 µ in diameter; cortical cells contain aggregations of calcium oxalate crystals measuring 10-15 µ in size; Cortex also contains 3-4 rows of aggregations of sclereids, each group containing three to fifteen sclereids; sclereids polygonal or tangentially elongated, 80-125 µ in diameter, wall has branched simple pits; Vascular bundles numerous, arranged in the form of a ring, conjoint, collateral, open and endarch;

Phloem 300-450 µ wide, contains sieve cells, companion cells , phloem parenchyma and phloem fibres; Each vascular bundle contains 60-120 vessels, xylem parenchyma and xylem fibres; vessels 300-450 µ long and 30-45 µ wide and contain scalariform and reticulate thickenings; xylem fibres scanty; Medullary rays 15-25 cells wide and 60-120 cells long, parenchymatous, cells radially elongated, 20-30 µ long and 15-20 µ wide, compactly arranged, cells contain aggregations of calcium oxalate crystals measuring 10-15 µ in size; Pith parenchymatous, contains patches of xylem and aggregations of calcium oxalate crystals measuring 10-15 µ in size.

Powder characteristics

Brownish, free floats on the surface of water, shows sclereids with branched simple pits, vessels with scalariform thickenings and aggregations of calcium oxalate crystals.

Physico-Chemical Standards

The Physico-Chemical Parameters data expressed here as mean values of the three readings calculated. The foreign matter composed of not more than 2 gm%; Total ash and acid insoluble ash not more than 2 gm%; where as Water soluble ash not more than 10 gm%. Alcohol soluble matter in terms of %w/w is found to be 22.83 and water soluble matter as 33.12; Successive extract values in terms of %w/w found to be Pet. Ether (60-80^{0}C) 12.0; Alcoholic extract 15.0; Distilled water extract 24; The moisture content i.e., Loss of weight on drying at 105^{0}C found to be 9.2 gm%. P^H of the 1% aqueous solution measured as 4.46-4.52 and 10% aqueous solution measured as 3.60-3.98.

Chemical Analysis: (HPTLC analysis, UV spectrum, Heavy metals, Fluorescence behavior)

Chromatogram was developed and detected using the 5% methanolic Sulphuric acid and heated at 105^{0}C for 5 minutes has clearly shown four spots in the UV region of 254 nm and 366nm and also in the visible region. HPTLC studies of

methanolic extract of Ood-e-Saleeb reveal four spots (Components) in the densitogram. The corresponding R_f values of the four components are 0.35, 0.54, 0.84 and 0.91 which are correspondingly positioned at 34.8mm, 51.6mm, 77.5mm, 82.9mm and the area corresponds to each peak determines the concentration of the component in the solution applied on the TLC Plate as shown in the table-1. UV spectrum for the spots obtained in the densitogram was carried out corresponding to their positions. The bands resulting in the spectrum for the spots at 34.8mm shows one band at 275nm; where as at 51.6mm shows two bands at 254nm and 320nm; at 77.5mm shows two bands at 260nm and 385nm; and at 82.9mm shows one band at 254 nm approximately as shown in the figure 7a-7b. The study carried out on heavy metals such as lead is present with in the permissible limits of WHO and FDA; other elements cadmium and mercury were found below the detection limit as shown in table-2. Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight i.e, visible region and under ultra-violet light were noticed and are presented in the table-3. Fluorescence analysis of powdered drug extracts in different solvents was observed and reported in the table-4.

Table-1. Rf values of various spots of methanolic extract of Ood-e-saleeb

Lane: 1 Type: Standard 1 Name: Ood-e-saleeb X-Position: 10.0mm

Peak	Component name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf.
1	:	34.8	1890.24	36.3	354.93	b	0.35
2	:	51.6	979.02	18.8	104.90	b	0.54
3	:	77.5	1338.77	25.7	267.11	f	0.84
4	:	82.9	1005.94	19.3	385.26	f	0.91

Table-2. Heavy metal Quantitative analysis of Ood-e-Saleeb:

S.No.	Name of the Element Analyzed	WHO & FDA Limits of Detection	Result obtained
1.	Cadmium	0.3 ppm	(BD) Below detection value
2.	Lead	10 ppm	(0.051) Below detection value
3.	Mercury	1 ppm	(BD) Below detection value

Table-3. Fluorescence analysis of powdered drug:

S.No.	Reagents	UV light		Visible light
		Short 254nm	Long 366nm	
1.	Powder as such	Dark green	Light yellow	Light brown
2.	Powder treated with 1N NaOH in Methanol	Dark brown	Light green	Dull green
3.	Powder treated with 1N NaOH in Water	Blackish brown	Dark green	Reddish brown
4.	Powder treated with 1N HCl	Black	Pale yellow	Light brown
5.	Powder treated with 50% HNO3 aqueous	Dark green	Black	Yellow
6.	Powder treated with 50% H_2SO_4 aqueous	Black	Black	Reddish brown
7.	Powder treated with Glacial Acetic acid	Black	Light blue	Light brown

Table-4. Fluorescence analysis of powdered drug extracts in different solvents:

S.No.	Extraction Solvent	UV light		Visible light
		Short 254nm	Long 366nm	
1.	Acetone Extract	Blackish brown	Light blue	Colourless
2.	Alcoholic Extract	Blackish brown	Pale yellow	Pale yellow
3.	Benzene Extract	Light blue	Dark brown	Colourless
4.	Chloroform Extract	Light blue	Light brown	Colourless
5.	Petroleum ether extract	Dark brown	Dark brown	Colourless
6.	Methanol	Dark brown	Pale yellow	Yellow
7.	Ethyl Acetate	Light brown	Light blue	Colourless
8.	Distilled water	Light green	Light blue	Light yellow.

Table-5. Antimicrobial activity studies for organisms v/s zone of inhibition values in extracts as shown.

S.No.	Organisms	Zone of Inhibition (mm)	
		Methanolic extract	Aqueous extract
1	*E.coli*	13.5 mm	6.5 mm
2	*Staphylococcus aureus*	25 mm	7.5 mm
3	*Pseudomonas aeruginosa*	25.5 mm	20 mm
4	*Salmonella paratyphi*	23.5 mm	6.5 mm
5	*Proteus vulgaris*	34.5 mm	20 mm
6.	*Klebsiella pneumonia*	10 mm	6.5 mm
7.	*Shigella spps.*	18.5 mm	6.5 mm
8.	*Bacillus subtilis*	32 mm	25.5 mm

Antimicrobial Activity

The methanolic and aqueous extracts of *Paeonia emodi* Wall. were screened for its antimicrobial activity against eight strains of bacteria and the results were shown in the table 5. Methanolic extract was found to be more active than aqueous extract, Proteus vulgar was found to be more sensitive with inhibition zone of 34.5mm in methanolic extract Whereas in aqueous extract, *Pseudomonas aeruginosa* and *proteus vulgar* was shown similar sensitivity with a zone of inhibition of 20mm. *Klebsiella pneumonia* was less sensitive to both the extracts with a zone of inhibition ranges from 10mm to 6.5mm. In aqueous extract *Bacillus subtilis* shown more sensitivity with an inhibition zone of 25.5mm.

Conclusion

The drug under study was subjected to Physico - chemical analysis, which is very much supportive in establishing the standard along with the other parameters such as macroscopic, microscopic, fluorescence behavior as reported in the present investigation including heavy metal analysis resulting in the permissible limits of WHO guidelines. HPTLC studies were correlated with the UV spectrum data illustrating the number of individual components present in it. Consequently the drug was brought up in determining and ascertaining its quality standard**.** The evaluation of antimicrobial activity on methanolic extract was found to be more active than aqueous extract with evidence to the data given for the corresponding result. Thus, the study is likely to help in the quality assurance of drug used in the Indian System of Medicine and in development of standard parameters.

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Effect of Kaknaj (*Physalis alkekengi* Linn Fruit) on Gentamicin-Induced Acute Renal Impairment in Rats

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Abstract

Kaknaj the fruit of *Physalis alkekengi*, Linn is an important drug of Unani Medicine. It has been described to be mainly effective in various renal diseases on account of its diuretic, anti inflammatory, nephroprotective, kidney tonic, healing, and analgesic effect etc. In the present study 70% ethanol extract of *Physalis alkekengi* fruit was investigated for its protective and curative effects against gentamicin (40 mg/kg) induced acute renal injury in Wistar rats. Elevation of blood urea, serum creatinine and appearance of histopathological features of acute tubular injury were taken as the indicators of nephrotoxicity. In the preventive regimen, the extract caused significant reduction in the elevated blood urea and serum creatinine and almost normalized the structural disintegration of kidney. In the curative group too both biochemical markers of kidney function decreased significantly and definite signs of regenerative changes were observed in histological examination. The findings suggest that the ethanol extract of *Physalis alkekengi* fruit possesses marked nephroprotective and curative activity and could offer a promising role in the treatment of acute renal injury caused by nephrotoxins such as gentamicin.

Key Words: *Physalis alkekengi*, Gentamicin, Nephroprotective, Unani Medicine, Kaknaj

Introduction

Many safe and effective drugs are in use in Tibb-e-Unani (Unani Medicine) since ancient times, in various renal disorders. A number of drugs studied in recent years for the effects that can be useful in the management of renal diseases demonstrated promising results. Bisehri Booti (*Aerva lanata*, Juss) (Amin *et al.,* 1994; Shirwaikar *et al.,* 2004), Revand Chini (*Rheum officinalis*) (Yokozawa *et al.,* 1991), Zanjabeel (*Zingiber officinale*) (Narora *et al.,* 1992), Asgand (*Withania somnifera*) (Panda *et al.,* 1997), Kharekhasak (*Tribulus terrestris*) (Nagarkatti *et al.,* 1994), Haleela (*Terminalia chebula*) (Yokozawa *et al.,* 1995), Banadequl Buzoor (a poly herbal formulation) (Anwar *et al.,* 1999) and Jawarish Zarooni Sada (a poly herbal formulation) (Afzal *et al.,* 2004) have been investigated for nephroprotective effect against different nephrotoxins, and also for certain related effects such as diuretic, analgesic, anti inflammatory and anti oxidant activity etc. These reports indicate that the drugs used in Unani system of medicines in the management of renal diseases have great potential to combat the effect of different types of toxins on kidney and also possess some associated effects that help improve the renal function. However, many important drugs used extensively in renal diseases by Unani physicians have still not been investigated for their reported effects.

Physalis alkekengi Linn, family Solanaceae, commnly known, as Kaknaj is an important Unani drug used frequently by physicians in the management of different

renal diseases. It has been described in Unani literature to be anti-inflammatory (Aawan, 1993; Ghani 1920), diuretic (Aawan, 1993; Aziz, 1948; Ghani 1920; Husain, 1872) lithotryptic (Kabiruddin, ynm), and nephroprotective (Kabiruddin, ynm), useful in kidney stone (Aawan, 1993), urinary tract infection (Aziz, 1948; Ibn Sina 1906), wound of urinary tract (Ibn Sina 1906; Ghani 1920; Husain, 1872), wounds of kidney and urinary bladder (Aawan, 1993; Aziz, 1948; Ghani 1920), and other diseases of kidney and urinary bladder (Ghani 1920). In ethnobotanical literature too, it has been described to be anti-inflammatory (Dymock, 1891; Nadkarni, 2000), and diuretic (Anonymous 1992; Anonymous, 1996; Chopra, 1956; Dymock, 1891; Khory & Katrak, 1985; Lindley, 1981; Nadkarni, 2000; Singh, 1974; Trease and Evan, 2002), useful in bladder stone (Anonymous, 1996), kidney stone (Anonymous, 1996; Nadkarni, 2000), and kidney diseases (Chopra, 1956; Dymock, 1891; Nadkarni, 2000). Some of the constituents isolated from *Physalis alkekengi* such as physalin A, B and C, histonin (Rastogi & Mahratra, 1998), lime, mineral salt, iron mingled with manganese (Nadkarni, 2000), Vit C, citrone and glyco alkaloids etc (Anonymous, 1992, 1996) have shown related pharmacological effects. While it's aqueous extract has been reported to show reproducible antineoplastic activity (Dornberger, 1986). However, in spite of having some important pharmacological actions as mentioned in Unani literature and having a long history of successful therapeutic use by the physicians of Unani Medicine, and also few related effects reported in its aqueous extract and someconstituents, no scientific study has been carried out so far, to investigate its effects related to the renal system. Therefore, in the present study the ethanol extract of *Physalis alkekengi* fruit was studied for its nephroprotective activity against gentamicin induced renal damage in albino rats at a dose which is used in Unani medicine for the treatment of different renal disease both in a preventive and curative paradigm i.e. before and after the induction of kidney damage.

Materials and Methods

Preparation of ethanol extract

The fruits of *Physalis alkekengi* Linn were procured from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh, India. Prof. S. H. Afaq and Dr. M. Inamuddin (Pharmacognosists), Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh confirmed the identity of the drug. A voucher specimen No. WA/2005/3 has been deposited in the museum of the department of Ilmul Advia.

The fruits were dried at room temperature and reduced to a coarse powder by grinding. Powdered drug was then immersed in 70% ethanol and left for 12h at room temperature and then extracted for 6 h in Soxhlet apparatus at 82 ± 2^0C. 100 g of powdered drug was extracted in 400 ml of solvent. The filtrate obtained after filtration of liquid extract through filter paper, was concentrated, using a water bath. The yield of the extract was found to be 30% of crude drug (w/w). 70 % ethanol was used because the highest yield percentage was found at this concentration.

Experimental Animals

Wistar Albino rats of either sex weighing 100-150 g were divided into four groups of six animals each. These four groups represented the Plain Control, Control, Preventive and Curative groups, respectively. Animals were maintained on standard diet and water *ad libitum* unless stated otherwise, and housed in clean polypropylene cages at room temperature (25-30ºC) with a 12h light: 12h dark cycle.

Treatment Schedule

Dose of the test drug for albino rats was calculated by multiplying the human therapeutic dose, described in the literature of Unani Medicine (Aawan, 1993; Ghani, 1920; Husain, 1872; Singh, 1974) by the conversion factor of 7 (Freirich *et al.,* 1968) and the animals were treated as follows:

Group	Treatment	Dose	Duration
Group I (plain control)	Normal saline	30 ml/kg	1^{st}-12^{th} day
Group II (control)	Gentamicin	40 mg/kg	1^{st}-12^{th} day
Group III (preventive)	Gentamicin + ethanol extract	40 mg/kg 450 mg/kg	1^{st}-12^{th} day
Group IV (curative)	Gentamicin ethanol extract	40 mg/kg 450 mg/kg	1^{st}-5^{th} day 6^{th}-12^{th} day

Gentamicin-induced renal damage

The animals in Group I were administered 30 ml/kg of 0.9% normal saline, intragastrically by a gastric canula, twice a day for 12 days. The animals in Group II were treated with gentamicin only in the dose of 40 mg/kg, twice a day for 12 days. Gentamicin was administered by intramuscular injection at quarters (Anwar *et al.,* 1999; Afzal *et al.,* 2004). Concentrated ethanol extract of the fruit of *Physalis alkekengi* was reconstituted in suspension form by using normal saline (450 mg/3ml, w/v) and 2% gum acacia and the animals in Group III serving as preventive group, were treated with a dose of 450 mg/kg by oral route, once a day along with gentamicin. While those in Group IV and served as curative group, were administered a daily dose of gentamicin (40 mg/kg) intramuscularly, twice a day for the first 5 days followed by the oral administration of extract at a dose of 450 mg/kg from the 6^{th} day onwards for the next 7 days (i.e. until the 12^{th} day).

On the 13^{th} day all the animals were sacrificed by overdosing of anaesthetic ether, administered by inhalation and blood was collected by decapitation. Serum was

separated from the blood and the level of urea and creatinine was estimated by the diagnostic kit supplied by J. Mitra Pvt. Ltd., New Delhi. Kidneys were dissected out after blood withdrawal and immersed in 10% formalin for histopathological studies. The tissue samples were embedded in paraffin, sectioned and stained with haematoxylin and eosin. Elevation of urea and creatinine level in the serum (Afzal *et al.,* 2004; Anwar *et al.,* 1999) and presence of features of acute tubular necrosis in the histopathological sections of the kidneys (Bennit *et al.,* 1982; Ali *et al.,* 2001; Shirwaikar, *et al.,* 2004) were taken as the indices of nephrotoxicity.

Statistical Analysis

The results were given as mean ± S.E.M. Significance was determined by using the Student's't' test. P-value equal to or less than 0.05 was considered significant.

Results

Rats treated with gentamicin only (Group II) showed definite signs of nephrotoxicity (Table 1-2 Figure-2), as compared to the plain control (Table 1-2, Figure-1) as evidenced by the significant increase in two serum markers of the kidney function, viz. blood urea and serum creatinine. The presence of peritubular and glomerular congestion, tubular casts, epithelial degeneration, interstitial edema, blood vessel congestion and infiltration by inflammatory cells, which are features of acute tubular necrosis, were observed in the histopathological sections of the kidneys in this group (Table-2, Figure-2). These features are indicative of the type and extent of damage done at the tissue level by the nephrotoxin (gentamicin). The animals in Group III and Group IV showed a significant decrease ($p<0.005$) in the level of serum markers of the kidney function (Table-1) when compared with group II, demonstrating significant nephroprotective and nephrocurative effects, respectively. Although, the histopathological sections of the kidneys of Group III (Table-2, Figure-3) and Group IV (Table-2, Figure-4) continued to show slight glomerular

Table-1. Effect of Kaknaj on blood urea and creatinine

Groups	Blood urea (mg/dl) (Mean ± S.E.M.)	Serum creatinine (mg/dl) (Mean ± S.E.M.)
Group I (plain control)	36.06±0.558	0.742±0.435
Group II (control)	66.05±0.606	2.287±0.302
Group III (preventive)	46.66±0.606*	1.470±0.423*
Group IV (curative)	48.78±0.558*	1.652±1.104*

n = 6
* = P < 0.005

congestion, peritubular congestion, blood vessel congestion, interstitial edema and inflammatory cell infilteration, nevertheless the damage was found to be markedly decreased as compared to group II.

Discussion

The study demonstrated gentamicin induced severe renal toxicity/injury which was evidenced by the elevated blood urea and serum creatinine levels (Table 1) in animals treated with gentamicin only. It indicated tubular cell damage which was further confirmed by the histopathological studies where the features

Table-2. Effect of Kaknaj on kidney histology

Histopathological features	Group I	Group II	Group III	Group IV
Glomerular congestion	–	+++	–	+
Tubular casts	–	+++	–	–
Peritubular congestion	–	+++	+	+
Epithelial desquamation	–	+++	–	–
Blood vessel congestion	–	+++	+	++
Interstitial edema	–	+++	+	–
Inflammatory cells	–	+++	+	+

(-): Normal; (+): Little effect; (++): Appreciable effect; (+++): Severe effect

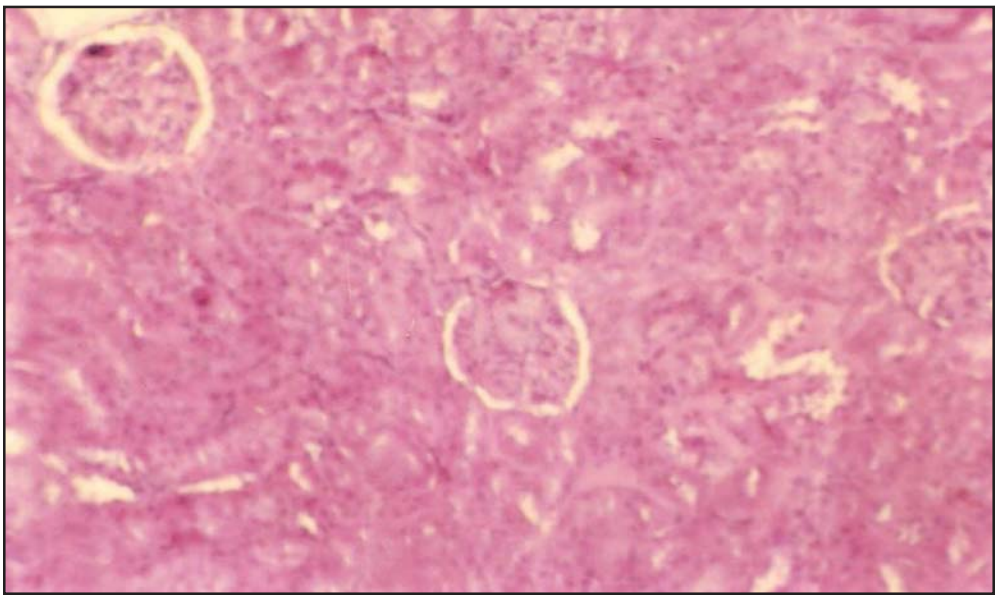

Fig. 1. Photomicrograph of Group-I (Plain Control)

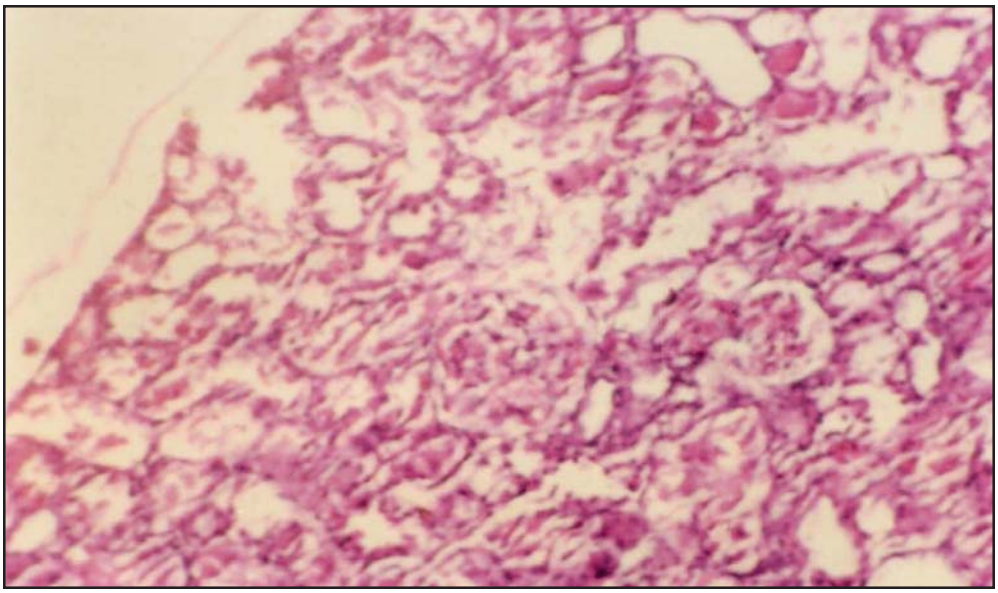

Fig. 2. Photomicrograph of Group-II (Control)

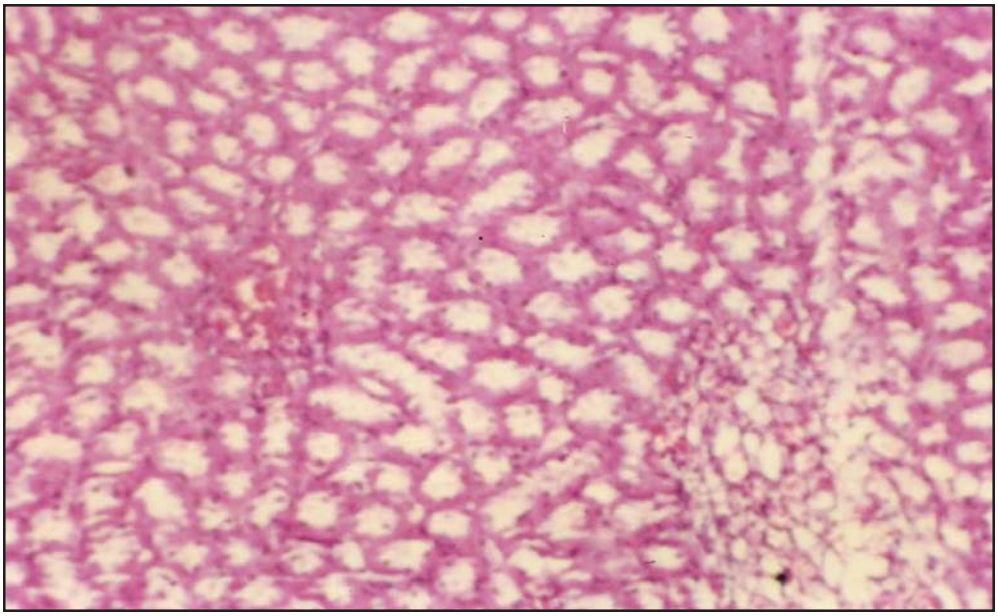

Fig. 3. Photomicrograph of Group-III (Preventive)

of acute tubular necrosis were observed (Table 2). Elevation of blood urea and serum creatinine has been considered as one of the important manifestations of severe tubular injury of kidney (Gilman *et al.,* 1992; Bennit *et al.,* 1982; Ali *et al.,* 2001) especially if the elevation in both is proportional (Braum, *et al.,* 1975). Gentamicin has been reported to produce nephrotoxicity even at normal therapeutic dose level (Smith *et al.,* 1980) mainly because it accumulates in proximal tubular cells and causes local damage and impairs the function as well as the structure of proximal tubular cells, (Leitman and Smith, 1983; Bennit *et al.,* 1982) and aggravates the toxicity and the degree of damage further at

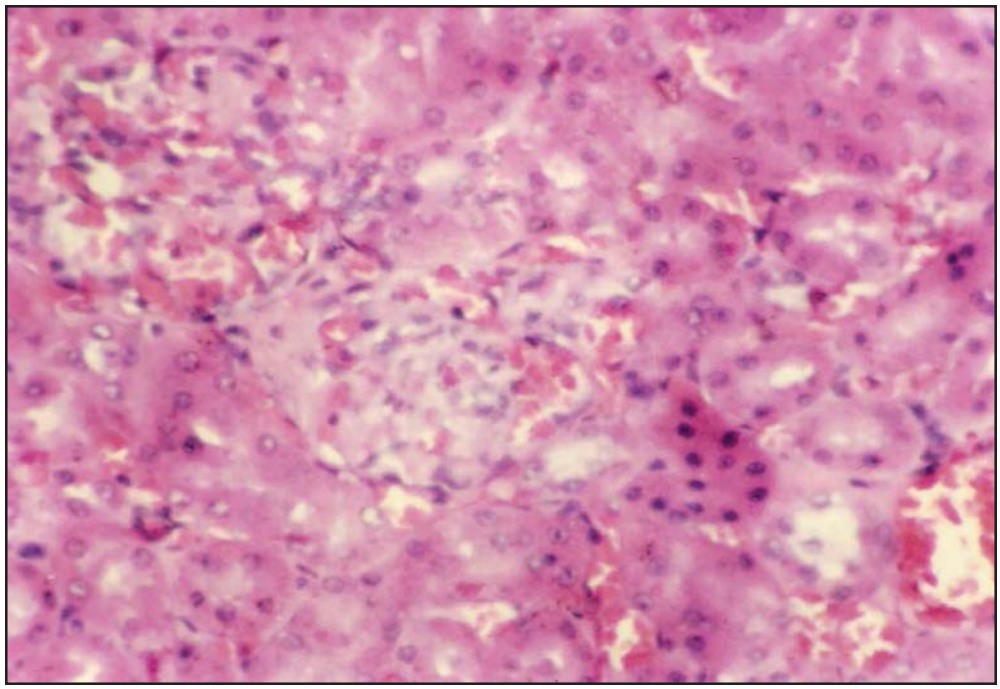

Fig. 4. Photomicrograph of Group-IV (Curative)

high dose level (Aronoff *et al.,* 1983). Tubular damage caused by gentamicin leads to loss of urinary concentration power and low glomerular filtration rate (GFR) etc. It has also been suggested that gentamicin and other aminoglycosides interfere with the production and metabolism of prostaglandins in the kidney and that is actually related to reduced-GFR (Humes *et al.,* 1984), which is considered as a sensitive index of functional nephron mass (Newman and Price, 1999). It has been further demonstrated that morphological change takes place in glomerular endothelial cell in animals that receive aminoglycosides causing reduction in glomerular capillary ultrafiltration coefficient (Baylis, *et al.,* 1977). The administration of ethanol extract of Kaknaj (*Physalis alkekengi* fruit) at the dose level of 450 mg/kg along with a high dose of gentamicin in preventive and curative groups blocked nephrotoxicity completely, as the blood urea and serum creatinine levels were found within the normal limits. The marked recovery produced by the test drug in damaged state of kidney is also evidenced from the microscopic examination. Curative group also showed definite signs of normalization in deranged functions and the texture of kidney. In microscopic examination regenerating blood vessels, few leucocytes, periglomerular fibrosis and infiltration of large number of mononucleus cells were observed, which clearly indicated the regenerative development. It is clear that the test drug did not allow two important serum markers of kidney function to elevate significantly above the normal level and showed marked protection of kidney when administered along with gentamicin. It also demonstrated significant level of improvement and onset of regeneration in damaged tissues in curative group. Therefore it may be inferred that the test drug has strong protective effects

against toxic and aversive effects of gentamicin and also the curative effect in case the damage has taken place by the effect of nephrotoxins. As far as the presence of slight glomerular congestion, peritubular congestion, blood vessel congestion, interstitial edema and inflammatory cell infiltration in histopathological slides of Group III and IV is concerned, it can be seen markedly lesser than the severely damaged condition of kidney in control group. Thus the test drug can be used as a protective agent to check the genesis of renal diseases and as a curative agent in cases of established injury and toxicity or a diseased condition. The study validated comprehensively that the therapeutic dose of Kaknaj described in Unani literature and used by Unani physicians is sufficient to produce significant degree of nephroprotection in chemically challenged kidney. However, it may be further studied at different dose levels and for a longer duration especially for its curative potential.

Although, *Physalis alkekengi* has not been studied earlier for nephroprotective and other related effects but some of its phytochemicals are reported to possess related effects. Quercetin isolated from *Physalis alkekengi* (Masao, *et al.,* 1988) has been shown to produce nephroprotective effect against cisplatin induced nephrotoxicity (Priya and Devi, 1999). Vit. C and citrone are known for their antioxidant properties while its aqueous extract has been reported to show reproducible antineoplastic activity (Dornberger, 1986). These activities may also be considered important in improving the chemically challenged kidney and complement the findings of the present study.

Since gentamicin accumulates in renal tubules particularly in S_1 and S_2 segment of the proximal tubules in lysomal and endosomal vacuoles (Vandewalle, *et al.,* 1981) and elicits an array of morphological and functional alteration of increasing severity through phospholipidosis (Tulken, 1989), the findings of the study therefore provide a clue for the site of action i.e. the cortical region of kidney and the likely mechanism of action i.e. phospholipase–like activity.

Conclusion

Based on the findings of present study we can conclude that the fruits of *Physalis alkekengi* possess marked protective and curative effect against the toxic effect of gentamicin and it may be used as a potential drug for protective and preventive application in the management of nephrotoxin induced renal injury and other related conditions.

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Anti-oxidant Activity of Zafran (*Crocus sativus* L.) with Vitamin E as Referent – An Experimental Study

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Abstract

Many Natural Drugs of Traditional Medicines have been shown to possess anti-oxidant effect. Unani Medicine, practiced from Morocco to India, is likely to possess outstanding antioxidants. So, the Unani drug, Zafran (*Crocus sativus*), was studied for effect on Lipid Peroxidation (TBARS), SOD and Catalase. After 7-day oral treatment with 175 mg/kg of 50% ethanolic extract of Zafran and Vit.E as the standard antioxidant for comparison, the rats were subjected to immobilization stress and the post-mitochondrial supernatant of liver was used for estimating the test parameters. The MDA concentration was decreased and SOD and Catalase activity were increased not only vis a vis the untreated and Vit.E – treated stressed animals but even the unstressed animals. So, the Unani drug Zafran is shown to possess a striking antioxidant effect. The remarkable increase in both SOD and Catalase, that effectively block the generation of Reactive Oxygen Species (ROS) in two successive steps, indicates that the test drug's antioxidant effect is exerted mainly by ROS generation blockade.

Key Words: *Crocus sativus,* Anti-oxidant, Saffron, Unani Medicine, Lipid peroxidation, SOD, Catalase

Introduction

Oxidative stress is being incriminated in a growing list of serious and common diseases such as inflammatory conditions, neurodegenerative diseases and malignancies as well as in ageing (Stadman & Berlet, 1999; Ames, 1983). Correspondingly, Antioxidant Drugs such as Vit.E, Vit.C etc are being increasingly used as prophylactic and therapeutic agents (Rice-Evans & Arif, 1999). Most of the effective and safe antioxidants are of Natural origin, so Traditional Medicines, mainly employing Natural drugs are being explored for better antioxidants (Farnsworth & Soejarto, 1985). Tibb-e-Unani (Unani Medicine), also known as Islamic or Arab Medicine, practiced as a rational and sophisticated medicine from Morocco to Malaysia over fourteen centuries and moored in both Greek and Indian Medicine of antiquity, is officially recognized and actively researched in the Indo-Pak subcontinent. It is likely to possess effective antioxidant agents. However, the Unani pharmacopoeia has not been systematically explored for these sought-after drugs. In the present study, the Unani drug Zafran (*Crocus sativus*) was studied for antioxidant activity. It was selected chiefly on the basis of 'translation' or matching of the characteristics of antioxidants and of Unani drug groups, short-listing drugs described as Tonic, Memory Enhancer etc. followed by selecting the most commonly used, scientifically studied and phytochemically suggestive agent. Unani classical literature describes it as Muqawwi Ruh (Tonic to Pneuma) (Ibn Baitar; Pub. 1272 H) Muqawwi Qalb (Cardiac Tonic) (Ibn Sina; Pub 1906), Muqawwi Dimagh (Brain Tonic) (Kareem, 1879), Muqawwi Jigar (Liver Tonic) (Razi, Pub.1968), Mufarrih

(Exhilarant) (Attar, Pub. 1888), Mufarrih Quwa (Exhilarant to Faculties) (Antaki, Pub. 1924), useful in Nisyan (Amnesia) (Hussain, 1872), Waj' al Mafasil (Arthrites) (Momin, 1272), Khafqan (Palpitation) (Mohammad Azam Khan, 1313H) and Laqwah (Bell's Palsy) (Hussain, 1872). These Af'al (Pharmacological Actions) and Isti'malat 'Ilaji (Therapeutic Uses) may correspond to Anti-Oxidant Activity. In scientific studies, Saffron has been demonstrated to possess Anti-Tumour Activity (Kumar *et al.,* 2001; Nair *et al.,* 1991 & Jagdeeswaran *et al.,* Anti-Inflammatory Activity (Zadeh & Younusi, 2002) and Nootropic Activity (Zhang *et al.,* 1994). Since, Anti-Inflammatory and Nootropic effect may be due to Anti-Oxidant Activity, the suggestions of Zafran possessing this activity from Unani descriptions are reinforced and it can be hypothesized that Zafran is a potent antioxidant. However, it has not been tested for this activity. So, in the present study Zafran was studied for Anti-Oxidant Activity.

Since Lipid Peroxidation is the best-characterized type of oxidative damage that also plays a crucial role in cell destruction (Wiseman & Ridgway, 2000), so, the antioxidant effect was evaluated in terms of protection against lipid peroxidation by using the TBARS Test.

Some mechanistic elucidation was also attempted. Antioxidant effect may be exerted by many mechanisms, operating alone or in combination. Most studies have focused on Scavengers, agents that react with 'Reactive Oxygen Species' (ROS) to neutralize them. But ROS generation blockers are likely to be not only better prophylactics but more effective curatives as well, because Scavengers only remove oxidative agents without necessarily repairing the damage already caused by them, while blockade of generation would preclude or greatly minimize the damage itself (Gutteridge and Halliwell, 1999). The Superoxide dismutase (SOD) enzyme plays the most fundamental role in generation blockade by converting the Superoxide Anion (O_2^-) into the less active Hydrogen Peroxide (H_2O_2) (Fridovich I., 1977). Though O_2^- is itself not so damaging (Sies H., 1985), it however serves as one of the chief progenitor of the highly reactive oxidative agent ie the hydroxyl radical ($OH^\bullet$). However, even H_2O_2 damages biomolecules, again by producing $OH^\bullet$ (Blake *et al.,* 1987), so, generation blockade is completed only by the consequent breakdown of H_2O_2 into water and O_2, which is chiefly catalyzed by Catalase (Haliwell & Gutteridge, 1985). In this light, any possible antioxidant effect of the test drug could be due to augmentation of SOD and Catalase activity and the consequent blockade of 'ROS' generation, so, Zafran was also studied for effect on the activity of these enzymes.

Materials and Methods

Test Drug

Zafraan (*Crocus sativus* L) was procured from a private supplier in Aligarh, Messrs Mohan Lal. Its identity was confirmed in the light of Unani descriptions and morphological study by a Pharmacognosist in the Departments. The sample of the

above batche of Zafraan were preserved in the museum of the Department. of Ilmul Advia, AMU Aligarh vide voucher No A-137.

Chemicals

All the chemicals and reagents were of analytical grade and were obtained from various sources. Tetra-methoxypropane (Sigma Aldrich,USA), ∝-tocopherol acetate, Amino Acid kit (Loba Chemie, India), Sodium dodecyl sulphate (SRL, India), Thiobarbituric acid, Bovine serum albumin (Ottokemi, India), Tris (Hydroxymethylaminomethane), Succinic acid, Pyrogallol, (S. D. Fine, India). Trichloroacetic acid (CDH, India), Follin Phenol Ciocalteu Reagent (Merck, India). All other chemicals and reagents were of the highest purity commercially available.

Physicochemical Analysis

Before carrying out the investigation for its antioxidant property, the Spectrophotometry, Thin Layer Chromatography and Colour Reactions was doneto characterize the sample.

Investigation for Antioxidant activity

Preparation of Test Drug

Zafraan (*Crocus sativus*. L) powder was prepared in Teflon Homogeniser at 2000 rpm.The test drug wase suspended in double distilled water while µ-tocopherol acetate was suspended in 5% Gum Acacia, for administration. The dose was obtained by multiplying the Unani clinical dose with appropriate conversion factor (Dhawan, 1982) and found to be 15 mg/Kg BW for µ-tocopherol acetate and 35 mg/Kg BW forZafraan.

Animals and Treatment

Twenty eight male albino rats (Wistar strain), weighing 120-130 gm, were divided into 4 groups of 7 animals each. All the animal procedures were performed according to the CPCSEA norms. The Institutional Animal Ethics Committee approved the experimental procedures. The animals were provided with standard diet (Purina) and tap water *ad libitum* and maintained at 20-25° C with 12 hour light and dark cycle. The animals were deprived of food for 12 hours before the administration of treatment, water was provided throughout the study. The animals in all the groups were administered with the treatment by oral route once a day for 7 days. The animals in all the groups except Gp I (Plain Control), were subjected to stress on the 7th day, as described later. The animals in Gp I & II that served as Plain Control and Stressed Gp, respectively, were administered with only the vehicle i.e. distilled

water, while animals in Gp III serving as the Standard Gp were administered with ∝-tocopherol acetate (15 mg/Kg), the animals in Gp IV, serving as test group, were administered with Zafran (35 mg/Kg BW). On the 7th day, immediately after giving the treatment, all the animals were subjected to immobilization stress in individual cages of their size for 6 hours (Hasan *et al.,* 1980, modified by Zaidi *et al.,* 2003). The animals were then removed from the cages and post-stress treatment was given as above. Forty-five minute after the post-stress treatment, the animals were sacrificed by cervical dislocation.

Preparation of post-mitochondrial supernatant

The liver was removed, washed in cooled 0.9% normal saline, kept in ice, blotted on filter paper, weighed and homogenized at 2500 rpm in cold 0.15M KCl for TBARS and SOD estimation and in cold phosphate buffer (50mM, pH 7) for Catalase estimation, separately, using Elvehjen homogeniser. The homogenization procedure was performed as quickly as possible under completely standardized conditions. The homogenates were centrifuged at 10,000 x g for 20 minutes at 4^0C and the post-mitochondrial supernatant was kept on ice until assayed.

TBARS Test

Malondialdehyde (MDA) levels were assayed - as the index for lipid peroxidation - in terms of a coloured product formed by the reaction of MDA with Thiobarbituric acid (TBA), according to the method of Ohkawa *et al.,* (1979). The deproteinised post-mitochondrial supernatant was reacted with 0.8% TBA, boiled in water bath at 100ºC and extracted with n-butanol. The absorbance of clear butanol supernatant was read at 532 nm in spectrophotometer using 1,1,1,3,3, tetramethoxypropane as standard. Results were expressed as nano-moles of Malondialdehyde per milligrams of protein.

Estimation of SOD activity

The SOD activity in post-mitochondrial supernatant of liver was assayed according to the method of Marklund *et al., (*1974). The assay was based on the ability of SOD to inhibit spontaneous autoxidation of pyrogallol. The reaction was started by the addition of 8 mM pyrogallol and the change in absorbance due to pyrogallol was recorded at 420 nm, spectrophotometerically. Results were expressed as unit (U) of SOD activity/mg protein. One unit of SOD activity was 50% inhibition of the rate of the auto-oxidation of pyrogallol under standard conditions.

Estimation of Catalase activity

Catalase activity in post mitochondrial supernatant of liver was estimated by the method of Aebi *et al.,* (1984). The Assay was based on the ability of Catalase to

induce decomposition of hydrogen peroxide followed by decrease in absorbance at 240 nm spectrophotometrically. The specific activity of the enzyme was expressed as ìM of H_2O_2 decomposed/min/mg protein.

Protein estimation

Protein estimation was made since the concentration of all the test parameters, namely, MDA, SOD and Catalase, was expressed per mg of Protein. The estimation was carried out by the method of Lowry *et al.,* (1951).

Statistical Analysis

The concentration of each parameter in various animal groups (Gp I - IV) were statistically compared for determining significance of difference by one-way ANOVA Test followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website, *www.analyseit.com.*

Results and Observations

TBARS Test

Six hours immobilization stress in the Stressed Gp (Gp II) resulted in significant increase in MDA to 8.29±0.21 n mole /mg protein ($p<0.001$). In Gp III, administered with the Standard drug, the MDA level was significantly decreased to 4.97±0.14 n mole /mg protein, in comparison to the Stressed Gp ($p<0.001$). In the Test Gp (Gp IV), the MDA level was significantly decreased to 1.03±0.03 n mole /mg protein, in comparison with the Stressed Gp ($p<0.001$). (Table-1).

Estimation of SOD activity

SOD was significantly decreased to 1.78 ± 0.09 U/mg protein in the Stressed Gp in comparison to the unstressed Plain Control Gp ($p<0.001$), while it was significantly increased to 3.22 ± 0.09 U/mg protein in the Standard Gp in comparison with the Stressed Gp ($p< 0.001$). In the Test Gp, it was significantly increased to 6.55 ± 0.11 U/mg protein in comparison with the, Stressed Gp ($p<0.001$) and the Standard Gp ($p<0.001$). (Table-1).

Estimation of Catalase activity

Catalase was significantly decreased to 163.45±8.55 U/mg protein in the Stressed Gp in comparison to the unstressed Plain Control Gp ($p<0.001$), while it was significantly increased to 485.07±11.32 U/mg protein in the Standard Gp in comparison to the Stressed Gp ($p<0.001$) as well as the Plain Control Gp ($p<0.001$). In the Test Gp, it was significantly increased to 527.46±10.29 U/mg protein in

Table-1. Effect of Zafraan and α-tocopherol acetate (Standard) on Lipid Peroxidation in Liver of Rats subjected to restraint stress.

Group	ηmole of MDA/ mg of Protein
Plain Control	4.64±0.15
Stress	8.29±0.21 b*
Standard	4.97±0.14 a*
Zafraan	4.22±0.13 a*

(n=7)

a = Against Stress, b = Against Plain Control, c = Against Standard

* =P < 0.001

Table-2. Effect of Zafraan and α-tocopherol acetate (Standard) on the activity of Superoxide Dismutase(SOD) in Liver of Rats subjected to restraint stress

Group	U/ mg protein
Plain Control	4.2 ± 0.09
Stress	1.78 ± 0.09 b*
Standard	3.22 ± 0.09a*
Zafraan	6.55 ± 0.11 a*b*c[8]

(n=7)

a = Against Stress, b = Against Plain Control, c = Against Standard

*= P < 0.001

Table-3. Effect of Zafraan and α-tocopherol acetate (Standard) on the activity of Catalase in Liver of Rats subjected to restraint stress

Group	μ mole of H_2O_2 decomposed /min/mg of protein
Plain Control	291.19±8.23
Stress	163.45±8.55b*
Standard	485.07±11.32a*b*
Zafraan	527.46±10.29a*b*

(n=7)

a = Against Stress, b = Against Plain Control, c = Against Standard

* = P < 0.001

comparison with the Stressed Gp ($p<0.001$) and the Plain Control Gp ($p<0.001$) (Table-1).

Discussion

Malondialdehyde (MDA) is one of the many products of Lipid Peroxidation caused by 'ROS', therefore, the decrease in MDA concentration in the animals treated with Zafran indicates that it protects against Lipid Peroxidation, hence, possesses antioxidant activity. Since, the Test Drug decreases MDA to levels which are not significantly different to that seen in the unstressed Plain Control Gp and the Gp treated with the standard antioxidant agent Vit E, it is shown to possess a striking antioxidant activity.

Oxidative Stress, i.e. increased generation of 'ROS', that can not be fully antagonized by physiological antioxidants, results in oxidative damage to all biomolecules (Massaki *et al.,* 1989). However, lipid damage is the most important and takes the form of Lipid Peroxidation (Kappus, 1985; Larson, 1997). So, the increase in Lipid Peroxidation shows that the Immobilization Stress applied by the method of Hasan *et al.,* (1980), modified by Zaidi *et al.,* (2003), successfully causes oxidative stress and damage. The expected decrease in MDA concentration shown in Vit E – treated animals indicates the integrity and validity of our experimental procedure.

As mentioned, lipid peroxidation, though quantitatively and clinically important, is only a part of overall oxidative damage, which involves all types of biomolecules e.g. proteins, nucleic acids, carbohydrates etc (Kappus, 1985). So, strictly speaking, the positive results seen in the TBARS Test indicate protection of only the lipids. But Lipid Peroxidation is also used as an indicator of the total oxidative damage (Kappus, 1985; Larson, 1997). Therefore, it is justified to infer that the test drugs, protect against overall oxidative damage, thus, possessing comprehensive antioxidant activity.

Antioxidant studies, in addition to including TBARS Test, also consist of the estimation of endogenous physiological antioxidants, both enzymatic eg SOD, Catalase etc. and non-enzymatic eg Glutathione, Uric acid etc. Short-term oxidative stress usually leads to a decrease in the activity of anti-oxidant enzymes while long-term insult is associated with its increase (Torres *et al.,* 2004). Non-enzymatic anti-oxidants are obviously decreased in both situations. So, with acute oxidative stress, as adopted in the present study, antioxidant agents usually increase the concentration of both enzymatic and non-enzymatic physiological antioxidants (Al-Qirim *et al.,* 2002, Sosnovsky & Kozlov, 1992). This could be due to the direct antioxidant activity of the test agents that 'spares' and thereby increases the concentration of the physiological antioxidant or the test agent may produce an actual increase in the physiological antioxidant by various mechanism such as, increased synthesis of an antioxidant enzyme due to gene activation etc. (Gutteridge and Halliwell, 1999). The increase in concentration of physiological antioxidants, therefore, indicates

antioxidant activity and confirms the antioxidant activity shown by decreased lipid peroxidation in TBARS Test. Secondly, these studies are also of mechanistic value. The increase in a physiological antioxidants indicates that the test agent may be producing its antioxidant effect through the mediation and manner of that physiological antioxidant.

The mechanism of antioxidant effect of endogenous substances can be classified as scavenging and quenching, ROS-generation blockade, repair etc. As mentioned above, ROS-generation blockade, seems to be a privileged mechanism, likely to be clinically more useful than others. The SOD and Catalase enzymes, acting successively block 'ROS' generation in a big way (Blake *et al.,* 1987). Therefore, in the present study the effect of the Test Drug was studied on SOD and Catalase, not only as indices of antioxidant activity, but also as markers of ROS-generation blockade.

The Test Drug significantly increased SOD to a level greater than that seen in the stressed animals, the animals treated with the potent antioxidant agent, Vit E , and even the unstressed animals and increased Catalase to a level greater than that seen in the stressed animals, and even the unstressed animals. Thus the study provides further evidence for a striking antioxidant effect of the Test Drug by demonstrating its ability to augment two powerful antioxidant enzymes in animals subjected to oxidative stress to levels greater even than that of unstressed, normal animals.

As mentioned, SOD converts O_2^- to the less reactive H_2O_2, which however can give rise to oxidative damage, particularly by forming one of the most reactive radical, $OH^{\bullet}$. So, Catalse completes the job of 'ROS' generation blockade by converting H_2O_2 (Haliwell & Gutteridge, 1985). The striking increase in both SOD and Catalase activity produced by Silk Cocoon therefore indicates that it exerts a rather complete 'ROS' generation blockade which seems to be the main mechanism for its antioxidant activity.

Due to the common incidence of adulteration of the costly drug, Zafraan, it is important to apply some rapid and easy methods for checking the purity and genuineness of the sample. For this, Zafraan was reacted with Conc. H_2SO_4 and it was found that the sample obtained from Mohanlal, Aligarh (Sample M) released immediate pinkish to deep blue colour, while the sample obtained from Dawakhana Tibbiya College, AMU, Aligarh (Sample D) showed light blackish to dark colour indicating the purity of former as described in literature of Ethnopharmacology. Further, since the method of spectral analysis of Zafraan, described in Indian Materia Medica of single drugs is quite unclear and ambiguous, therefore, we used probably for the first time, visible light Spectral Analysis of different samples of Zafraan. The pattern of spectra was compared with the pattern of chromium trioxide. The peak maxima of sample D, M and Chromium Trioxide were found to be 2.45 at 439.6 nm, 2.894 at 490.6 nm and 3.017 at 488.8 nm, respectively (Afaq *et al.,* 2006). The

findings clearly showed the close resemblance in the pattern of spectra of the sample M with that of the Chromium Trioxide and the deviation of sample D is indicating it to be exhausted or adulterated. Further, thin layer chromatography confirmed the findings of spectral analysis as TLC showed an extra pinkish colour band in sample D, whereas sample M contained only four yellow colour bands. These observations indicated that sample D was firstly exhausted and then adulterated with dye (probably aniline) and thus sample D was found to be substandard. Therefore, sample M was selected for pharmacological study.

Zafraan is reported to be Muqawwie (Tonic) (Razi, Pub. 1968, Ibn Sina, Pub. 1906) and Muhallil (Resolvent /Anti-inflammatory) (Razi, Pub. 1968; Ibn Baitar; Pub. 1291H; Ibn Sina, Pub. 1906; Antaki, Pub. 1924). The findings of the present study provide scientific support to these descriptions and offer an explanation by showing antioxidant activity in the drug. This is also in consonance with reports regarding Antitumour Activity (Nair *et al*. 1991) Memory Enhacing Activity (Zhang *et al.,* 1994) Adaptogenic Activity (Anonymous, 2001) and Anti-inflammatory activity (Zadeh & Younusi, 2003) in various fractions and compounds of Zafraan and the demonstration of Antioxidant Activity in the stem of Zafraan plant (Anonymous, 2001). Zafraan has been subjected to extensive phyto-chemical study and has been shown to possess various carotenoids like picrocrocin, Crocin -1, Crocin-2, and Crocin-3 (LI-N *et al*. 1999; Anonymous, 1999), and Flavonoids, such as Phytoene, Phytofluene, Tetrahydrolycopene and Zaxanthene etc. (Pfander *et al.* 1982). Carotenoids are likely to possess strong antioxidant activity e.g. β-Carotenoids. Flavonoids are also likely to have antioxidant activity (Larson, 1997). So, the antioxidant activity demonstrated in whole Zafraan in the present study, and in its fractions and compounds in earlier studies could be due to its carotenoids and flavonoids.

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Botanical and Physico-chemical Standardization of Sufoof-e-Bers – a Polyherbal Unani Drug of Repute

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Abstract

Sufoof-e-Bers, a powder drug of high therapeutic efficacy, widely used to cure several skin diseases, has been prepared from the prescribed ingredients of pharmacopoeial quality. Based on the preparation of three different batches, a standard operating procedure (SOP) of the drug is developed. In order to lay down certain features of diagnostic value, the formulation is scientifically standardized on the basis of standard pharmacopoeial parameters viz. the microscopy, physico-chemical analysis, thin layer chromatography, level of microbial and pesticidal contaminations, aflatoxins and the heavy metals. Present study, therefore, holds high significance not only for identification of genuine ingredients but also to detect the adulteration or substitutions, if any.

Key Words: Sufoof-e-Bers, SOP, Microscopy, Physico-chemical standards, TLC, Aflotoxins, Heavy metals

Introduction

Safoof-e-Bers is a compound polyherbal unani drug, classified under sufoofs as per NFUM-I and consists of four raw ingredients, all from herbal origin. Therapeutically, it is a good blood purifier (Musaffi-e-Dam) but known best for its property to cause the pigmentation of skin (Humrat-e-Jild). It is therefore prescribed extensively in the unani system of medicine to cure leucoderma (Bers), a disease of unknown aetiology (Anonymous, 1983, 1987; Kabiruddin, 1967). 10-12 g of fine powder soaked overnight in 50 ml of water and the infusion decanted is administered orally in the morning. The sediment is mixed with vinegar to make a paste and applied locally on the affected parts which are exposed to the sun rays.

Although the consumption of Unani drugs both as a raw drug as well as in compound preparation has increased tremendously during last few decades because of a speedy awareness about its safe and quick efficacy under a low cost, the authenticity of crude drugs being used is still not up to full satisfaction. In order to maintain the quality and efficacy, proper standardization of drugs is therefore an indespensible task. In this direction SOPs of a number of compound unani formulation viz. Itrifal-e-Kishnizi, Itrifal Zamani, Jawarish-e-Hazim, Jawarish Podina, Majoon Ispand Sokhtani, Majoon-e-Rewandchin, Majoon-e-Yahya Bin Khalid, Sharbat-e-Aijaz and Sunoon-e-Tambaku have been developed by Negi *et al.,* (2009), Siddiqui *et al.,* (1991), Meena *et al.,* (2009), Aminuddin and Siddiqui (2007), Goel *et al.,* (2007), Ramaswamy *et al.,* (2009), Mageswari *et al.,* (209), Bagul *et al.,* (2006) and Zuberi *et al.,* (2008) respectively. Continuing with the ongoing efforts an attempt has been made to standardize the unani formulation – 'Sufoof-e-Bers' as per the set pharmacopoeial parameters, thus far remained unstudied and the findings are presented in this communication.

Material and Methods

This formulation contains four raw ingredients but all belong to herbal origin (Plate 1). The details of composition of Sufoof-e-Bers as per NFUM-I (Anonymous, 1983) is given as under.

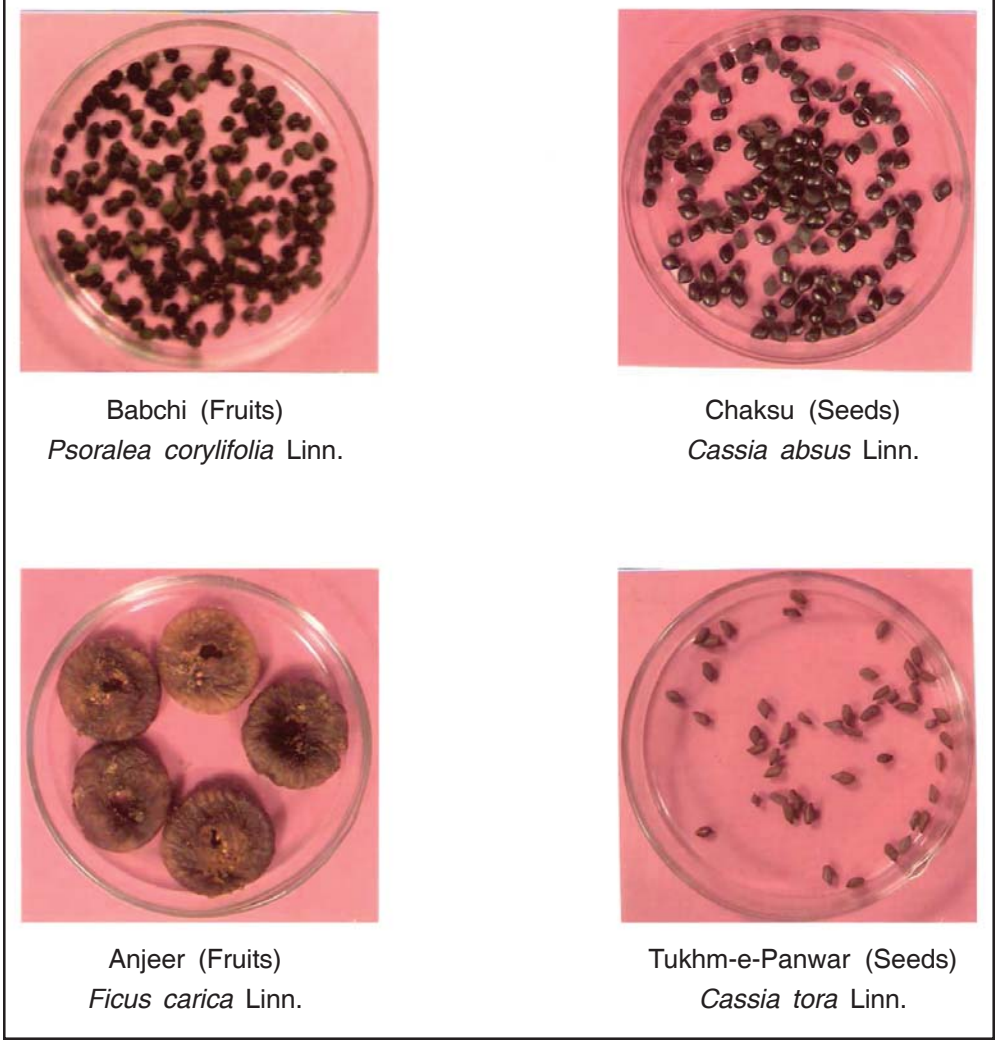


Plate 1. Ingredients of Sufoof-e-Bers

Formulation composition

S.No.	Unani Name	Botanical name	Part	Quantity
1.	Babchi API-I	*Psoralea corylifolia* L.	Fruit	1 part
2.	Chaksu UPI-II	*Cassia absus* L.	Seed	1 part
3.	Anjeer khushk UPI-II	*Ficus carica* L.	Fruit	1 part
4.	Tukhme Panwar UPI II	*Cassia tora* L.	Seed	1 part

In order to prepare the three batches of Sufoof-e-Bers, sufficient quantity of all the four raw ingredients were procured from three different sources i.e. one from Dawakhana Tibbiya College, AMU, Aligarh and other two from authorized druggists (ie M/s Mohan Lal Vinod Kumar and M/s Taj Trading Co.) of the locality. All these raw drugs were identified using the pharmacognostical methods according to Wallis (1967) and Trease & Evans (1972) and cleaned to remove all impurities.

Since, chaksu seeds are to be used after detoxication (Mudabbar) so these were kept in a cloth bag and then boiled for half an hour in fennel water. The cloth bag was opened and the seeds rubbed gently with hand so as to remove the seed coat and finally dried under shade. The Anjeer fruits being quite fleshy, were broken into smaller pieces and dried. Each of the four ingredients were then powdered separately and sieved through mesh No. 60. Equal quantity of all the four ingredient's powder was then taken and mixed thoroughly in a clean bowl. All the three batches of drug were thus prepared by the same procedure.

The drug was standardized with reference to all the parameters of Unani pharmacopoeia of India. The microscopic study of drug was carried out according to Johansen (1940), and Wallis (1967), physico-chemical analysis as per Indian Pharmacopoeia (Anonymous, 1966), thin layer chromatography following Wagner *et al.,* (1984) and Stahl (1996) and aflatoxin and pesticide estimation as per standard methods (Anonymous, 2000).

Observations

Sufoof-e-Bers is a yellowish brown dry powder drug having a strongly bitter taste and an aromatic odour.

Microscopic features

Careful examination of different mounts of the powder drug revealed that it is characterized by the presence of certain cell types only. Accordingly presence of abundant thick walled palisade epidermal cells (60-80 m long), I-shaped bearer cells of hypodermis and thin walled as well as polyhedral and elongated cotyledonary parenchymatous cells filled with small aleurone grains (4-12 μ) confirmed the three leguminous ingredients i.e. Babchi, Chaksu and Panwar (Plate 2). The epidermal cells of receptacle (40 x 50 μ – 25-35 μ) having occasionally raised stomata (Anomocytic type), some polyhedral collenchymatous hypodermal cells often containing cluster of calcium oxalate crystals and the pitted stone cells of endocarp however, indicate the presence of another syconus fleshy ingredient i.e. the Anjeer fruits.

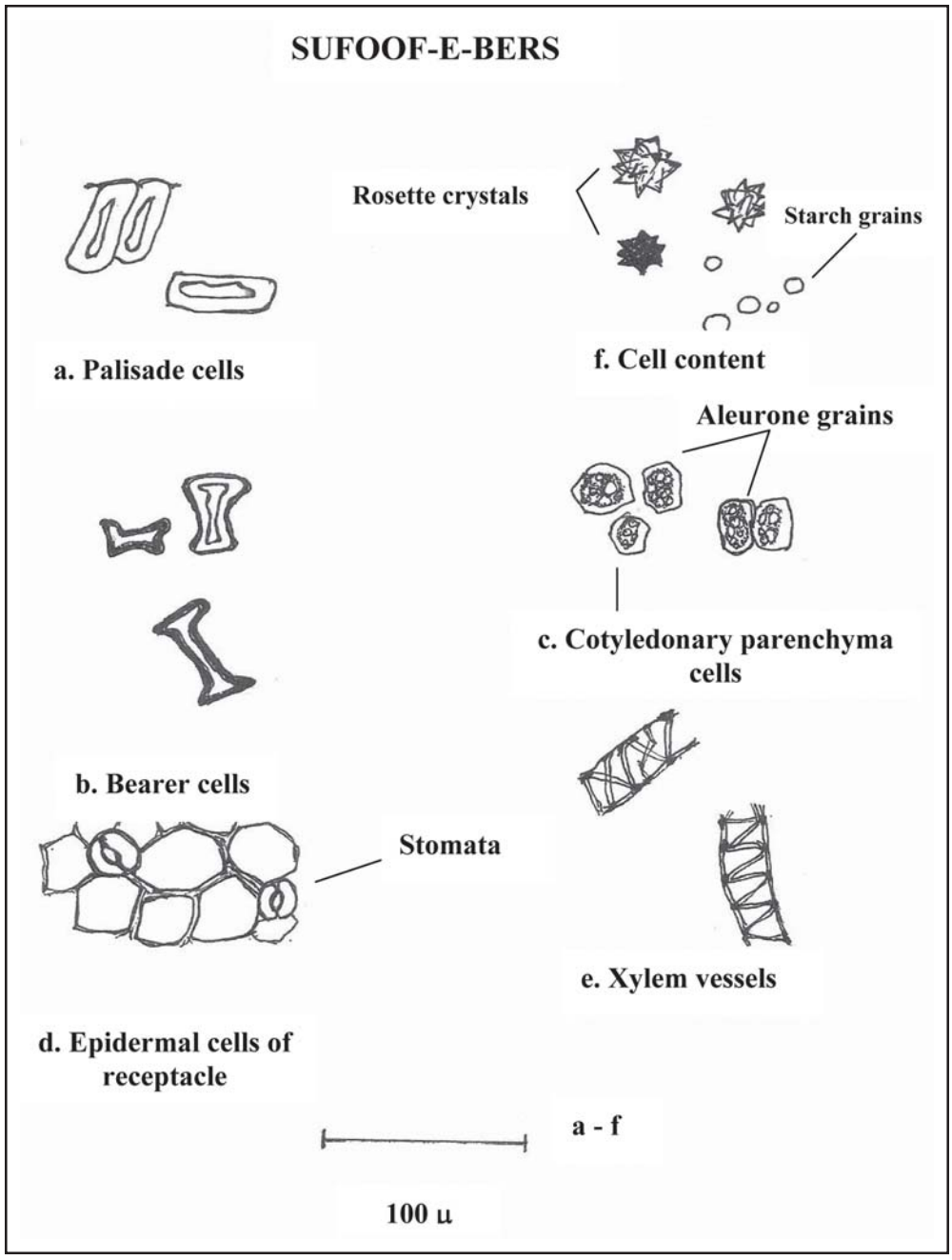


Plate 2. Microscopic examination of powder showing various cellular structures and cell content.

Physico-chemical Analysis

Preliminary physico-chemical investigations of drug including the ash values, solubility, pH values and loss on drying have been carried out (Table 1). The extractive values of all the three batches of drug (in the five different solvents) are shown in Table 2. The estimation regarding microbial load and heavy metal contamination is indicated in Table 3, while the pesticide and aflatoxin contamination limit in Table 4.

Table 1. Physico-chemical Analysis of Sufoof-e-Bers

Parameters	Batch Number			
	I	II	III	Range
Alcohol soluble matter (%, w/w)	15.32 15.38 15.35	15.88 15.84 15.90	15.50 15.51 15.58	15.32-15.88
Water soluble matter (%, w/w)	21.85 21.82 21.80	22.15 22.18 22.21	21.92 21.88 21.96	21.80-22.21
Total Ash (%, w/w)	6.27 6.25 6.29	6.33 6.36 6.30	6.31 6.34 6.29	6.25-6.36
Water Soluble Ash (%, w/w)	0.57 0.54 0.59	0.85 0.82 0.87	0.68 0.65 0.61	0.54-0.87
Acid Insoluble Ash (%, w/w)	3.25 3.28 3.21	3.78 3.75 3.72	3.53 3.50 3.55	3.21-3.78
pH (1% aqueous solution)	4.92	4.70	4.72	4.70-4.92
pH (10% aqueous solution)	4.83	5.0	5.04	4.83-5.04
Bulk Density	1.39 1.38 1.36	1.45 1.43 1.42	1.42 1.43 1.40	1.36-1.45
Loss on drying at 105°C (%, w/w)	14.82 14.80 14.86	15.28 15.25 15.21	14.64 14.62 14.65	14.62-15.28

Thin Layer Chromatography

The methanol extract was prepared and applied as bands on precoated Aluminium plates, silica gel 60 F_{254} using Toluene-Ethyl acetate-Methanol-Formic acid (6-3-1-1-) v/v as solvent system, and the plate was developed in ascending mode to 8 cm, dried at room temperature (32°C) and was visualized under uv (365 nm) without any chemical treatment. Twelve spots were seen at Rf 0.19(light pink), 0.25 (light sky blue), 0.32 (sky blue), 0.38 (light pink), 0.51 (light brown), 0.55 (light fluorescent

Table 2. Successive Extraction of Sufoof-e-Bers

Extractive solvents	Extractive values			Mean value	Range
	I	II	III		
Petroleum ether	7.64	8.13	7.92	7.89	7.64 – 8.13
Benzene	2.24	2.40	2.45	2.36	2.24 – 2.45
Chloroform	0.26	0.27	0.28	0.27	0.26 – 0.28
Ethyl alcohol	18.62	17.88	17.94	18.14	17.88-18.62
Water	17.45	17.24	17.13	17.27	17.13-17.45

Table 3. Microbial & Heavy metal contamination of Sufoof-e-Bers

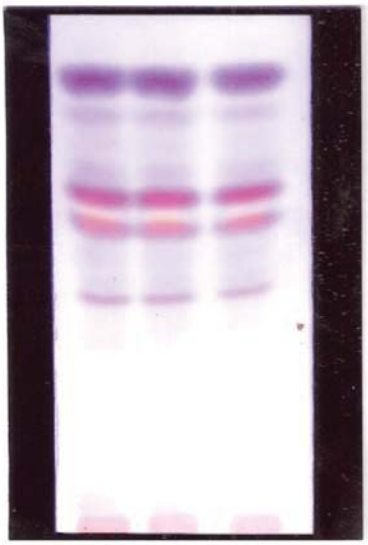
S.No.	Parameters	Results	WHO Limit
	Microbial Load		
1.	Total Bacterial Load	Nil	Not more than 10^5/g
2.	*Salmonella* spp.	Nil	Nil
3.	*Escherichia coli*	Nil	Nil
4.	Total Fungal count	Nil	Not more than 10^3/g
	Heavy Metals		
5.	Arsenic	Nil	Not more than 3.0 ppm
6.	Cadmium	Traces	Not more than 0.30 ppm
7.	Lead	Traces	Not more than 10.0 ppm
8.	Mercury	Nil	Not more than 1.0 ppm

pink), 0.62 (dark brown), 0.64 (light pink), 0.67 (black), 0.71 (sky blue), 0.77 (grey) and 0.84 (orange) as shown in Plate 3-A.

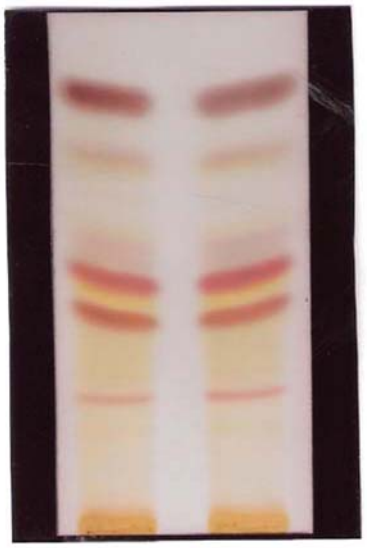
The chromatogram was sprayed with 10% Ethanolic H_2SO_4, and was left for a couple of mt at room temperature for complete carbonization, and then inspected under UV (365 nm). Fourteen spots were visualized at Rf 0.03, 0.19 (light yellow), 0.25 (light sky blue), 0.42 (light orange), 0.45, 0.51, 0.58 (light yellow), 0.61, 0.63 (sky blue), 0.64 (blackish), 0.68 (light pink), 0.74 (grey), 0.77 (violet) and at 09.84 (light orange red). The sprayed chromatogram when heated at 110°C for optimal colour development, ten spots were seen at Rf 0.19 (violet grey), 0.25 (light brown),

Table 4. Pesticide & Aflatoxin Contamination of Sufoof-e-Bers

S.No.	Parameters	Results	WHO Limit
	Pesticides		
1.	Chloropyriphos	Nil	Not more than 0.2 mg/kg
2.	DDT	Nil	Not more than 1.0 mg/kg
3.	Endosulfan	Nil	Not more than 3.0 mg/kg
4.	Malathion	Nil	Not more than 1.0 mg/kg
5.	Parathion	Nil	Not more than 0.5 mg/kg
	Aflatoxins		
6.	B1	Nil	Not more than 0.5 ppm
7.	B2	Nil	Not more than 1.0 ppm
8.	G1	Nil	Not more than 0.5 ppm
9.	G2	Nil	Not more than 0.1 ppm



A : Derivatized with 10% Ethanolic H_2SO_4



B : Under ultraviolet radiation (UV 254nm)

Plate 3. TLC of Methanolic Extract of Sufoof-e-Bers

0.45 (violet), 0.55 (light violet grey), 0.58 (light brown), 0.64 (light grey), 0.67 (orange brown), 0.71 (grayish brown), 0.80 (light red) and at 0.84 (grayish brown) as shown in plate 3-B.

Discussion and Conclusion

Screening of few latest publications however revealed that SOP's of several compound unani formulations have already been developed recently as reported by Bagul *et al*. (2006), Aminuddin and Siddiqui (2007), Goel *et al*. (2007), Mageswari *et al*. (2009), Meena *et al*. (2009), Negi *et al*. (2009), Zuberi *et al*. (2008) and the key characters established for their correct identification.

The present study as per developed SOP of Sufoof-e-Bers has similarly brought out many key characters such as the presence of cell types and cell contents, various physico-chemical, extractive and the Rf values. Estimation of other parameters such as the microbial load, aflatoxin level, heavy metals and pesticide residue further indicates that this preparation is totally free from any such contamination. The above botanical features, various physico-chemical standards and the Rf values in combination would thus facilitate a uniformity in its large scale manufacture and the criteria for easy identification of the drug Sufoof-e-Bers, if adulterated.

Acknowledgement

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Standardization of a Nervine Unani Formulation – Habb-e-Hudar

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Abstract

The basic and essential requirement for the development of Unani and other traditional systems of medicine and to match the International standards is the standardization of single drugs and compound formulations. Habb-e-Hudar is a formulation contains two herbal constituents; Azaraqi Mudabbar (*Strychnos nux-vomica Linn.*) and Aab-e-Zanjabeel Tar (*Zingiber officinale Rosc.*). Until now no physico-chemical standards are available to assess the quality of the formulation. The present study is designed to fix the various physicochemical and pharmacognostical standards to assess the quality of the formulation, which can be used as quality control tool.

Key Words: Standardization, Habb-e-Hudar, Azaraqi, Aab-e-Zanjabeel.

Introduction

India has about 45,000 plant species; medicinal properties have been assigned to several thousands. The Government of India has formal structures to regulate quality, safety, efficacy and practice of herbal medicine (National Policy on Indian systems of Medicine and Homeopathy-2002). Even though India is the goldmine of herbal medicine, 80% of its exports to the developed countries are of crude drugs and not finished formulations (Sapna and Ravi, 2007). The basic and essential requirement for the development of Unani and other traditional systems of medicine and to match the International standards is the standardization of single drugs and compound formulations. In case of poly-pharmaceutical preparations the use of adulterated drugs and use of drugs less than the prescribed quantity or total absence of the costly ingredients like musk, amber and Saffron affect the quality of formulation. Keeping in view the above, it becomes evident that the standardization of a compound formulation is very necessary to check the purity, genuiness and optimum therapeutic efficacy of a preparation. Therefore, in the present study Habb-e-Hudar was investigated for physicochemical characteristics. Habb-e-Hudar is a compound formulation of Unani Medicine, which is used in the treatment of nervous disorders as a nervine tonic (Muqawwi-e-Asab) and in the treatment of inflammatory diseases, mainly in arthritis and other diseases of joints (Anonymous, 2001). In this pill Azaraqi Mudabbar (*Strychnos nux-vomica)* has been used as the chief ingredient and the other ingredient is the Aab-e-Zanjabeel Tar. The earliest description of Habb-e-Hudar is found in the pharmacopoeia that is largely based on the compounds used by physicians of Delhi and particularly by Khandan-e-Sharifi, such as 'Delhi Ke Muntakhab Murakkabat' (Lubhaya, 1979). The important pharmacopoeias written after the period of Ajmal Khan such as Beyaz-e-Kabir (Kabiruddin, 1967) and Unani Advia Murakkaba (Nigrami, 1995) have included this compound but there is no mention of it in the books written before. Thus it appears that this compound was introduced most probably by Hakim Ajmal Khan or some other Tabib of 'Sharif'

family. We have selected this compound Habb-e-Hudar for study which is mentioned in the National Formulary of Unani Medicine for standardization.

Materials and Methods

The method mentioned in N.F.U.M (Anonymous, 2001) was followed for the preparation of Habb-e-Hudar. Azaraqi was procured from the local market and after checking the identity and purity, detoxified by the method given in National Formulary of Unani Medicine. The voucher specimens (SC-0105/09-Z & SC-0106/09-L) have been kept in our museum for future references. The seeds were covered with yellow clay (Peeli mitti) in an earthen pot for 10 days, and irrigated daily. After 10 days seeds were taken out, washed and boiled in milk then the outer cover were removed with the help of sharp knife. The embryo was also removed, situated in between the cotyledons. The remaining parts were dried in shade then powdered (Anonymous, 1983). The fresh Ginger was obtained from the market and the juice was taken out with the help of Juicer and stored in a fridge for further use.

Formulation Composition

Azaraqi Mudabbar (Detoxified Nux vomica)	–	10 gram
Aab-Zanjabeel Tar (Fresh juice of Ginger)	–	Q.S

Preparation of Pill

The powder of Azaraqi was made in the form of a paste (lubdi) by adding sufficient quantity of Aab-e zanjabeel. With the help of a cutter this lubdi was cut into small pieces equivalent to the prescribed dose of the pill. The pieces so obtained were put in to a rolling machine which rotates at a fixed speed to give the round shape to the pills. The pills were dried in an oven at 60º C ± 5ºC.

Physicochemical Parameters

The prepared pills were evaluated for Alcohol and Water soluble matter, Total ash, water soluble ash and acid insoluble ash (Anonymous, 1968), Moisture content (Jenkins *et al*, 1967), Loss of weight on drying, pH value of 1% solution and pH value of 10% solution (Anonymous, 1987). Uniformity of diameter was performed by picking three pills randomly and the diameter was measured individually by using a Vernier caliper and expressed in mm (Dandagi *et al*, 2006). Ten pills were selected randomly and weighed individually to check for weight variation and average weight of the pill is expressed in mg (Dandagi *et al*, 2006). The rate of disintegration was measured by a Disintegration-testing apparatus using the two media, the aqueous as well as in the acidic medium. Simulated Gastric Fluid (pH about 1.2) was prepared without enzyme by dissolving 1gm of NaCl in 500 ml of deionized

water, adding 7 ml of concentrated HCL, and diluting the volume to 1000 ml with water. For measurement in aqueous medium Double Distilled water was taken (Anonymous, 1989 and modified by Afaq *et al.,*). Friability of the pills was determined using Friability test apparatus (Friabilator) of Macro Scientific Works, Delhi and expressed in percentage (Vijaya and Mishra, 2006).

Thin layer Chromatography

Thin layer chromatography was carried out on T.L.C. pre coated aluminum plates, silica gel 60 F 254 (layer thickness 0.25 mm) for alcoholic extract of Habb-e-Hudar and its ingredients in various mobile phases, later sprayed by different spraying reagents. The R_F values of the spots were calculated by the following formula (Anonymous, 1968).

$$R_F \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

Results and Discussion

H. Hudar has Characteristic Blackish brown colour, circular in shape, appeared like a pill, hard in texture and found to be bitter in taste (Table 1)

Physico-chemical Analysis

The physico-chemical data of the drug are shown in the Table-2. Weight variation test was conducted because a good quality pill should be accurate and uniform in weight. The % weight variation of the lab samples was within the prescribed pharamacopoeial limits of ± 7.5%. The mean weight value was found to be 248.2 ± 2.16 mg. The diameter of a pill can vary without any change in its weight hence the uniformity of diameter of the circular hand made pills is also measured. As a standard, the mean value of the diameter was found to be 6.9 ± 0.06 mm. After administration the pill should disintegrate readily, therefore, the pills were also subjected for the evaluation of disintegration time. The disintegration time in the water and in simulated gastric fluid were found to be 40 ± 1.16 minutes and 17.3 ± 0.88 minutes respectively. It is mentioned that plain tablets / pills pass the test

Table 1. Organoleptic Description of Habb-e-Hudar

Colour	Blackish brown
Appearance	Pills
Texture	Hard
Taste	Bitter

Table 2. Physicochemical Parameters

Parameters	Values of Samples	Mean Value
Uniformity of Diameter (mm) (n=3)	6.8 6.9 7.0	6.9±0.06
Weight Variation (mg) (n=10)	–	248.2±2.16
Disintegration time in the water (minutes) (n=3)	40 38 42	40±1.16
Disintegration time in the simulated gastric fluid (minutes) (n=3)	16 17 19	17.3±0.88
Friability (%)(n=3)	0.06 0.06 0.05	0.06±0.00
Alcohol soluble content (%)(n=3)	18.42 18.40 18.41	18.41±0.006
Water soluble content (%)(n=3)	17.40 17.36 17.38	17.38±0.01
pH (1% solution) (n=3)	4.72 4.71 4.74	4.72±0.009
pH (10% solution) (n=3)	5.20 5.19 5.20	5.20±0.003
Moisture content (%)(n=3)	7.20 7.48 7.73	7.47±0.15
Total Ash (%)(n=3)	2.87 2.93 2.74	2.85±0.06
Acid insoluble ash (%)(n=3)	1.29 1.25 1.27	1.27±0.01
Water soluble ash (%)(n=3)	0.44 0.40 0.41	0.42±0.01
Loss on drying (%)(n=3)	8.18 8.10 8.16	8.15±0.02

if each of the six plain uncoated tablets disintegrates in not more than 45 minutes (Anonymous, 1989). It was also noticed that as the disintegrant was changed from water to simulated gastric fluid the time taken for disintegration was reduced. Friability test is done to evaluate the ability of tablets / pills to withstand abrasions. For Friability, a loss of less than 1% is considered acceptable by industrial standard. All the pills were found well within the approved range (< 1%) in the lab samples. The mean percentage of friability tested with Friabilator was found to be 0.06 ± 0.00. The percentages of alcohol and water soluble contents were found to be 18.41 ± 0.006 and 17.38 ± 0.01 respectively. pH of the pills were determined and was found to be acidic. The values were 4.72 ± 0.009 in 1% aqueous solution and 5.20 ± 0.003 in 10% aqueous solution. On account of having high acidic pH, the drugs get ionized in stomach because pH of stomach is acidic; it means the pill is not absorbed in the stomach but some where in intestines. The percentage of moisture content and loss of weight of Habb was found to be 7.47 ± 0.15 and 8.15 ± 0.02 respectively and the percentage of total ash, acid insoluble ash and water soluble ash was found to be 2.85 ± 0.06, 1.27 ± 0.01 and 0.42 ± 0.01 respectively.

Thin layer Chromatography

Studies of the extracts of test drug and all the ingredients were carried out using different organic solvent systems. The solvent system for Habb-e-Hudar, Azaraqi and Aab-e-Adrak was Toluene, Ethyl acetate, Benzene and Acetic acid (4: 1: 2 drops : 2 drops). The plate was sprayed with freshly prepared vanillin sulphuric acid. The plate was heated for 5 min at 110^0C. The R_f values of spots were calculated which are presented in Table 3 and Fig 1.

Table 3. TLC profile of Habb-e-Hudar and its ingredients

Extract	Solvent system	Spraying reagent	Detection / Observations					
			Habb-e-Hudar		Azaraqi Mudabbar		Aab-e-Adrak	
			Colour	R_f Value	Colour	R_f Value	Colour	R_f Value
Alcohol	Toluene: Ethyl acetate: benzene: Acetic acid (4:1+2 drops + 2 drops)	Vanillin sulphuric acid	Yellow	0.24	Yellow	0.25	–	–
			Yellow	0.51	–	–	Yellow	0.51
			Yellow	0.90	–	–	Yellow	0.89

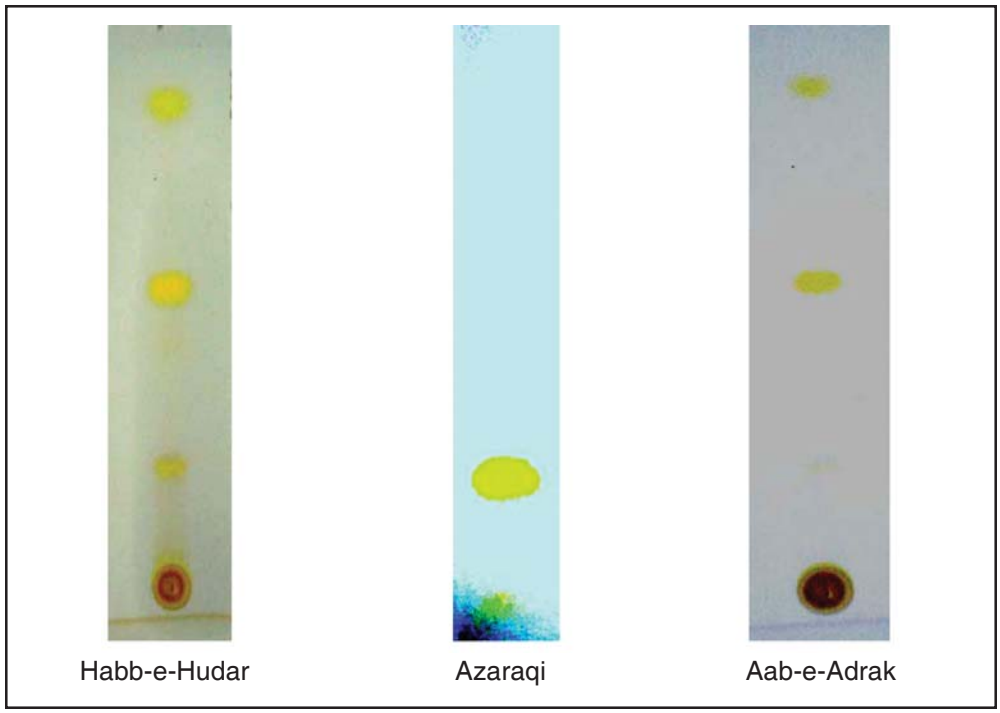

Fig. 1. TLC Profile of Habb-e-Hudar and its ingredients

Acknowledgement

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Morpho-anatomical Studies on *Malaxis acuminata* D. Don – An Endangered Medicinal Orchid

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Abstract

Malaxis acuminata D. Don (a terrestrial orchid), commonly known as 'Jeevak', belongs to the tribe Malaxideae (subfamily Epidendroideae) of family Orchidaceae. It is a perennial herb distributed in temperate and sub-tropical Himalayas, Andaman Islands and the Annamalai Hills in South India. The pseudobulbs of the plant are of medicinal value and form an important ingredient of classical herbal preparations viz. '*Chyawanprash'* and other similar patent and proprietary preparations using '*Astavarga'* (eight specific herbal ingredients). The morphology and vegetative anatomy of the plant has been investigated. The roots lack velamen, the vascular cylinder is polyarch and the inner few layers of the parenchymatous cortex have fungal hyphae. The pseudobulb has scattered vascular bundles embedded in a ground tissue comprised of aerenchyma, mucilaginous cells and raphidal cells. The leaf is unifacial with sessile glandular trichomes and tetra to pentacytic stomata. The role of the morphological and anatomical features in the correct botanical identity of the species, checking its adulteration, substitution and planning conservation strategies have been discussed.

Key Words: Anatomy, *Malaxis acuminata*, Orchidaceae, Pseudobulb, Raphide, Stomata.

Introduction

Malaxis acuminata D. Don (syn. *Microstylis wallichii* Lindl.) is a terrestrial orchid belongs to the tribe Malaxideae (subfamily Epidendroideae) of family Orchidaceae (Dressler, 1993). It is a perennial herb distributed in temperate and sub-tropical Himalayas at an altitude of 1800-2300 m, from Shimla to Sikkim, Khasia Hills, Andaman Islands and the Annamalai Hills in Travancore in South India (Hooker, 1882; Deva and Naithani, 1986). The pseudobulbs of the plant are of medicinal value and constitute an important herbal drug called 'Jeevak'. It is used in treatment of wide range of health disorders, such as pitta and vata, seminal weakness, emaciation, tuberculosis and general weakness (Varier, 1996). The pseudobulbs of this species have also been reported to possess aphrodisiac and antipyretic activity. 'Jeevak' is one of the main ingredients of herbal preparations '*Chyawanprash'* and '*Astavarga'* (cf. Handa, 1986).

Though significant studies have been made in the anatomy of the different taxa of family Orchidaceae (Cheadle, 1982; Kaushik, 1983; Williams, 1979; Pridgeon, 1987; Pormbski and Barthlott, 1988; Mohana Rao *et al*., 1989; Stern and Judd, 2000, 2001; Carlsward *et al*., 1997, 2006), but there is no adequate information on the anatomical details of *Malaxis acuminata* D. Don. Further, due to its high medicinal value, this species have been overexploited to meet the demand of herbal industries and traditional medical practitioners in recent years and which caused destruction to its habitat and threat of extinction. Consequently, this species have been included

in Appendix II of the CITES regulation and Red Data Book of Indian Medicinal Plants. Hence, the conservation and commercial cultivation measures of the *Malaxis acuminata* D. Don are necessary for its sustainable use.

Thus, considering the high medicinal value, endangered status and lack of adequate information on morpho-anatomical features of *M. acuminata* D. Don (Jeevak), the present investigations has been carried out with a scope to provide data which may be useful in correct botanical identity of this species, checking its adulteration and substitution and planning conservation strategies.

Material and Methods

Malaxis acuminata plants were collected from wild population in Shimla (2300m, Himachal Pradesh) during the months of July to September. The whole plants were killed and fixed in formalin - acetic acid - alcohol (FAA) for 48 hours and later stored in 70 % ethyl alcohol until use. The material was dehydrated in ethyl alcohol-*tert*-butyl alcohol series, and embedded in paraffin blocks following Johansen (1940). Serial sections cut at 5-12 mm, were stained in Safranin-Fast green combination and mounted in Canada balsam. Hand cut sections of root, pseudobulb and leaf were also made to observe the anatomical details. For studying the shape, size of the epidermal cells and type of stomata, the leaf epidermal peels were isolated by treating the 1-2 cm^2 leaf segments with 10% alkaline solution of NaOH for 1–2 h. The isolated peels were mounted in 10% glycerine solution to make semi-permanent mounts. The prepared sections and the epidermal peels were subjected for microscopy and microphotography. Standard prescribed procedures for histochemical studies (Johansen, 1940; Youngken, 1951; Cromwell, 1955, Trease and Evans, 1978) were adopted.

The commercial samples of dried pseudobulbs of *Malaxis acuminata* (Jeevak) were also procured from the markets of Khari Baoli, Delhi, India, for studying the morpho-anatomical characters of the drug. The voucher specimens for the species studied have been deposited in the herbarium-cum-museum of the Department of Botany at Panjab University Chandigarh, INDIA. The herbarium of different species of *Malaxis* housed in the herbarium-cum-museum of the Department of Botany, Panjab University were consulted for the taxonomic studies.

Observations

Taxonomic Attributes

Malaxis, a genus of about 300 species of terrestrial, lithophytic or rarely epiphytic orchids, is distributed in the warmer parts of the world and extended in the north temperate regions. Maximum numbers of species are found in Asia, Oceania and in Western hemisphere (Bose *et al.*, 1999). The generic name *Malaxis*, established

by Olof Swartz in 1788, is a Greek word meaning softening and refers to the soft texture of its leaves. *Malaxis* is a very complex genus and highly variable in vegetative form. The stems are creeping, fleshy; leaves are few to many, broad and thin, often paired, present towards the apex; inflorescence is erect, terminal, a few to many flowered, umbel-like condensed raceme; flowers are small, complex; the sepals are free and spreading, the lateral ones more or less connate; petals are ovate-lanceolate; lip is sessile, superior, erect or spreading, entire or trilobed; column is terete, very short, hollow on top, often toothed at apex; pollinia four in number.

In India, 19 species of *Malaxis* are distributed and six of them occur alone in Arunachal Pradesh. Some of the important Indian species are *Malaxis acuminata, M. andamanica*, *M. cylindrostachya, M. khasiana*, *M. muscifera, M. rheedii* and *M. versicolor* (Chowdhery, 1998). Only a few species of *Malaxis* are medicinally important (Table 1) and used in traditional system of medicines as therapeutic agents (Hegde, 1988; Chauhan, 1990).

Table 1. Medicinal uses of genus *Malaxis*.

S.No.	Species	Part used	Medicinal Uses
1.	*Malaxis acuminata* D. Don	Pseudobulb	Tonic, spermatogenesis, tuberculosis
2.	*M. muscifera* (Lindl.) O. Ktze.	Bulb	Tonic, rejuvenator of tissues
3.	*M. cylindrostachya* (Lindl.) O. Ktze.	Root	Tonic

These species grow as mixed community in the natural habitat and collectors gathered either one specific species of *Malaxis* or another species or mixture of different species for trade. On the basis of forgoing taxonomic key, the individual medicinal species subjected for trade can be indentified on the basis of morphological features.

Key to Identification:

1. Leaves three; sides of the apical part of the labellum with straight edges from the base to the apex, without any indentation and separation of the apical part from the basal part, apex slightly notched to bilobulate .... ***M. acuminata***

2. Leaves two; labellum three lobed with a notch near the centre, side lobes obscure, apex produced in to a long narrow beak with acute to acuminate tip ..... ***M. muscifera***

3. Leaf one; labellum not lobed, margin thickened, entire to denticulate, apex produced in a short fleshy beak with almost rounded tip .... ***M. cylindrostachya***

Morpho-anatomical Characteristics

a) Macroscopic

The plant is 30-40 cm high pseudobulbous terrestrial herb (Figure 1A, B). The pseudobulbs are conical in shape, fleshy, green, smooth, shining, 1 to 9 cm long and 1 to 3 cm thick (Figure 1F). The roots arising at the base of the pseudobulbs are thin and fibrous (Figure 1B). Leaves are usually three, membranous, ovate with undulate margin, upper sessile, lower one with sheathing base, arranged alternately and having parallel venation (Figure 1B). The inflorescence is raceme (Figure 1C) and flowers are yellowish-green or purple (Figure 1D). The bracts are lanceolate. Sepals are oblong, the laterals being broad and oblong, 3-5 nerved while the dorsal one is shorter than the laterals, 1-3 nerved and sub-acute; the petals are linear, 3-nerved and longer than sepals; the lip or labellum is shield-like, narrowly ovate and slightly convex with notched or bi-lobulate tip, auricles are straight and slightly overlapping; the columns have fleshy rounded arms (Figure 1E). The anthers are sub-terminal, bilocular; pollinia are 4, ovoid or obovoid.

Dried pseudobulbs (Figure 1G) conical, reddish-brown in colour, measuring 2 to 5 cm in length and 0.25 to 1 cm in thickness, covered with papery white remnants of leaves, fracture hard; transversely cut surface dark brown, coarsely granulated with irregular margins and white spots; odour pleasant; taste astringent, slightly mucilagenous.

b) Microscopic

Root: A transverse section of the root has numerous root hairs. It is circular or oval in outline and it exhibit epidermis, followed on inner side by an exodermis, cortical region, an endodermis and the central vascular cylinder (Figure 2A, B).

There is single layered epidermis of isodiametric, rectangular or radially elongated cells with unicellular root hairs here and there (Figure 2B). Below the epidermis is an exodermis consisting of comparatively large, transparent, polygonal, radially elongated parenchyma cells with thick-walls except passage cells (Figure 2B). Cortex composed of round or oval parenchyma cells with intercellular spaces at a few places (Figure 2B-E). Some of the cells of the cortex exhibit raphides of calcium-oxalate (Figure 2D). The innermost layer of the cortex is the endodermis and it is composed of rectangular or barrel-shaped cells (Figure 2D). The casparian strips are absent. One or two layers of parenchyma cells or thick-walled cells in the vascular tissue represent the pericycle. A cylinder of 6–15 vascular bundles lies

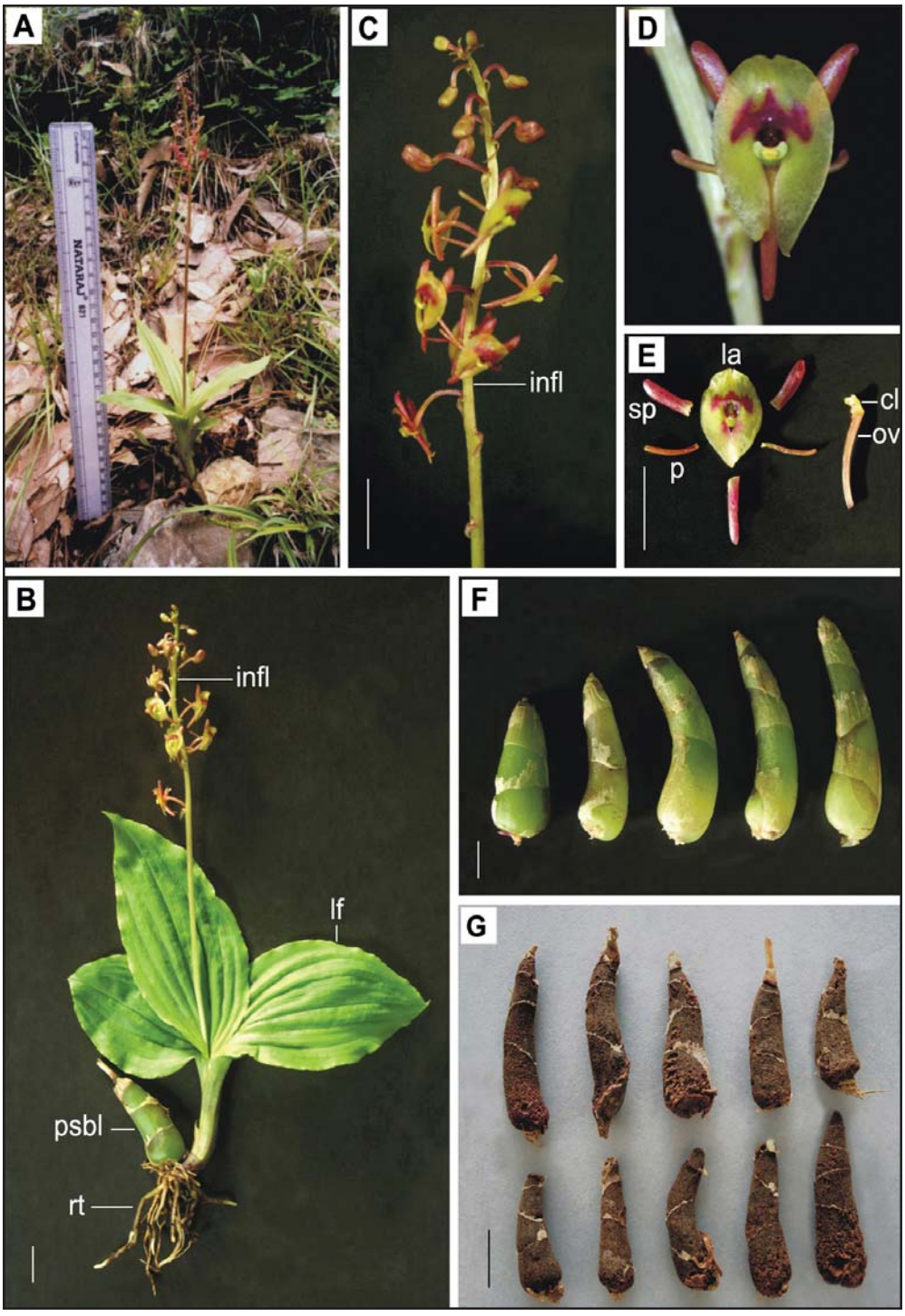

Fig. 1. Morphology of *Malaxis acuminata* D. Don. **A**, A flowering plant growing in wild; **B**, An uprooted plant showing mother pseudobulb and roots; **C**, Inflorescence; **D**, Magnified flower; **E**, Dissected flower showing petal, sepal, labellum, column and ovary; **F**, Fresh pseudobulbs; **G**, Dried pseudobulbs (as available in market). cl, column; Infl, inflorescence; la, labellum; lf, leaf; ov, ovary; p, petal; psbl, pseudobulb; rt, root; sp, sepal. Scale bar = 1 cm.

next to the pericycle (Figure 2B, D). The xylem is composed of tracheids, a few fibres and xylem vessels with pitted walls and spiral thickenings (Figure 2F) and it occurs close to the pith cells. The phloem consists of sieve tubes and companion cells and it occurs next to cells of the pericycle. The pith composed of tightly packed, large, oval to round parenchyma cells (Figure 2B, D, E). The hyphae of mycorrhizal fungus which entered through root hairs, epidermis form knots in many of the cortex cells (Figure 2B, C).

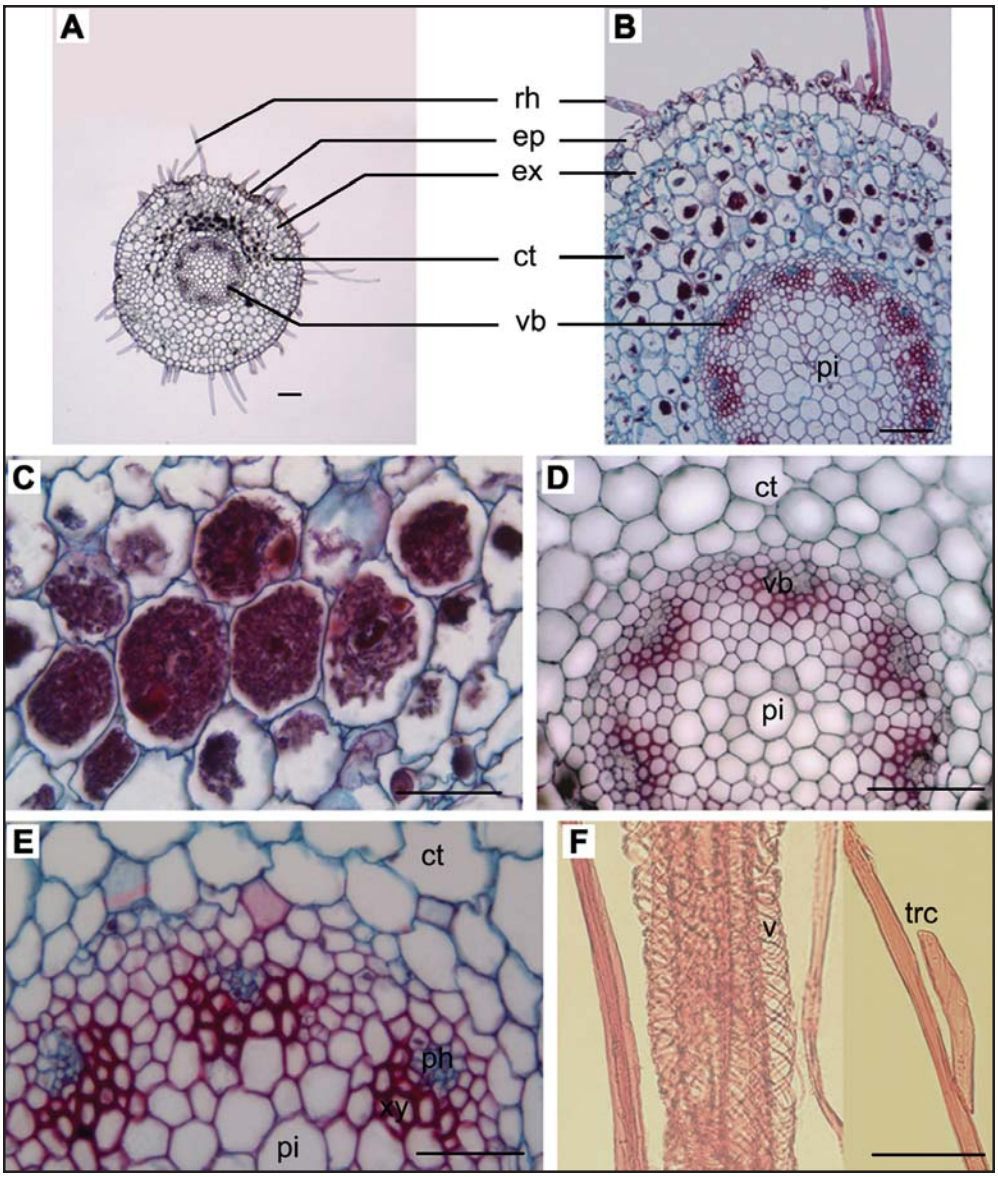

Fig. 2. Anatomy of *Malaxis acuminata* D. Don root. **A**, TS of root; **B**, TS potion of root showing root hair, cortex, exodermis and central vascular cylinder; **C**, Magnified cortex showing fungal hyphae; **D**, TS root showing central cylinder and pith; **E**, Magnified view of central cylinder showing xylem, phloem and pith cells; **F**, Isolated tracheids and group of spiral vessels. ct, cortex; ep, epidermis; ex, exodermis; ph, phloem; pi, pith; rh, root hair; trc, tracheids; v, vessel; vb, vascular bundle; xy, xylem. Scale bar, **A**, **B**, **D** = 100 µm and **C**, **E** = 50 µm.

Pseudobulb: The transverse section of the pseudobulb is circular to oval in outline with irregularly crenate margins (Figure 3A). It has parenchymatous ground tissue with scattered vascular bundles and large number of mucilage cavities.

The epidermis is composed of tangentially flattened cells of irregular size and a thin cuticle covering their outer tangential walls (Figure 3B-D). The ground tissue is parenchymatous traversed by aerenchyma cells and cells with large size containing mucilage, few raphidal cells and scattered vascular bundles (Figure 3B). The outer vascular bundles are larger than the inner. The vascular bundle is surrounded by bundle sheath (Figure 3E). The xylem is composed of few tracheids and vessels with spiral thickenings (Figure 3 E, F) while the phloem consists of sieve tubes and phloem fibres.

Leaf: Surface preparations of both upper and lower epidermal peel shows polygonal cells (Figure 4A,B). The glandular sessile trichomes present on both surfaces while stomata are present only on abaxial surface. Thus the leaf is hypostomatic. Subsiadiary cells are prominent and the stomata are tetra to pentacytic (Figure 4B).

Transverse section of the leaf is isobilateral with protruded midrib on ventral side (Figure 4C). Epidermis composed of isodiametric or rectangular parenchyma cells covered with thin cuticle. Glandular, sessile trichomes present on both surfaces while the stomata occur only on lower surface (Figure 4D). The glandular trichomes are sessile, bi-celled with funnel-shaped head and elongated basal cell (Figure 4E, F). A single vascular bundle occurs in the midrib with phloem on abaxial side (Figure 4C). Phloem composed of sieve cells, companion cells and phloem parenchyma while the xylem consists of groups of polygonal xylem vessels, fibres. The vascular bundle remains surrounded by closely packed oval to round parenchyma cells (Figure 4C). Lamina exhibits homogenous mesophyll and it consists of 4 or 5 closely packed layers of oval to round parenchyma cells filled with chloroplasts and few containing raphides of calcium oxalate (Figure 4D).

Histochemistry

Observations and results pertaining to histo-chemical tests ascertaining the presence of major groups of phytoconstituents and other ergastic contents in plant tissues are presented in Table-2.

Discussion

Orchids are commercially attributed high value for medicinal and floricultural importance. A number of orchid species are used in the different traditional system of medicine worldwide. But, the correct botanical identities of commercially exploited species are still ambiguous viz. the herbal drug 'Jeevak' (*Malaxis acuminata*) often substituted with allied species *M. muscifera*. 'Salampanja' (*Dactylorhiza hatagirea*) is often substituted with *Gymnadinea orchidis*. The over exploitation of orchids from

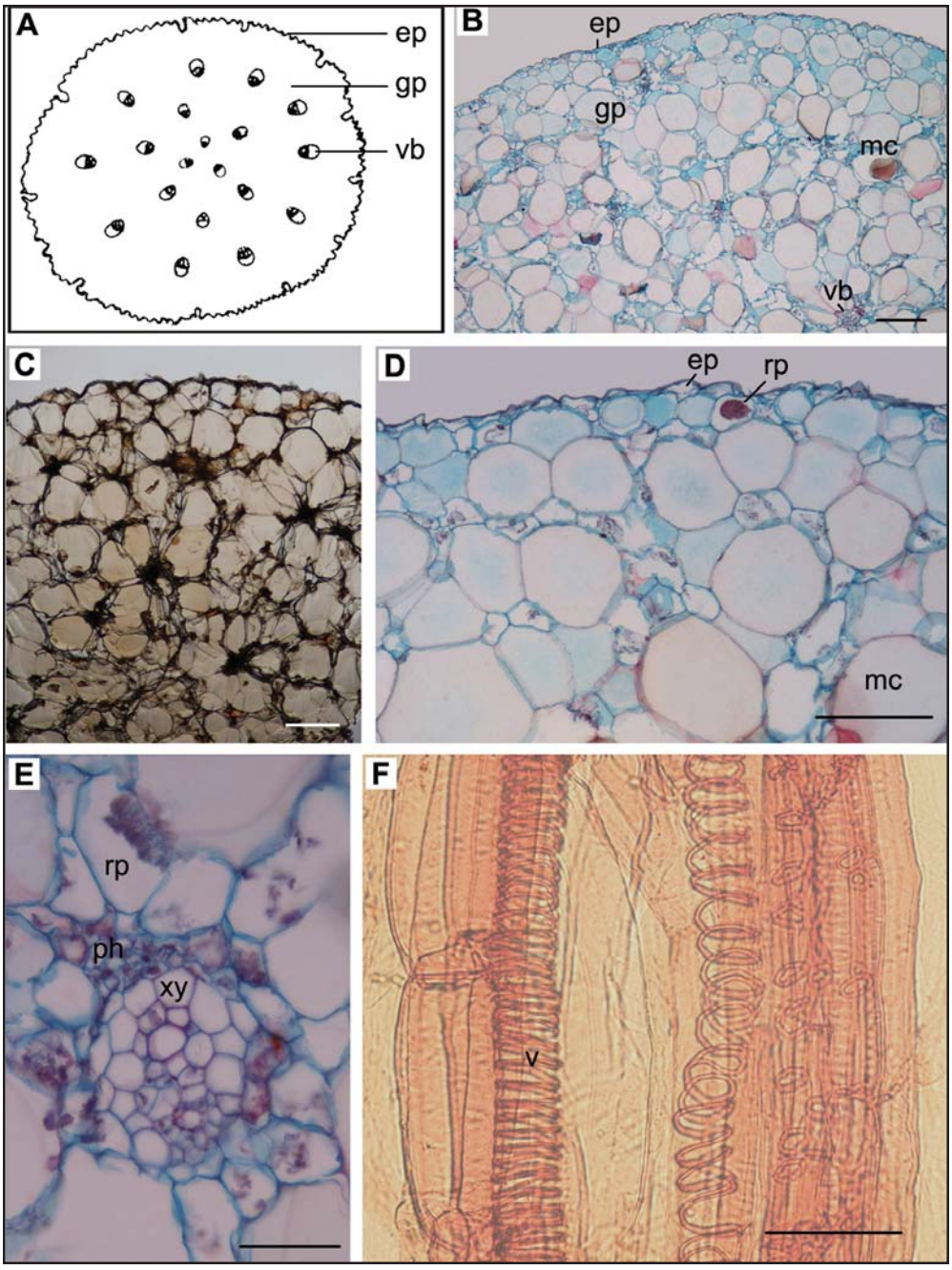

Fig. 3. Anatomy of *Malaxis acuminata* pseudobulb. **A**, Diagrammatic TS of pseudobulb; **B**, TS potion of fresh pseudobulb showing scattered vascular bundles, aerenchyma and mucilage cells; **C**, TS potion of dried pseudobulb showing mucilage cells and vascular bundles; **D**, Magnified TS portion of pseudobulb showing large aerenchyma cells and raphide in subepidermal layer; **E**, Magnified vascular bundle showing xylem and phloem; **F**, Macerated tissue from pseudobulb showing spiral thickening in vessels. ep, epidermis; gp, ground parenchyma; mc, mucilage cell; ph, phloem; v, vessel; vb, vascular bundle; xy, xylem. Scale bar, **B-E** = 100 µm and **F** = 50 µm.

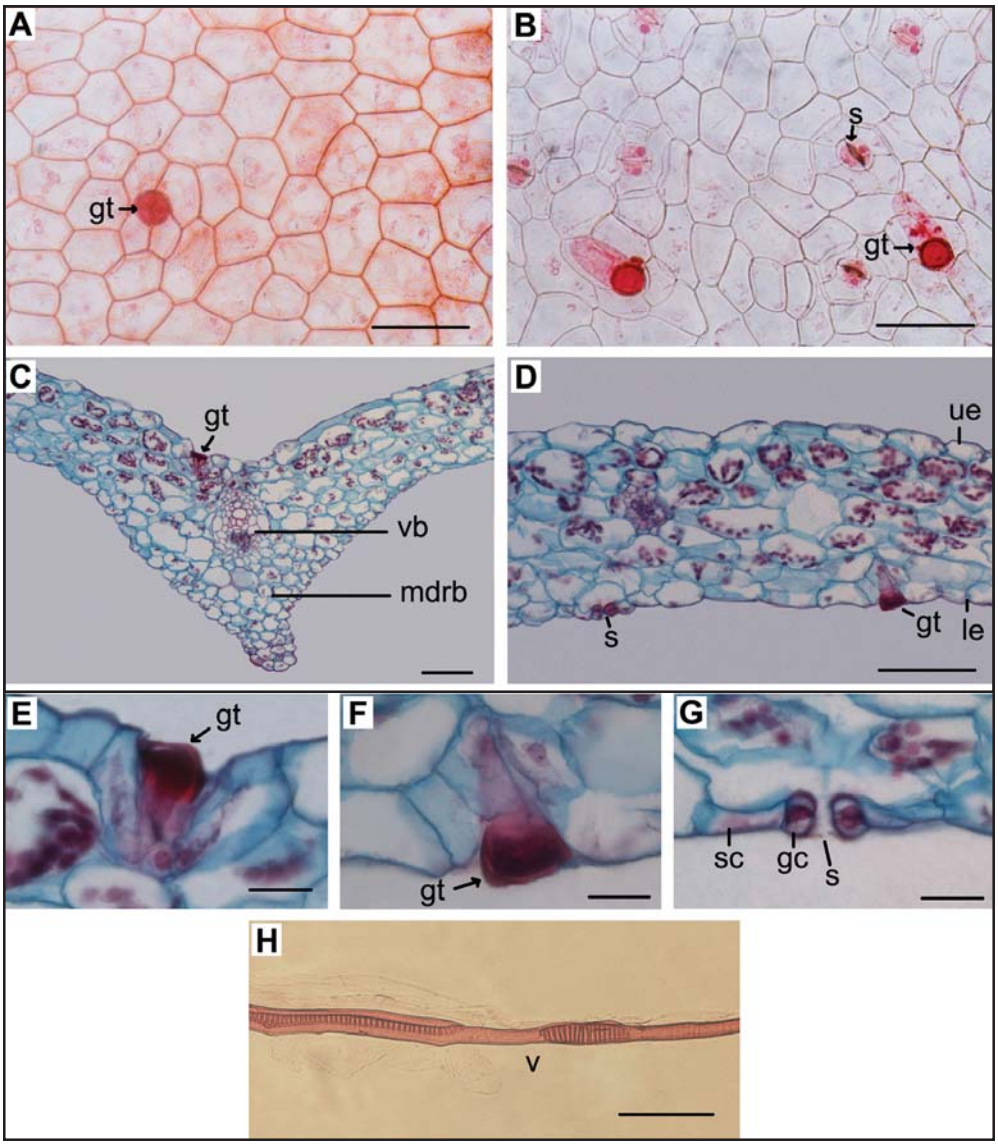

Fig. 4. Anatomy of *Malaxis acuminata* D. Don Leaf. **A**, Upper epidermis in surface view; **B**, Lower epidermis in surface view; **C**, TS of leaf through midrib; **D**, TS potion of leaf through lamina showing stoma and sessile glandular trichome on ventral side; **E**, **F**, Sessile glandular trichomes on upper and lower epidermis; **G**, Stoma in transectional view; **H**, Isolated vessel. gc, guard cell; gt, glandular trichome; le, lower epidermis; mdrb, midrib; s, stoma; sc, subsidiary cell; ue, upper epidermis; v, vessel; vb, vascular bundle. Scale bar, **A-D** = 100 µm and **E-H** = 50 µm.

the wild habitats to meet the ever increasing demand of herbal industries and traditional medical practitioners has caused destruction to their habitats and threats of extinction. Consequently, many orchid species have been included in Appendix II of the CITES regulation and Red Data Book of Indian Medicinal Plants. Thus, for the use of appropriate species, sustainable availability of medicinal orchids, the

Table 2. Micro-chemical tests and behaviour of specific reagents towards plant tissues and cells contents.

Sl.No.	Reagent	Test for	Histological zone/cell contents responded		
			Root	Pseudobulb	Leaf
1.	Dragendorff's reagent	Alkaloids	Not Responded	Cells of ground tissue	Not Responded
2.	Marme's reagent	Alkaloids	Not Responded	Same as above	Not Responded
3.	Wagner's reagent	Alkaloids	Not Responded	Same as above	Not Responded
4.	Potassium hydroxide solution (5% w/v)	Anthocynin	Not Responded	Not Responded	Not Responded
5.	Sulphuric acid (66% v/v)	Anthocynin	Not Responded	Not Responded	Not Responded
6.	Acetic acid	Calcium oxalate	Raphides of calcium oxalate crystals	Raphides of calcium oxalate crystals	Raphides of calcium oxalate crystals
7.	Potassium hydroxide solution (5% v/v) + Hydrochloric acid	Calcium oxalate	Same as above	Same as above	Same as above
8.	Sulphuric acid	Calcium oxalate	Same as above	Same as above	Same as above
9.	Kedde reagent	Cardiac glycoside	Not Responded	Not Responded	Not Responded
10.	Iodine Solution followed by Sulphuric acid	Cellulose	Cells of cortex and pith	Cells of ground tissue	Cells of mesophyll
11.	Sudan III	Fixed oil and fats	Not Responded	Not Responded	Not Responded

Sl.No.	Reagent	Test for	Histological zone/cell contents responded		
			Root	Pseudobulb	Leaf
12.	Ruthenium Red S	Mucilage	Not Responded	Cells of Ground Tissue	Not Responded
13.	Chlor-zinc-Iodine Solution	Latex	Not Responded	Not Responded	Not Responded
14.	Aniline sulphate Solution followed by Sulphuric acid	Lignin	Xylem vessels, tracheids, fibres	Xylem vessels, tracheids, fibres	Xylem vessels, tracheids, fibres
15.	Phloroglucinol HCl	Lignin	Same as above	Same as above	Same as above
16.	Lugol's solution	Protein	Cells of cortex and xylem	Cells of ground tissue	Cells of ground mesophyll
17.	Millon's reagent	Protein	Not Responded	Not Responded	Not Responded
18.	Picric acid	Protein	Not Responded	Not Responded	Not Responded
19.	Heating with KOH (5% w/v) + H_zSO_4	Suberin	Not Responded	Not Responded	Not Responded
20.	Sudan III	Suberin	Not Responded	Not Responded	Not Responded
21.	Weak Iodine solution	Starch	Not Responded	Not Responded	Not Responded
22.	Potassium hydroxide solution (5% w/v)	Starch	Not Responded	Not Responded	Not Responded
23.	Sulphuric acid	Starch	Not Responded	Not Responded	Not Responded

basic scientific information on their morphological and anatomical features is necessary which leads to correct botanical identity of the drug and to plan conservation strategies.

The present investigation provide basic scientific information on the morphological and anatomical features of *Malaxis acuminata* D. Don commonly known as 'Jeevak' which is often substituted with its allied species *M. muscifera* (commonly known as 'Rhisbhak'). The morphological characters of *Malaxis acuminata* (such as conical pseudobulb; 2-3, membranous, ovate leaves with undulate margin; shield-like, narrowly ovate and slightly convex labellum with notched or bi-lobulate tip and slightly overlapping straight auricles) and anatomical features (such as lack of velamen tissue, single layered exodermis, 6-15 vascular bundle in root; large aerenchyma, mucilage cells in ground parenchyma of pseudobulb; sessile, bi-celled glandular trichomes and tetra or pentacytic stomata in leaves) can be used as diagnostic features of the species. These morphological and anatomical features of *Malaxis acuminata* may be helpful in checking adulteration and substitution of herbal drug 'Jeevak' which is often adulterated and substituted with pseudobulbs of *M. muscifera* (Rhisbhak) and other terrestrial orchids. In addition, the anatomical features may be helpful in understanding ecological adaptations of the species and planning conservation strategies for its sustainable use.

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Hudar: Rheumatoid Arthritis in Unani System of Medicine, a Review

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Abstract

Hudar (Rheumatoid arthritis) is a disease as its name refers mainly associated with the (arth) joint(s), otherwise it effects to all connective tissues as an auto- immune disease. The affection produces inflammation and signs and symptoms like pain, swelling, stiffness mostly raised on the joints. The pain is the first one to make attention towards this disease that's why in Unani system of medicine this ailment is discussed under *Waja'-ul-Mafasil* (Pain in the joints) in general and in particular subjected as *Hudar*.

Hudar sustains from ancient times. Hippocrates (460-377 BC) also left his observations for the same. Presently WHO reports that it is 31st nonfatal discomfort and it is in picture for 0.8% of world population. Unani medicine covers this disease with an excellence and best possible time tested remedies are mentioned there. A general and brief coverage of Hudar is the theme of this paper.

Key Words: *Waja'-ul-Mafasil*: Arthritis, *Hudar*: Rheumatoid arthritis

Introduction

Waja'-ul-Mafasil (Arthritis)

In Unai literature *Waja'-ul-Mafasil* is discussed with the time of Hippocrates (d.377 BC) in his book *"Kitab-al-Mafasil",* followed by the description of all eminent physicians of the time like Dioscoride (d.70 AD) in his book *"Kitab al-Hashaish",* Roofas (d.117 AD) in his book *"Kitab al-Ajwa-li Mafasil",* Galen (d.200 AD) in his book *"Kitab al Elaj wal Amraz",* Gharyoos (d. 465) in his Books *"Risal fee Irqun Nisa"* and *"Risal fee Waj'a-ul-Niqras",* Sabit Bin Qurrah (d. 836 AD) in his book*"Kitab al-Awj-al-Mafasil",* Rabban Tabri (d.898) in his book *"Firdaus al-Hikmat",* Aili Bin Abbas Majusi (d.930) in his book *"Kamilussana"* Rhazes (d.923 AD)in his book *"Kitab Al-Hawi",* Abu Sahel Masihi (d. 1010 AD) in his book *"Kitab al-Meah",* Avicenna (d.1037) in his book *"Al-Qanoon"* and Najeebuddin Samarqandi (d.1232) in his book *" Al Asbaab wal Alamat"*. The gist of all that follows:

Waja'-ul-Mafasil: when the joints suffer by pain the condition refers to *Waja'-ul-Mafasil*. As the onset of any disease concerned Unani medicine classifies all the disorders in three categories according to the causes i.e. *Su-i- Mizaj*, *Su-i-Tarkeeb* and *Tafarruq-wa-Ittissal*. *Waja-ul-Masfasil* is a combination of all these. It is mentioned that when the *akhlat* like *balgham* and *safra* come under *su-i-mizaj* (abnormal state), the condition becomes prone to *waj-ul-mafasil*. Under the condition joints loose normal architecture, in the result their articulation disturbs, which is *su-i-tarkeeb* and *tafarruq-wa-Ittissal* respectively. In co- efficient causes of all these three immediate causes many things are described as age, sex, diet, habits, occupation, and climate etc.

Pain, swelling, redness and restricted movements are the main signs and symptoms. Pain takes lead as an alarming as well progressive symptom that's why referring to pain (*waja*') this disorder is labeled as *waja'-ul-mafasil*.

Unani medicine has sub categorized *waja'-ul-mafasil* according to the causes and appearance of the symptoms like *Hudar* (Rheumatoid Arthritis), *Niqras* (Gout), *Tahajjar-ul-mafasil* (Osteo-arthritis). The most common type is *Hudar*, commonly called *Gatthia* in our region. Here, this commonly occurring disorder *Hudar* is being taken to describe in an interest of a common man.

Hudar (Rheumatoid Arthritis)

The literature shows *Hudar* is the most common amongst all types of *waja'-ul-mafasil*. It generally appears in an age group of 35, may affect to a person of 20 years. It also occurs as juvenile. Females suffer three times more than males. Bulky and fatty persons remain more in danger than the light and thin. Inhabitants of cold habitants are more prone to disease. Its graph is high in eating lovers and less active persons. Variety of diet plays an important role as predisposing factors. Heredity characters are also reported for the occurrence.

Rheumatoid Arthritis is a constitutional disease, in which there are inflammatory changes throughout connective tissues of the body. Most characteristic feature is the poly-arthritis with a predilection for smaller joints such as proximal, interphalangeal and metatarsophalangeal, with a tendency for symmetric distribution after the disease is established.

Arthritis is caused due to chronic proliferative inflammation of the synovial membrane, which has the potentiality of producing irreversible damage to the joint capsule and articular cartilage, as these structures are replaced by granulation tissue. Both the constitutional manifestation and activity of synovial inflammation are subject to variation in severity with a strong tendency to unexplained remission with exacerbation.

Its occurrence comes into picture by the signs and symptoms mentioned like: pain, swelling, redness, restriction in movement, deformity of joints. The onset symptoms are weakness, loss of weight and vasomotor disturbance with numbness and tingling of hands and feet.

Any type of acute infection, exposure to excessive cold, exertion, worry and emotional stain promote progress of the disease.

Immediate findings of the onset are like one feels pain and stiffness in one or few joints, followed by swelling. Initially any of the joint may involved, usually smaller joints suffer first. In acute onset pain and swelling appear suddenly in multiple joints with addition of chill, fever and prostration. An episodic onset may also happen with pain, swelling, stiffness in one or many joints with fever and chill. After few days and

weeks all condition go down to reoccur by passing few weeks or months. The disease also occurs with a gradual advancement, in this situation fever and chill usually absent. Whatever be the mode of onset, the disease sooner or later sets in a characteristic chronic form.

These characteristics of the disease in respect of acute and chronic have been classified in Unani system of medicine by its own way. There sings and symptoms vary person to person based on the variety of matter involved distinct as *khilt* (matter which holds *su-i-mizaj*). There are four *akhlat* to cause four different types of *Hudar*, distinguished by identical marks as follow:

1. *Hudar-i-Damawi* (Redness much marked, Swelling conspicuous, Pain severe)
2. *Hudar-i-Safravi* (Redness over shed by yellow, Swelling moderate, Pain moderate supper added with itching)
3. *Hudar-i-Balghami* (Swelling soft but tender, Pain on movement, marked nodules)
4. *Hudar-i-Saudavi* (Swelling hard, skin dry with slight blue hue, marked nodule, structural deformity)

In this classification *Hadar-i-Damawi* and *Hadar-i-Safrawi* count for acute and *Hadar-i-Balghami* and *Hadar-i-Saudawi* refer for chronic form of the disease.

Differential diagnosis

Rheumatic fever should be differentiating from Rheumatoid arthritis, as these two diseases resemble closely. It is now easily possible by ruling out Rh factor.

In chronic stages Rheumatic arthritis has to be differentiated from Osteo-arthritis. This differentiation based on clinical findings with X-ray evidence, by bony overgrowth in the latter one

Rheumatoid arthritis has also to be differentiated from Gout. It is very easy by urine and blood investigations.

Investigations for Rheumatoid Arthritis

Common investigations:

- Radiological, X-ray findings
- MRI
- Blood investigations: TLC, DLC, ESR, PCV, Platelet Count, VDRL, Rh factor
- Urine: R & M
- Stool: Ova & Cyst

Unani Diagnostic Parameters: to rule out kind of Hudar and grade of the disease

- General physical Examination
- History of the case

Following findings may be used as a gist to confirm the case of Rheumatoid arthritis in general

1. Morning stiffness in the joint(s)
2. Pain in Joint(s)
3. Swelling on the symmetrical joist
4. Radiological features
5. Positive serological tests
6. Subcutaneous nodules
7. Morphological changes in the synovial membranes

If all above findings present that is a case of classical arthritis. If five findings are there that is usual arthritis. If less then five, arthritis may be may not be.

Treatment

Unani treatment very much depends on the *mizaj* (temperament) of the patient as well as variety of the disease, which are identified as mentioned above.

Then there are four methods of Treatment: Regimental Therapy, Diet Therapy, Drug Therapy and Surgery.

- **Regimental Therapy:** It is to suggest rest or movement for the joints according to the type of the disease and condition of the patient. Massage with a certain method and recommended oils are very useful for subsiding the signs and disperse the matter. Fomentation is also applied for relief; particularly *Hammam* a peculiar method of hot and wet thermo therapy is very effective to evacuate morbid substances.
- **Diet Therapy:** In advanced researches diet has no role but experience of the physicians suggests some recommendations and restrictions in diet to limit the onset and progress of the disease.

  Recommended food articles are: Wheat, Indian millet, Pulses, Broad beans, French beans, Spinach, Onion, Beet root, Carrot, Chilies (Red & Black), Bird's Flesh, Maize, Figs, Almonds, Pistachio nuts, Walnut, Dates, Mango, Apricot, Sweet Grapes, Potatoes, Pure Ghee, Amaranth, Fenugreek, Drumsticks, Turnip, Apple, Papaya.

  Some food supplements mostly contain chief ingredients like Aloe Vera and Amla namely *Halwa-i-Ghekwar* and Amla Candy are the best to check this autoimmune disease.

Restricted food articles are: Red meat, Beef, Brinjal, Cauliflower, Tomato, Tamarind, Buttermilk.

Mostly variety of proteins in the diet is checked. Red meat is restricted in particular.

- **Drug therapy:** There are number of single and compound time tested drugs for the treatment of *Hudar*. Most of them are used to check the causative factor in terms of *Khilt.* To excavate the matter *Munzij* and *Mushil* therapy applied. Particular drugs are used as a *mushil* and *munzij* for a particular variety of *Hudar* according to the *Khilt*. Effective and safe analgesics and ant inflammatory drugs are also available for internal and external use.
- **Surgery:** There are some special methods of treatment like *Fas'd* (Venesection), *Hijamat* (Cupping), *Ta'leeque* (Leeching) and *Kai* (Cauterization). These all are mentioned to treat *Hudar*. Venesection and Cupping usually applied and found effective.

Most recommended single drugs to treat Hudar

Asgand *(Withania sominifera*), Azraqi *(Strychnos nux vomica),* Bozidan *(Pyrethrum indicum)*, Chadela *(Parmilia perlata)*, Chob chini *(Smilax china)* Dar Hald *(Berberis vulgaris),* Gul-i-Babuna *(Matricoria chamomilla)*, Hina *(Lawsonia inermis),* Kaifal *(Myrica nagi)*, Khulanjan *(Alpinagalanga* Wild*)*, Majeeth Irani *(Rubia cordifolia)* Muqil *(Comminiphora mukul)*, Saad Kufi *(Cyperus rotundus)*, Suranjan Shirin *(Colchicum autumnale),* Turbud sufaid *(Operculina tuperthum)*, Ushba *(Smilax aristoloiaefolia),* Zanjbeel *(Zinjber officinale),*Zaitoon *(Olea europia).*

Most recommended compound formulations to treat Hudar

Aujai, Kushta Gowdanti, Majun Aujai, Majun Chob chini, Majun Chob chini ba nushka Kalan, Majun Surajan, Majun Falaspha, Jawarish Zanjbeel, Habb-i-Asgand, Habb-i-Suranjan, Halwa Ghekwar, Majun Seer, Majun Ushba, Sharbat Masaffi Murakkab Khas.

Most recommended oils for external use

Roghan-i-Kuchla, Roghan-i-Babuna, Roghan-i-Suranjan, Roghan-i-Hina, Roghan-i-Seer, Roghan-i-Malkangni

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