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• Instructions to Contributors

Editorial

Recent advances in experimental methods in phytochemistry and pharmacology have brought out new researches in traditional medicines. And, in view of growing demand of herbal medicines in India and abroad, issues of their quality, efficacy and safety have, of recent, received renewed attention of scientists. All these ongoing investigations have generated lot of new research data in recent times and there is an enormous need for exchange of this information amongst academicians and researchers engaged in the scientific validation of traditional drugs, particularly the Unani medicine. In this context, Central Council for Research in Unani Medicine, through its clinical, drug research, literary research, survey & cultivation of medicinal plants programme is contributing significantly for over three decades. *Vitiligo, sinusitis, filariasis, eczema, malaria, infective hepatitis, asthma* are some of the conditions where Unani therapies have earned recognition.

The Council has been publishing the peer reviewed *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 concpts and strengths of Unani medicine.

In view of an overwhelming response, the journal earlier published twice a year, its periodicity had 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 12 original and review papers in the areas of *clinical research, literary and fundamentals of Unani medicine, drug standardization, ethnobotany* 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.

September 10, 2015

(Prof. Rais-ur-Rahman)
Editor-in-Chief

Clinical Study of a Unani Formulation 'Sharbat Zoofa Murakkab' in the Management of Sual Ratab (Productive Cough)

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Abstract

The objective of the study was to evaluate the efficacy and safety of a Unani formulation Sharbat Zoofa Murakkab in the management of Sual Ratab (productive cough). 'Sharbat Zoofa Murakkab' in a dose of 10 ml, thrice daily was administered orally to the patients for 14 days. The Mean ± S.E.M. scores of signs and symptoms of Sual Ratab (productive cough) i.e. frequency, intensity, quantity, sore throat, hoarseness of voice and chest tightness were found decreased by 44.0% ($p<0.001$), 53.62% ($p<0.001$), 23.96% ($p<0.001$), 39.89% ($p<0.001$), 49.42% ($p<0.001$) and 58.09% ($p<0.001$) respectively as compared to in baseline findings. After treatment, the variation in biochemical parameters of Liver and Kidney Function Tests were found non-significant. No adverse effect was found in the patients. 'Sharbat Zoofa Murakkab' was found effective and safe in the treatment of Sual Ratab (productive cough).

Keywords: Sharbat Zoofa Murakkab, Sual Ratab, Unani formulation.

Introduction

Cough is a physiologically useful protective reflex that clears the respiratory tract by removing accumulated mucus and foreign substances (Sharma *et al*., 2011; Brunton et al., 2007). It occurs due to stimulation of chemo receptors in throat, respiratory passages or stretch receptors in the lungs (Tripathi, 2007). Traditionally cough is classified as either productive *(*producing mucus usually with expectoration) or non-productive (dry) (Harvey et al., 2008). Productive coughs are treated by the expectorants that enhance the bronchial secretion or reduce the viscosity of phlegm to facilitate its removal by coughing (Canning *et al*., 2004). It should be suppressed only when it is exhausting the patient or is dangerous (Karisson, 1996).

According to Unani Scholar Ibn Sina, sual (cough) is an act by which tabiyat removes aziyat (irritating substances) from the lungs and adjacent structures (Kantoori, 2007). Ismail Jurjani has described that Sual is movement of lungs to remove or reduce the painful stress on the lungs (Khan, 1903), it eliminates the irritating substances from the lungs and its associated structures (Kirmani, 1926). Most of the Unani scholars, while describing the pathogenesis of the disease have mentioned Asbabe badiyah (extrinsic factors) i.e. smoke, dust, fumes cold air and Asbabe wasila (intrinsic factors) i.e. sue mizaj as causative factors of cough. Asbabe badiyah cause inflammation in the airways and produces ratoobat (mucus hyper-secretion) that result in narrowing of the airways. According to them, cough is produced due to narrowing of the airways caused

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by accumulation of secretion (Khan, 1903; Ibn Sina, 2007; Tabri 1997) and is more prevalent in the persons of balghami mizaj (phlegamatic temperament) (Ibn Sina, 2007; Khan, 1903; Arzani, 2002). Some Unani scholars have described that Sue-mizaj ratab of lungs produce cough (Ibn Sina, 2007; Arzani, 2002, Khan, 2003). According to the nature of the cause, Sual har maddi (cough of hot humours) and Sual barid maddi (cough of cold humours) are collectively known as Sual Ratab (Productive Cough) (Khan, 1903). Sual Ratab (Productive cough) is caused by the fluids (Ratubat) of lungs and Chest. It is mainly found in elderly people and the people with wet temperament. The symptoms are amount of discharge are excessive, hoarseness of voice are present during the sleep and after awaking (Arzani, 1903)

Since the drugs available in modern medicine produce varying adverse effects in the human body, therefore natural, herbal or traditional medicines including Unani medicine are now being seen by the people with an eye of great interest and hope. Unani medicine claims to possess effective treatment for the management of sual and suggest an array of medicament for the purpose. Shabali 2 of Murakkab is one of the important drugs used to improve the condition of wet cough and other respiratory diseases (Arzani, 2002; Khan, 2003). Therefore, present study has been designed to study the efficacy and safety of Sharbat-Zoofa Murakkab in patients of Bronchial cough.

Material and Methods

Study Drug

The study drug 'Sharbat Zoofa Murakkab' is a Unani pharmacopoeial formulation, having 9 single drugs of plant origin (Table 1). The drug was manufactured by Central Research Institute of Unani Medicine, Hyderabad, and supplied to the Regional Research Institute of Unani Medicine, Patna.

Place of the Study

An open level clinical study, approved by the Institutional Ethics Committee (IEC), was carried- out on the patients of Sual Ratab (productive cough) in the O.P.D. of Regional Research Institute of Unani Medicine, Patna, for two years from 2012 to 2014.

Selection of Patients

The screened patients presenting one or more symptoms of productive cough, who met the inclusion and exclusion criteria of the study, were selected for this

study. Diagnosis of each case was made with the help of detailed history of selected patients, physical and systemic examinations as well as the laboratory investigations.

Inclusion Criteria

- Patients of either sex in the age group of 18-65 years.
- Cases of cough with the expectoration.
- Complaints of cough with history of more than 3 days duration.
- Patients willing to sign informed consent form to participate in the study.
- Patients willing to comply with various demands of study.

Exclusion Criteria

- Cases of non-productive cough.
- Cases of concomitant disease that may affect the evaluation of response to protocol therapy (such as Pneumonia, Bronchiectasis, Bronchial Asthma, pulmonary tuberculosis and lung carcinoma)
- Known cases of renal / hepatic/ cardiac impairment or the ailments needing long term therapy.
- Diabetes mellitus excluded by taking the history and blood sugar fasting examination.
- Pregnant or lactating women.

Treatment of Patients

All selected patients as per the inclusion/exclusion criteria were treated with Sharbat- Zoofa-Murakkab in the dose of 10 ml with lukewarm water thrice daily for 14 days.

Clinical Evaluation

The effects of Sharbat Zoofa Murakkab were assessed on subjective and objective parameters of the productive cough. Subjective parameters included, sore throat, hoarseness of voice and chest tightness; frequency and intensity of cough. As, these clinical parameters differ in severity (such as absent, mild, moderate or severe) from patient to patient therefore severity of the clinical parameters were graded as absent=0, mild=1, moderate=2 and severe=3 for appropriate assessment and statistical evaluation of the efficacy of Unani compound formulation. The patients were followed up on 7^{th} and 14^{th} day and at every visit, they were clinically examined and asked about the improvement or worsening of their symptoms.

Safety Assessment

The safety was assessed by monitoring adverse events when reported by the patients or elicited by the investigator by clinical as well as laboratory investigations before and after the treatment. The laboratory tests included Hematological Test (Hb, TLC, DLC, ESR), Liver Function Test (serum bilirubin, SGOT, SGPT, alkaline phosphatase) and Kidney Function Test (blood urea, serum creatinine).

Statistical Analysis

All data were statistically analyzed by applying paired't' test to evaluate the efficacy and safety of the drugs. Probability level of less than 5% was considered as statistically significant.

Results

A total of 109 subjects with signs and symptoms of Sual ratab (productive cough) completed the study. Means age of the patients was found to be 32.87 years. The distribution of the characteristics / demographic data of the selected patients for the study is summarized in table 2.

Effects of Sharbat-Zoofa Murakkab on Clinic Parameters

After 14 days treatment with Sharbat Zoofa Murakkab, the clinical parameters of productive cough i.e. sore throat, hoarseness of voice, chest tightness, frequency, intensity and quantity decreased significantly by 39.89%, 49.42%,

Table 1: Composition of 'Sharbat Zoofa Murakkab' (Kabiruddin, 1935)

Constituents	Latin name	Parts used	Quantity
Injeer	*Ficus carica* Linn.	Fruit	10 pieces
Tukhm-e-Khatmi	*Althaea officinalis* Linn	Seed	10 gm
Aslus Soos	*Glycyrrhiza glabra* Linn.	Root	10 gm
Irsa	*Iris ensata* Linn.	Root	10 gm
Badian	*Foeniculum vulgare* Mill	Fruit	15 gm
Tukhm-e-Karafs	*Apium graveolens* Linn	Seed	15 gm
Persiao Shan	*Adiantum capillus-vereris* Linn.	Whole Plant	20 gm
Zoofa Khushk	*Hyssopus officinalis* Linn.	Whole plant	20 gm
Muveez Munaqqa	*Vitis vinifera* Linn.	Fruit	90 gm

Table 2: Effect of Sharbat Zoofa Murakkab, on clinical parameters of Sual-Ratab (productive cough).

Presenting Symptoms		Mean ± SEM	Percentage Decrease	t-value	df	p-value
Sore Throat	BT	1.83 ± 0.05	39.89	11.581	108	<0.001
	AT	1.10 ± 0.06				
Hoarseness of Voice	BT	1.72 ± 0.06	49.42	11.788	108	<0.001
	AT	0.87± 0.06				
Chest Tightness	BT	1.36 ± 0.07	58.09	10.688	108	<0.001
	AT	0.57 ± 0.06				
Presenting Signs		Mean ± SEM	Percentage Decrease	t-value	df	p-value
Frequency	BT	2.50 ± 0.05	44.00	16.911	108	<0.001
	AT	1.40 ± 0.06				
Intensity	BT	2.07 ± 0.06	53.62	15.723	108	<0.001
	AT	0.96 ± 0.06				
Quantity	BT	2.17 ± 0.05	23.96	7.7880	108	<0.001
	AT	1.65 ± 0.06				

Paired 't' test, $p<0.001$ (Highly significant), $p<0.05$ (Significant), n=109

58.09%, 44.0%, 53.2% and 23.96%, respectively as compared to baseline findings (Table 2).

Effects of Sharbat-Zoofa Murakkab on Safety Parameters

The effect of test drug on haematological parameters (HB, ESR, TLC and eosinophils) and Biochemical parameters (Liver Function Test parameters and Kidney Function Test parameters), as assessed by laboratory investigations are depicted in table 5 and table 6, respectively.

After completion of treatment, erythrocyte sedimentation rate (ESR), total leukocyte count (TLC) and eosinophils were found decreased by 38.68% ($p<0.001$), 1.73% ($p>0.05$) and 54.29% ($p<0.001$) respectively. Heamoglobin was found significantly increased by 2.74 % ($p<0.001$) as compared to baseline value (Table 5).

Biochemical parameters of the Liver Function Test and Kidney Function Test were found within the normal range. After treatment percentage difference in Biochemical parameters as compared to baseline were found non-significant (Table 3 & 4).

Table 3: Effect of Sarbat Zoofa Murakkab on the Haemological Parameters.

Pathological Tests			Mean± S.E.M.	Percentage Increase(↑)/ Decrease(↓)		t- value	df	'p' value
Hemoglobin (gm/dL)		BT	11.66±0.08	2.02	↑	-3.48	108	P<0.001
		AT	11.90±0.11					
ESR (mm/hr)		BT	16.57±1.20	38.68	↓	6.884	108	p<0.001
		AT	10.16 ±0.91					
Total Leukocyte count 1000/cu.mm		BT	6.34 ± 0.14	1.73	↓	0.770	108	P=0.440
		AT	6.23 ± 0.07					
Differential Leukocyte Count								
DLC	Polymorphs (%)	BT	58.47 ±0.60	1.83	↓	1.475	108	p>0.05
		AT	57.40 +0.39					
	Lymphocytes (%)	BT	33.53 ±0.60	9.60	↑	-5.331	108	p<0.001
		AT	37.09 ±0.32					
	Monocytes (%)	BT	0.93 ± 0.07	52.77	↑	-3.89	108	p<0.001
		AT	1.96 ± 0.27					
	Eosinophils (%)	BT	6.69 ± 0.31	54.29	↓	12.686	108	p>0.001
		AT	3.06 ± 0.15					

Paired 't' test, n=109, p <0.001 Highly Significant (H.S.), p>0.05 Non-Significant (N.S.)

During the study neither the adverse effect was reported by the patients nor it was detected during clinical examination and laboratory investigation.

Discussion

The study demonstrated that Sharbat-Zoofa Murakkab is effective in relieving the productive cough as it caused significant decreaase in almost all the signs and symptoms of cough.

After two weeks of the treatment with Sharbat Zoofa Murakkab, improvement was recorded in sore throat (39.89%), hoarseness of voice (49.42%), chest tightness (58.09%), frequency (44.0%), intensity (53.62%) and quantity of sputum (23.96%). The ingredients contained in Sharbat Zoofa Murakkab have been ascribed to possess some of the pharmacological effects which are effective directly or indirectly in improving the cough and expectoration. Irsa, Persiosham, Badian and Tukhme Karafs etc. have been described to be anti-inflammatory, Asl-us-Soos and Persioshan are expectorant and Gule Zoofa, Tukhme Khatmi

Table 4: Effect of the Sharbat Zoofa Murakkab on Liver and Kidney function.

Laboratory Tests	Parameters	Day of Measurements	Mean ± S.E.M.	Percentage Reduction	p value
Liver Function Tests	Serum Bilirubin, (mg/dL)	Baseline	0.72 ±0.05	6.94 %	>0.05*
		After Treatment	0.67 ± 0.01		
	SGOT (IU/L)	Baseline	15.63 ±0.33	3.01 %	>0.05*
		After Treatment	15.16 ±0.36		
	SGPT (IU/L)	Baseline	21.90 ±0.56	0.59 %	>0.05*
		After Treatment	21.77 ±0.40		
	ALP (KAU/dl)	Baseline	6.99 ± 0.49	0.72 %	>0.05*
		After Treatment	6.94 ± 0.45		
Kidney Function Tests	Blood Urea (mg/dL)	Baseline	23.05 ±0.39	0.95 %	>0.05*
		After Treatment	22.83 ±0.37		
	Serum Creatinine (mg/dL)	Baseline	0.80 ± 0.05	0.03 %	>0.05*
		After Treatment	0.78 ± 0.01		

*p>0.05 (Non-significant); **p<0.05 (Significant), (Statistical analysis by paired't' test), n=109.

and Asl-us-Soos have mucolytic property. Due to anti-inflammatory, expectorant and mucolytic properties of the ingredients of Sharbat Zoofa Murakkab, appears to control the inflammation in respiratory track and modify the consistency of the mucous to enable it to be expectorated easily. The collective response of various ingredients actually translated into the improvement of production cough.

At the end of the study, ESR, which indirectly measures inflammation, was decreased. Percentage reduction in ESR (38.68%) and Eosinophil (54.26%) were found highly significant. They indicated that the test drug also possesses anti-allergic response. Increase in heamoglobin (2.74%) as compared to baseline were found highly significant (p<0.001). (Table 5). Biochemical parameters of liver function test, kidney function test (Serum Bilirubin, SGOT, SGPT, Alk. phosphatase) and kidney function (Blood Urea, S.Creatinine) were found within the normal range indicating that the test drug is safe.

Conclusion

On the basis of above observations, it can be concluded that Sharbat Zoofa Murakkab is clinically effective and safe in relieving the symptoms and signs of

sual ratab (productive cough) and hence it can be safely prescribed to the patients.

Acknowledgement

The authors are indebted to Director General, Central Council for Research in Unani Medicine, New Delhi, for sponsoring the trial drug for this study. We are also thankful to Dy. Director, RRIUM, Patna for providing facilities to conduct the study.

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Evaluation of Antidepressant Activity and the Possible Mechanism of Action of *Majoon Najah* in Experimental Models

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Abstract

The present study has been carried out to evaluate the antidepressant activity of a pharmacopoeal Unani drug *Majoon Najah* (MN) in experimental animals. Tetrabenazine antagonism test and Yohimbine toxicity enhancement test were used to study the antidepressant activity in mice divided into 4 groups of 6 animals each. Animals in Group I, II and III were treated with distilled water, 50% alcoholic extract of MN in 260 mg/kg (single dose) and 520 mg/kg (double dose), orally, respectively. Group IV was treated with standard drugs Imipramine (20 mg/kg per oral) and Desipramine-Hcl (10 mg/kg i.p.) in both the tests, respectively. The effect of test drug was observed on duration of catalepsy, degree of ptosis and the mortality rate of the animals.

MN demonstrated antagonist effect in Tetrabenazine induced catalepsy and ptosis. Cataleptic score and degree of ptosis were significantly reduced ($p<0.001$) in Group II and III in a dose dependent manner, and no significant difference was found between Group III and IV. In Yohimbine toxicity enhancement test, the mortality rate increased significantly ($p<0.001$) in Group II & III; and at 24 hr significant difference was observed when mortality rate was compared among the groups, between I & III ($p<0.011$), and between I & IV ($p<0.05$), between II & III ($p< 0.011$). The mean time of mortality in group III was observed significantly less ($p<0.0001$) when compared with group I, II & IV.

The study demonstrated that the test drug possesses significant anti depressant. It has most likely produced its effect by inhibiting the monoamine uptake through adrenergic, serotonergic and monoamine oxidase inhibiting mechanisms.

Keywords: Antidepressant, *Majoon Najah*, Yohimbine, Catalepsy.

Introduction

Depression is a disorder of emotion rather than disturbance of thought. Major depression which affects approximately 20% of the population is classified as either unipolar or bipolar (Porth and Kunert, 2002). It is characterized by a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and physical well-being and is twice common in women than in men (Salman, 1997). Although, the currently prescribed molecules have shown signs of improvement in the clinical condition of the patients, but it is at the cost of having to bear the burden of their numerous adverse effects and chances of recurrence (Stahl, 1998).

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In Unani system of medicine the term "*Malikholia*" (Melancholia) is commonly used for depression. Melancholia is one of the often used words of psychiatry, while depression is the recent name of melancholia (Rao, 2004). Hippocrates (460-357 BC) described melancholia as a state of "aversion to food, despondency, sleeplessness, irritability and restlessness" (Kaplan and Sadock, 1995). It is defined as a disease in which there is derangement of thoughts and intellect. It is characterized by social isolation, loneliness, fear of objects an average person is not afraid of, negative thoughts and feelings, excessive grief, anxiety, delusions, hallucinations etc. The disease has been described to be caused mainly due to disproportionate (excessive) accumulation of black bile or deterioration in its quality (Jurjani, 1898; Tabri 1995; Garzooni, 1994; Ibn Sina, 2007; Razi, 2002). The symptoms of *Malikholia* as described in Unani literature, are withdrawal from the society, negative thoughts and feelings, inability to think and act rationally, excessive grief, hallucinations, delusions, feeling of worthlessness or excessive guilt, fearfulness without a cause, nervous exhaustion, sleeplessness, restlessness, loss of interest and enjoyment, fatigue and loss of energy etc (Jurjani, 1898; Ibn Sina, 2007). These symptoms have similarity with the symptoms of depression described in DSM-IV (Anonymous, 1994). Therefore, *Malikholia* has been taken by us to correspond to the depressive disorder. There are a number of drugs both single and compound preparations that are used in Unani medicine in depressive disorders since hundreds of years. One important pharmacopoel compound drug is Majoon Najah (MN) described in all major formulary books of Unani medicine. It is a semi-solid preparation obtained by mixing different powdered drugs as mentioned below (Table 1), in a *qiwam* (base) made of purified honey or sugar.It is an age-old and time tested polyherbal preparation which is commonly used in depression and related conditions (Kabiruddin, 1938). MN has also been investigated on scientific parameters and shown to be significant antidepressant, CNS stimulant, anxiolytic and antioxidant activities (Imran, 2008) using Gross Behaviour Test, Despair Swim Test, Reserpine Induced Hypothermia Test, Pentobarbitone Induced Narcosis Potentiating Test and Elevated plus Maze Tests etc. The present study was designed with an aim to assess the antidepressant effect of the test drug and also to explore the possible mechanism of action especially with reference to monoamine concentration. In depression, since there is a deficiency of neurotransmitters noradrenaline and serotonin in the brain, which can be altered by antidepressants therefore the drugs that effect depression, can modify amine storage release or uptake. In view of the above therefore two important tests i.e. Tetrabenazine antagonism test and Yohimbine toxicity enhancement test were used to determine its anti depressant effect and the likely mechanism of action. However, the extract of the ingredients sans sugar/honey was used.

Table 1: Ingredients of Majoon Najah

Ingredients	Parts used	Weight
Post Halila Kabli (Pericarp of *Terminalia chebula* Retz.)	Pericarp	50 g
Post Balela (Peel of *Terminalia belerica* Roxb.)	Pericarp	50 g
Amla Khushk (Fruit of *Emblica officinalis* Gaertn.)	Fruit	50 g
Halila Siyah (Unripe Fruit of *Terminalia chebula* Retz.)	Unripe Fruit	50 g
Turbud Mudabbar (Root & stem of *Ipomoea turpethum* Br.)	Root /stem	25 g
Bisfaij (Rhizome of *Polypodium vulgare* Linn.)	Rhizome	25 g
Aftimoon (Whole plant of *Cuscuta reflexa* Roxb.)	Whole plant	25 g
Ustukhuddus (Whole plant *Lavandula stoechas* Linn.)	Inflorescence	25 g

Materials and Methods

The present study was undertaken in the department of Ilmul Advia, National Institute of Unani Medicine (NIUM) Bangalore, Karnataka, India. Before starting the experiment the protocol was submitted to the Institutional Animal Ethics Committee (IAEC) of NIUM Bangalore, for ethical clearance. The proposal was approved vide Reg No. IAEC/V/05/IA dated 25/04/2010.

Plant Drug Material

All the ingredients of MN were procured from the local market of Bangalore. Professor Amthul Shukoor (Senior Botanist and Taxonomist, University of Mysore) authenticated the drug samples (vide letter No.D.Auth-01/2010-11 dated 25-06-2010). The specimens of the ingredients have been submitted to NIUM herbarium library for record and future reference.

Preparation of Extract

The dried plant materials were pulverized separately and the coarse powder obtained was mixed and stocked in plastic containers from which extracts were prepared. 100 g of powdered drug was extracted separately in 400 ml of ethyl alcohol (50%) along with water (50%) in Soxhlet apparatus at a temperature of 70°-80°C for 8 hours continuously. The extract obtained from each sample was filtered, cooled and evaporated on water bath till it dried. It was weighed and the yield percentage was calculated with reference to the crude drug. The average yield of the hydro- alcoholic extract of three samples of MN was found to be 39%.

Animals

The study was carried out in two different groups of male Swiss mice with one group weighing between 20-22 gm for Tetrabenazine Antagonism test and the other weighing between 25-28 gm for Yohimbine Toxicity Enhancement Test (Vogel, 2002). The mice were procured from Central Animal Research facility (CARF), National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. They were housed in polypropylene cages (6 animals per cage) and were maintained under standard laboratory conditions with temperature at 25 ± 2^0 c, relative humidity of 50%-60 % and 12 hours light/dark cycle at the animal house facility of NIUM. Mice were given standard pellet diet (Lipton-India ltd.) and tap water ad *libitum* under strict supervision and hygienic conditions.

Dosage of Drug

The dose of the Hydro alcoholic extract of MN for Swiss mice was calculated by multiplying the therapeutic dose of the test drug as describe in Unani literature, by conversion factor 12 (Frierich et al., 1968) and found to be 260 mg/kg. To evaluate the dose dependent response the double dose i.e. 520 mg/kg was also used in the study.

1. Tetrabenazine antagonism test

This test was carried out by the method of Vogel (2002). Swiss male mice weighing between 20-22 gm were used in this test. The animals were observed for catalepsy and ptosis induced by TBZ. The mice were divided into four groups of six animals each and treated per orally as follows:

Group-I: Control group was administered Distilled water 0.25 ml.

Group-II: Treated with MN in the dose of 260 mg/kg.

Group-III: Treated with MN in the dose of 520 mg/kg.

Group-IV: Treated with standard drug Imipramine in the dose of 20 mg/kg.

All the drugs and the vehicle were administered once in the morning. The time of administration of treatment was recorded. Sixty minutes after the administration of treatment, Tetrabenazine (TBZ) was mixed with a drop of glacial acetic acid and diluted with 0.9% saline and was administered in the dose of 40 mg/kg intraperitoneally, to all the animals in each group (Yamada, 1994; Fabio, 1999). 30 minute after the administration of TBZ, animals of all the groups were observed for Catalepsy individually. Each mouse was placed on a cork stair which was made of two cork stoppers having 2 steps of 3cm height each on which the animals were placed head downwards with their hind legs upon the top cork. Cataleptic effect was observed as long as TBZ exerts its cataleptic effect. The

duration of catatonic state of each mouse was recorded with the help of stop clock. Cataleptic effect was observed for a maximum of 60 seconds. Scores were allotted depending upon the duration of catalepsy produced in each and every mouse. Scoring pattern was adopted as follows:

Duration of catalepsy	Scores
>60 sec	5
Between 30 - 60 sec	4
Between 10 - 30 sec	3
Between 05 - 10 sec	1
<05 sec	0

In the above experiment TBZ – control were taken as 100%. Whereas standard drug Imipramine has shown its effect at a dose of 20 mg orally.

Soon after observation of catalepsy Mice were placed into normal position and placed into cages. After a gap of just 30 seconds the animals were again observed for ptosis which was produced due to the effect of TBZ administration. Ptosis was observed at an interval of 30 minutes up to a maximum of 150 minutes and the degree of ptosis was recorded in each and every mouse. The pattern of awarding the degree of ptosis was adopted as follows:

Eyes close ..... 4^0

Eyes ¾ closed ..... 3^0

Eyes ½ closed ..... 2^0

Eyes ¼ closed ..... 1^0

Eyes open ..... 0^0

2. Yohimbine Toxicity Enhancement Test

This test was carried out by the method of Vogel (2002). Swiss male mice of body weight between 25 to 28 g were used for this test. They were divided into four groups of ten animals each. The mice in group I were treated as a negative control with 0.25 ml of distilled water orally. Mice in group II were treated with MN in the dose of 260 mg/kg orally and group III were given MN in the dose of 520 mg/kg orally while group IV were treated with standard drug Desipramine-HCL at the dose of 10 mg/kg i.p. All the drugs and the vehicle were administered once in the morning 30 minutes before the conduction of test.

Exactly after 30 minutes of administration of test drug, a sub lethal dose of 25 mg/kg of Yohimbine-HCL was given, subcutaneously. Yohimbine-HCL occupies central α_2 receptor and prevents noradrenaline from binding to these receptors.

An anti depressant is known to inhibit physiological inactivation of noradrenaline and other biogenic amines by blocking the re-uptake at nerve terminal. Administration of antidepressant (Standard and the test drug) leads to an increase in noradrenaline concentration. Following the simultaneous administration of Yohimbine and an antidepressant, deaths of mice have been recorded due to noradrenaline poisoning which has exhibited the antidepressant activity of the drugs.

Mortality rate was assessed at every 1, 2, 3, 4, and 24 hrs. Lethality in Yohimbine negative control group has been mentioned as less than 10% and about 90% in standard drug of Desipramine HCL at the dose of 10 mg/kg. Death rate was also recorded by giving in two different doses of the test drug. This test has been proven as simple and critical assessment method to detect antidepressants with monoamine uptake inhibiting properties.

Statistical Analysis

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Kruskal Wallis test a non-parametric test has been used to find the significance of study parameters between three or more groups of animals, Kaplan Meir Function analysis is performed to find the significance of time to death in each group. Mann Whitney U test has been performed to find the pair wise significance. Fisher Exact test has been used to find the significance incidence of death in four different groups.

Observations and Results

Effect of MN on Tetrabenazine Induced Catalepsy and Ptosis

Catalepsy

The cataleptic effect in the mice was observed for a maximum of 60 seconds at a regular interval of 30, 60, 90, 120 and 150 min (max). The duration of catalepsy was recorded; the mean and median scores of catalepsy were obtained from the experimental data and were compared among the different groups by Kruskal Wallis test (Table 2).

During the first observation after 30 minutes, the mean and median cataleptic score in Group II was found to be 3 as compared to Group I which was 4.67. This shows that the cataleptic score was significantly reduced by 1.68 (Z=3.146). The mean and median cataleptic score of Group III was found to be 1 when

Table 2: Comparison of Mean Catalepsy Score in Four Groups

Catalepsy	After 30 min	At 60 min	At 90 min	At 120 min	At 150 min
Group I	4.67(5.00)	4.67(5.00)	4.67(5.00)	4.67(5.00)	4.67(5.00)
Group II	3.00(3.00)	3.00(3.00)	3.00(3.00)	3.00(3.00)	3.00(3.00)
Group III	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)
Group IV	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)	1.00(1.00)
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Pair wise difference					
Group I vs Group II	1.68	1.68	1.68	1.68	1.68
Group I vs Group III	3.67	3.67	3.67	3.67	3.67
Group I vs Group IV	3.67	3.67	3.67	3.67	3.67
Group II vs Group III	2.00	2.00	2.00	2.00	2.00
Group II vs Group IV	2.00	2.00	2.00	2.00	2.00
Group III vs Group IV	0.00	0.00	0.00	0.00	0.00
Pair wise Comparison (Z values)					
Group I vs Group II	3.146**	3.146**	3.146**	3.146**	3.146**
Group I vs Group III	3.146**	3.146**	3.146**	3.146**	3.146**
Group I vs Group IV	3.146**	3.146**	3.146**	3.146**	3.146**
Group II vs Group III	3.317**	3.317**	3.317**	3.317**	3.317**
Group II vs Group IV	3.317**	3.317**	3.317**	3.317**	3.317**
Group III vs Group IV	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)

Results are presented as Mean; Z- value is obtained by Kruskal Wallis test

compared with Group I, the cataleptic score was found to be decreased by 3.67 (Z=3.146), which was even more significant. When the mean and median cataleptic score were compared among the groups, it was found that, the cataleptic score in Group III was decreased significantly by (2.00) i.e. (Z=3.317). The cataleptic score in group- III and group IV were found to be similar and there was no statistically significant difference. This shows that the double dose of test drug and the standard drug have similar effect.

Similarly mean and median cataleptic score in group - II, at 60, 90, 120 and 150 min interval were found to be 3, 3, 3 and 3 when compare with group -I which were found to be 4.67, 4.67, 4.67, 4.67 respectively. When the mean and

median cataleptic scores were observed at 60, 90, 120 and 150 min interval and compared , the cataleptic score in group III was decreased by 2.00, 2.00, 2.00 and 2.00 i.e. ($Z=3.317^{**}$ sig), which was highly significant when compared with group- II. The cataleptic score in group- III and group IV at 60, 90, 120 and 150 min interval were again found to be similar and there was no statistically significant difference between these two groups, shows that the double dose of test drug and the standard drug have similar effect.

Ptosis

The animals were observed for ptosis at a regular interval of 30, 60, 90, 120 and 150 min (max). The degree of ptosis was recorded and ranged from 0^0-4^0 in which higher degree indicates augmentation in ptosis and lower degree indicates reduction in the degree of ptosis.

The mean and median degree of ptosis was obtained from the experimental data using Mann Whitney-U test (Table 3) and the overall degree of ptosis was found to be 4^0 in Group I, 2^0 in Group II, 0^0 in Group III and 0^0 in Group IV. When the first observation for ptosis was done at 30 min, during the experiment the degree of ptosis in the Control Group I was found to be maximum i.e. 4.00, while in Group II degree of ptosis was 2.33. When the mean degree of ptosis was compared among different groups at 30 min, it was found that the mean degree of ptosis of Group II was significantly less 1.67 (Z=3.146) than Group I; the mean degree of ptosis in Group III was found to be 0 which was highly significant (Z=3.317) as compared to Group I. However, no significant difference was observed between Group III and IV. When Group II was compared with Group III, the mean degree of ptosis was found to be significantly reduced (2.33, Z=3.146).

Observation for ptosis was also done at 60, 90, 120 and 150 minutes and it was found that the mean degree of ptosis in Control Group was maximum i.e. 4^0 throughout the recording of the experiment. While in Group II degree of ptosis was 2, 2, 2 and 1, at 60, 90,120 and150 min, respectively. The mean degree of ptosis of Group II when compared with Group I was found to be significantly less by1.83 (Z= 3.108), 2.17 (Z= 3.207), 2.33 (Z= 3.146) and 2.67 (Z=3.146), at 60, 90, 120, and 150 minutes, respectively, throughout the experiment. The mean degree of ptosis in Group III was found to be 0, 0, 0 and 0, at 60, 90, 120, and 150 minutes, respectively, when it was compared with Group I, it was found less by 4 (Z=3.317) at all the intervals as there was no degree of ptosis observed in the animals of Group III and when it was compared with Group IV the degree of ptosis was statistically similar between Group III & Group IV, and when Group II was compared with Group III the mean degree of ptosis was significantly reduced

Table 3: Comparison of Mean and Median Degree Ptosis in Four Groups

Ptosis	After 30 min	At 60 min	At 90 min	At 120 min	At 150 min
Group I	4.00(4.0)	4.00(4.0)	4.00(4.0)	4.00(4.0)	4.00(4.0)
Group II	2.33(2.00)	1.83(2.00)	1.83(2.00)	1.67(2.00)	1.33(1.00)
Group III	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
Group IV	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Pair wise difference					
Group I vs Group II	1.67	1.83	2.17	2.33	2.67
Group I vs Group III	4.00	4.00	4.00	4.00	4.00
Group I vs Group IV	4.00	4.00	4.00	4.00	4.00
Group II vs Group III	2.33	2.17	1.83	1.67	1.33
Group II vs Group IV	2.33	2.17	1.83	1.67	1.33
Group III vs Group IV	0.00	0.00	0.00	0.00	0.00
Pair wise Comparison (Z values)					
Group I vs Group II	3.146**	3.108**	3.207**	3.146**	3.146**
Group I vs Group III	3.317**	3.317**	3.317**	3.317**	3.317**
Group I vs Group IV	3.317**	3.317**	3.317**	3.317**	3.317**
Group II vs Group III	3.146**	3.108**	3.207**	3.146**	3.146**
Group II vs Group IV	3.146**	3.108**	3.207**	3.146**	3.146**
Group III vs Group IV	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)	0.00(NS)

Results are presented in Mean; Z- values are obtained by Pair wise comparison done by Mann Whitney U test

by 2.17 (Z=3.108), 1.83 (Z=3.207), 1.67 (Z=3.146) and 1.33 (Z=3.146) at 60, 90, 120, and 150 minutes, respectively.

Effect of Majoon Najah on Yohimbine Toxicity Enhancement

The effect of intervention of test drug based on mortality was assessed at 1, 2, 3, 4, 5 and 24 hrs of study period (Table 4 and 6). During the observation, the mean mortality rate was compared among the different groups by Fisher Exact test.

Table 4: Comparison of Mortality Rate in Four Groups

Mortality	1 hour	2 hours	3 hours	4 hours	5 hours	24 hours
Group I						
• Alive	10 (100.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	9 (90.0%)
• Death	0	0	0	0	0	1 (10.0%)
Group II						
• Alive	10 (100.0%)	10 (100.0%)	10 (100.0%)	9 (90.0%)	7 (70.0%)	6 (60.0%)
• Death	0	0	0	1 (10.0%)	3 (30.0%)	4 (40.0%)
Group III						
• Alive	8 (80.0%)	5 (50.0%)	2 (20.0%)	1 (10.0%)	1 (10.0%)	1 (10.0%)
• Death	2 (20.0%)	5 (50.0%)	8 (80.0%)	9 (90.0%)	9 (90.0%)	9 (90.0%)
Group IV						
• Alive	9 (90.0%)	8 (80.0%)	4 (40.0%)	2 (20.0%)	2 (20.0%)	2 (20.0%)
• Death	1 (10.0%)	2 (20.0%)	6 (60.0%)	8 (80.0%)	8 (80.0%)	8 (80.0%)
P value	0.595	0.001	<0.001	<0.001	<0.001	<0.001

2x4 Fisher Exact test

Effect of M N on Yohimbine Toxicity Enhancement

At 1 hr, the mean mortality rate of Swiss mice was found to be 0%, 0%, 20% and 10% in group I, II, III, and IV, respectively, which when compared among different groups it was found that there was no significant difference between these groups (Table 5).

At 2nd hr the mean mortality rate was 0%, 0%, 50% and 20% in group I, II, III and IV, respectively, which on comparison with different groups demonstrated that there was no difference between Group I, II and IV. However, Group I and II when compared with group III showed significant difference ($p<0.05$), while significant difference was observed between group III and IV.

Table 5: Comparison of Rate of Death in Four Groups

N=10	Incidence of Mortality			
	Group I	Group II	Group III	Group IV
1 hour	0	0	2(20.0%)	1(10.0%)
2 hours	0 c^1	0 a^4, c^1	5(50.0%) a^1,b^1,d^4	2(20.0%) a^4, c^4
3 hours	0 b^4,c^3	0 a^4, c^3	8(80.0%) a^3,b^3, d^4	6(60.0%) a^1,b^1 c^4
4 hours	0 c^3	1(10.0%) c^3, d^2	9(90.0%) a^3,b^3, d^4	8(80.0%) a^3,b^2 c^4
5 hours	0 c^3	3(30.0%) c^1	9(90.0%) a^3,b^1, d^4	8(80.0%) a^3 b^4 c^4
24 hours	1(10.0%) b^4,c^3, d^2	4(40.0%) a^4,c^3	9(90.0%) a^3,b^3, d^4	8(80.0%) a^2, b^4

(P value is obtained by Fisher Exact test, n = 10, mean ± SD (Min - Max) and results on categorized measurements are presented in number %)

1, 2, 3 & 4 = p < 0.005, p < 0.011, p d" 0.001 & N.S (Not significant)

a = Comparison with group -I (Control)

b = Comparison with group-II (Test drug A)

c = Comparison with group- III (Test drug B)

d = Comparison with group-IV (Standard drug)

At 3rd hr the mean mortality rate was 0%, 0%, 80%and 60% in group I, II, III, and IV, respectively. Significant difference was found when Group I and II were compared with Group III (p< 0.001), whereas Group I and II when compared with Group IV showed a significant difference (p<0.011). However, no significant difference was observed between Group I and II as well as between III and IV.

At 4th hr the mean mortality rate was 0%, 10%, 90% and 80% in group I, II, III, and IV, respectively. During inter group comparison the values of Group I were found significant (P <0.001) as compared to Group III and IV. Group III and IV (P<0.001) and Group II and IV (P<0.005) also demonstrated significant difference when compared with each other. No significant difference was found between Group III and IV.

At 5th hr the mean mortality rate was 0%, 30%, 90% and 80% in group I, II, III, and IV, respectively. Significant difference was found when Group I was compared with Group III and IV (P< 0.001); Group II showed significant difference (P<0.05)

Table 6: Prediction of the Time of Mortality (Kaplan Meir Function Test)

	Mean time of death	SE	95%CI
Group I	>24.00	0.0	-
Group II	18.20	3.23	11.86-24.54
Group III	4.50	2.07	0.43-8.56
Group IV	7.10	2.69	1.84-12.36
Inference	Time of death in hrs is significantly early in Group III (4.50 hrs), followed by Group IV (7.10 hrs) (P<0.0001) (Log rank test)		

when compared with Group III. No significant difference was observed between III and IV group.

At the end of the study i.e. at 24 hr the mean mortality rate was 10%, 40%, 90% and 80% in group I, II, III, and IV, respectively. A significant difference was observed between Group I and Group III, Group I and IV (P<0.001) and between Group II and III (P<0.001). However, there was no significant difference between Group I & II, group II & IV and group III & IV.

Kaplan Meir function test was performed to assess the mean time of mortality. The findings summarized in Table 6 indicate that the mean time of mortality in Group III was 4.5 hours which was significantly less than the mean time of Group I and II (P<0.0001). In Group I, the mean time of mortality was found to be more than 24.00 hrs and in group II, it was 18.20 hrs. However, the mean time of death in Group IV was found to be slightly higher i.e. 7.1 hours than Group III. Therefore, the early onset of deaths in group III when compared to other groups suggested that the double dose of test drug has better response.

Discussion

In the present study, hydro alcoholic extract of MN was evaluated for antidepressant activity on two experimental models of depression. These two tests are considered simple and reliable for the evaluation of classical antidepressant drug through which both Monoamin oxidase inhibitory (MAOIs) and Tricyclic antidepressant (TCAs) effects may be evaluated (Vogel, 2002). The findings of Tetrabenazine antagonism test suggested that there was a significant reduction of catalepsy and ptosis. In this experiment, the test drug ameliorated the catalepsy and ptosis caused by TBZ through noradrenergic, serotonergic and monoamine oxidase inhibition as the TBZ induces depletion of biogenic amines (eg. noradrenaline, serotonin and dopamine) from nerve terminals without affecting their de novo synthesis and prolongs reuptake into the granula.

Noradrenaline is degraded by monoamine oxidase, this depletion of monoamine actually produces catalepsy and ptosis (Vogel, 2002).

It has been reported that all clinically useful antidepressant drug potentiate, either directly or indirectly, the action of norepinephrine, dopamine and/or serotonin in the brain (Mary *et al*., 2000). The standard TCA drug Imipramine which was used in this test is a strong reuptake inhibitor of norepinephrine and serotonin (http://drugbank) and acts as an adrenergic and seratonergic (Fabio et al., 1999). It inhibits the reuptake of noradrenaline into nerve terminals and thereby increases its concentration at the receptor site (Vogel, 2002). By decreasing the degree of catalepsy and ptosis the test drug appears to antagonize the effect of TBZ. When the results were compared with that of control group, the two doses of test drug were found to reduce the cataleptic score of ptosis significantly ($p<0.001$), in dose dependent manner as the effect of double dose was found to be more significant than the single dose, while no significant difference was observed between the results of Group III Group IV. Therefore, the findings suggested that the test drug possesses striking antidepressant effect that is equable to standard drug Imipramine.

The findings of Yohimbine toxicity enhancement test suggested that there was a significant increase in the mean mortality rate of test drug. In this experiment, Yohimbine-Hcl occupies central α_2 receptors and prevents noradrenaline from binding to these receptors, thus allowing an increase in noradrenaline concentration. It has been reported that an anti depressant drug inhibits physiological inactivation of noradrenaline and other biogenic amines by blocking the reuptake at nerve terminals and consequently increasing the biogenic amines concentration (Mary, 2000). Desipramine which was used as the standard drug in this test is known to exhibits greater non adrenergic reuptake inhibition as compared to other TCAs (Fabio, 1999). Therefore, following the simultaneous administration of Yohimbine and an antidepressant, death of mice was recorded due to noradrenaline poisoning. Here the mechanism involves dual activity both by blocking the selective reuptake of noradrenaline from the neural synapse in the CNS by using an antidepressant and also by administration of Yohimbine which lead to high concentration of noradrenaline resulting in death of mice. When the results were compared with that of control group, the mortality rate was found significantly increased ($p<0.001$) at single and double dose of MN. At 24 hrs, when the mortality was compared among different groups it showed that the more number of animals died in less time after treatment with double dose of the test drug. This observation revealed that the test drug increased noradrenaline and other mono amine concentration by the similar mechanism as that of standard drug Desipramine-Hcl. This test has proved the antidepressant activity of MN via adrenergic reuptake inhibition, in a dose

dependant pattern. The findings of present study in respect of its anti depressant effect are in agreement with the findings of previous study (Imran, 2008).

Almost all the ingredients of the test drugs are described in Unani literature to possess *Munzije Sauda* (concoctive of black bile) and *Mushile Sauda* (purgative of black bile) properties, therefore they are able to improve a diseased condition where the *sauda* is accumulated in excessive amount or its quality is compromised, giving rise to certain pathological conditions. Since depression as discussed earlier, is mainly caused by the qualitative or quantitative imbalance of *sauda,* the improvement in depressive condition by the test drug therefore suggests that it possesses antidepressant effect because of its *Munzije Sauda/ Mushile Sauda* properties. *Munzij* and *Mushil* properties are mainly responsible to improve depression by removing the causative factor or improving its quality. Thus, the claim of Unani medicine that the drugs possess *Munzije Sauda/Mushile Sauda* activity can be used in the management of depressive disorders, has been validated in this study.

Conclusion

In view the findings of present study it can be concluded that *Majoon Najah* possesses significant antidepressant effect. It increases the concentration of noradrenaline at the receptor site probably through adrenergic reuptake inhibition and blocking the degradation of noradrenaline.

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Comparative Clinical Evaluation of Hijaamah (*Cupping Therapy*) in the Treatment of Knee Osteoarthritis

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Abstract

In Unani medicine, Hijaamah (Cupping Therapy) has been traditionally used for variety of applications including management of osteoarthritis, the most common form of arthritis and is a major cause of morbidity, limitation of activity, and healthcare utilization, especially in elderly patients. Although this treatment approach has been used for many centuries, there is little scientific data on its effectiveness. The aim of this study was to validate the efficacy of cupping therapy in knee osteoarthritis. This study was a randomized parallel group comparative trial conducted with the approval of Institutional ethical committee, to compare the combined efficacy of cupping therapy (Ilaj-bil-Hijamat) and the traditional Unani herbal formulations against the same traditional formulation alone. Intervention was carried out in 40 patients, 20 in each group completed the study over a period of 6 weeks. The outcome measures included; Visual Analogue Scale (VAS), Knee injury and osteoarthritis outcome score (KOOS), range of motion, and 15- meter walking time were used to assess clinical efficacy. The test group received cupping therapy along with a Unani formulation. The other group (control) received the Unani formulation only. The test group demonstrated highly significant improvements in evaluated parameters when compared with baseline values. Statistically significant differences were observed in KOOS total score and its sub scores (P<.001), VAS (P<.001) at the 6th week when compared with the control group. The Cupping therapy seems to be an effective treatment for reducing pain and other symptoms of knee osteoarthritis and improving physical function with no major adverse effects.

Keywords: Cupping therapy, Osteoarthritis, Hijamah, Unani medicine, Waja-ul mafasil.

Introduction

The word “hijama” is derived from “hajm” which means “sucking” (Ibn Manzur,YNM; Ahmad, 2006; Nayab, 2011). Hijaamah (Cupping Therapy) is the process of applying cups to various points on the body by removing the air inside the cups to form a vacuum (negative pressure) in order to treat certain diseases (Kamaluddin,2004; Ahmad, 2006). Cupping (hijama) has been practiced for over thousands of years and can be traced back to the ancient Egyptians, Babylonians and ancient Chinese civilizations. Cupping is an ancient mode of therapy for various ailments, practiced and recommended by ancient healers (Azam, 2007). Cupping therapy is a widely employed mode of treatment; classified in alternative

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medicine and gaining popularity worldwide. Physicians are practicing it and hundreds of patients of various diseases have been reported to be benefited from cupping therapy. It has some religious roots too (Ahmad, 2006). It was the most recommended medical remedy by the Messenger (sallallaahu alayhi Wasallam) who said, "Indeed the best of remedies that you have is cupping" (Bukhari) (Husaini, 2003; Ehsanullah, 2006). The Chinese expanded the use of the cupping technique to surgery. Other ancient cultures including the Egyptians and early Greeks are all embraced the therapeutic value of cupping. Hippocrates (400 B.C.) used cupping for internal disease and structural problems (alhijamah.com). Famous Unani scholars like Rhazes, Avicenna, Galen, Jurjani, Allama Kabeer-Uddin, Ibn-e-Habal Baghdadi practiced cupping therapy and mentioned this important treatment modality in their books. Rhaze quotes in his book Al-Hawai-al-Kabeer, "In the treatment of hip joint arthritis, when humors are thick and difficult to evacuate, the use of mahjama is advised and it is very beneficial" (shah, 1892). The Cupping technique soon spread throughout Asian and European civilizations. Each country is having their own name for cupping therapy and having their own methods of cupping (History-of-cupping; alhijamah.com). Presently, cupping therapy has been claimed to treat various disorders successfully, such as carpel tunnel syndrome, nonspecific low back pain, sciatica, arthritis, digestive, respiratory, skin diseases and menstrual disorders (Anjum, 2003; Alam, 2011). This therapy reduces inflammation, pain and stiffness and hence improves the joint function in diseases like osteoarthritis (OA). There is lack of scientific evidence of efficacy of cupping; hence this study was aimed to evaluate the significance of this unique technique.

Osteoarthritis (OA) refers to a clinical syndrome of joint pain accompanied by varying degrees of functional limitation and reduced quality of life (Louis, 2010). It is by far the most common form of arthritis and one of the leading cause of pain and disability worldwide (Royal College of physicians, 2008; Johanne, 2011). It was previously thought to be a normal consequence of aging, thereby leading to the term degenerative joint disease. Now it is realized that osteoarthritis results from a complex interplay of multiple factors, including joint integrity, genetics, local inflammation, mechanical forces, cellular and biochemical processes (Neuprez, 2007); Anjum, 2003; Rehman, 2009). The subcommittee on Osteoarthritis of the American college of Rheumatology Diagnostic and therapeutic criteria committee defined OA as "A heterogeneous group of condition that leads to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins" (Anonymous, 2000). Clinically the condition is characterized by pain, tenderness, crepitus, limited movements, and occasionally effusion and variable degree of local inflammation (Wall, 1994; Altman, 1986; Issel, 2001).

In literature of Unani medicine, osteoarthritis (OA) is not mentioned as such, instead it is described under the broad entity of Waja-ul-Mafasil which includes the entire joint disorders. Observations suggest that Waja-ul-Mafasil barid has obvious resemblance to osteoarthritis in such a way that the signs and symptoms of balghami and saudavi type of Waja-ul-Mafasil (Waja-ul-Mafasil barid) have more similar features with OA (Nayab, 2011; Faris, 2010; Shiffa *et al.*, 2013).

Unfortunately, there is no cure for osteoarthritis, although it may be possible to reduce cartilage loss and slow the progression of the disease (Faris, 2010). The major goals of treatment are pain control with minimal adverse effects, maintenance or improvement of joint mobility and function and improved health related quality of life. Treatment should be personalized to individual. A non-pharmacological intervention including physiotherapy, occupational therapy, weight loss and exercise can be used to alleviate the symptoms associated with osteoarthritis. These are often used in combination with pharmacological interventions. But the symptomatic treatment often fails to provide satisfactory relief. Furthermore in modern medicine, Non- Steroidal Anti-Inflammatory Drugs (NSAIDS) are the main stay of treatment of OA. Nevertheless, these NSAID have many adverse effects like gastric ulceration, gastro-intestinal bleeding and perforations (Shiffa *et al.*, 2013). Considering the large number of people suffering from OA, limitations in conventional medical management and the known adverse effects associated with NSAIDS and Glucocorticoids use, indicate a real need for safe and effective treatment of arthritis patients, for which unani medicine is the best answer because they have been used successfully on humans without any reported major adverse effects over centuries. These challenges drive us to explore alternative modes of treatment having the least or no side effects for this painful condition.

Material and Methods

This study was a randomized, parallel group, comparative trial carried out at Aligarh UP. The protocol was approved by Institutional ethical committee for clinical trials in Unani drugs of Dept of Moalejat, A.K. Tibbiya College, AMU, Aligarh. Patients were enrolled from Unani OPD_s in AKTC AMU Aligarh. Each participant was informed about the trial. They were further given a description of anticipated risks and discomforts. Then informed written consent was obtained from each participant in the prescribed format prior to performance of the study related procedures (i.e. Physical examination, laboratory screening and other investigational procedures) and before administration of any study related medication. The study included individuals aged >40 but <70 yrs, either sex, fulfilling the following criteria.

Inclusion criteria were as follows: diagnosed with OA of the knee of at least 6 months duration fulfilling American College of Rheumatology criteria; knee pain VAS- pain after walking (50 feet) in a flat surface >30 mm and < 90 mm, Kellgren-Lawrence Radiographic Grading Scale of Osteoarthritis; patients who were willing to discontinue all NSAIDs or other analgesic medication taken for any condition; patients who had given their written informed consent & agreed to follow the protocol voluntarily were included.

Exclusion criteria were as follows: Pregnancy and Lactation; patients who were on steroid drug therapy; history of surgery of the joint involved, tidal lavage or arthroscopy of either knee within the past 12 months; hypersensitivity/ allergy to food &/ drug; intra-articular (IA) corticosteroid injection of either knee; acute medical or surgical conditions which could affect the outcome of the study such as cardiac, renal, hepatic diseases. Ongoing use of prohibited medication including NSAID, other oral analgesic, muscle relaxant, or low-dose antidepressant for any chronic pain management; history of alcohol or drug abuse, excessive smoking (more than 10 cigarettes/day); established/ diagnosed neurological or psychiatric disorders and those who were not willing to be randomized are also excluded from the study.

Allocation of patients to study group

The total of 40 patients were randomly allocated to test and control groups containing 20 patients in each.

Interventions

The test group received cupping therapy (4 cups two in medial side and other 2 in lateral side of the knee joint around the patella of both knees, every weeks for four weeks along with a Unani formulation i.e. safoof (powder), 6 gm twice daily for 42 days. While the control group received the same Unani formulation alone in same dose for same period.

Unani formulations: It has the combination of seeds of four plants i.e Methi (*Trigonella foenum-graecum*), Haloon (*Lepidium sativum*), Kalonji (*Nigella sativa*) and Ajwain desi (*Trachyspermum ammi*) in equal quantity. All drugs were procured from Dawakhana Tibbiya College, Aligarh Muslim University Aligarh and after proper identification of the drugs were cleaned from all impurities and a safoof was prepared in pharmacy section of Ajmal Khan Tibbiya college Hospital.

Application procedure

The Cupping was performed weekly on the affected joints for 20 minutes; clinical sign, symptoms and relief were assessed on each visit. Basic cupping therapy

equipment was utilized including a hand suction pump, plastic cups set .The transparent plastic cup with a capacity of 200 ml was used four cups were applied on the knee on each side. During cupping, the skin was sucked up to the level of ¼ to ½ cups. The place where the Cupping was to be applied was cleaned of hairs and draped properly. Presence of hairs may cause leaking of air into the cup and loosening of the cup grip. The cups are placed on the skin that has previously been oiled and then moved along the meridians back and forth. up and down the main meridian until skin becomes red The back and forth movement promotes circulation, after that cups are placed and remain in place as long as the congestion is visible (indicated by reddening of the skin).

Outcome measures

Outcome measures were Visual Analogue Scale (VAS), Knee injury and Osteoarthritis Outcome Scores (KOOS), Active Range of Motion (AROM), 15 meter walking time and Kellegran-Lawrence radiographic grading scale.

VAS is a straight horizontal line of fixed length, usually 100 mm. The ends are defined as the extreme limits of the parameter to be measured (symptom, pain, health) orientated from the left (worst) to the right (best).

The Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire is an extension of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the most commonly used outcome instrument for assessment of patient-relevant treatment effects in osteoarthritis. It is intended to be used over short- and long-term time intervals; to assess changes from week to week induced by treatment (medication, operation, physical therapy) or over years following a primary injury or OA.KOOS consists of 5 subscales; Pain, other Symptoms, Activities of Daily Living (ADL), Sport and Recreation Function (Sport/Rec) and knee-related Quality of Life (QOL). KOOS has been used in patients 13-79 years of age. KOOS includes WOMAC Osteoarthritis Index LK 3.0 in its complete and original format. KOOS takes 10 minutes to complete. It uses simple language and similar one-word responses for each item.

Range of motion (ROM) is a description of how much movement exists at a joint. ROM was measured before and after the treatment, by Universal Goniometer. The subjects were positioned prone with Knee suitably stabilized and active range of motion was taken with the Universal Gonimeter. The stationary arm holding the protractor was placed parallel with a stationary body segment (pointing towards greater trochentar) and the moveable arm moves along a moveable body segment (pointing towards the medial malleolus). The final ranges were recorded.

Patients were asked to walk across 15-m distance at their natural speed. Three readings were taken and the mean was calculated and recorded.

Laboratory investigations were performed before treatment (at baseline) and after treatment (after 42 day), which included Hematological assessment; TLC, DLC, ESR, Hb %, LFT, KFT.

Results were analyzed by using Statistical analysis was done according to the type of data; paired-t-test was applied to evaluate the paired data within the group; Unpaired-t-test test was applied to find statistical difference between the groups. The analysis of the observational data was performed and presented in the form of graphs and tables by using *Graph Pad instat 3* and *Microsoft® Excel (2007)* software.

Results

Total 40 patients completed the study, 20 in each group.32 patients were female while 08 patients were male. Mean age of participants was 47.8 ± 8.3 years in control group and 48.75 ± 7.4 years in test group. Mean BMI of participants was 28.2 ± 3.3Kg / m^2 in group control group, while in group test it was 27.9 ± 3.3 Kg / m^2. Differences of baseline characters between two groups were not statistically significant (Table 1).

Table 1: Baseline characteristics of study patient

Variables	Test group (n = 20)	Control group (n = 20)
Age (Years)	48.75 ± 7.4	47.8 ± 8.3
Male	12	04
Female	08	16
BMI (Kg/ m^2)	27.9 ± 3.3	28.2 ± 3.3
KOOS Pain score	37.7 ± 7.8	36.5 ± 10.1
KOOS Symptoms score	42.05 ± 11.2	44.7 ± 14.0
KOOS ADL score	38.55 ± 14.3	37.3 ± 12.1
KOOS Sports / Rec. score	22.85 ± 9.9	24.5 ± 12.4
KOOS Quality of Life (QOL)	35.85 ± 11.9	36.6 ± 13.7
KOOS Total score	35.0 ± 10.4	35.9 ± 12.2
AROM Right Knee joint	120.3 ± 10.4	121.7 ± 9.9
AROM Left Knee joint	119.5 ± 11.0	121.30 ± 9.7
Visual Analogue Scale (VAS)	66.0 ± 9.9	65.5 ± 10.2
Walking time (s)	25.9 ± 4.2	24.9 ± 2.7
K-L grading scale	1.8 ± 0.8	1.9 ± 0.8

Values are expressed in means ± SEM

Test group had extremely significant improvement in KOOS pain score, symptoms score, activities of daily living, sport/recreational score, quality of life score, and KOOS total score at 3rd week and 6th week. Visual Analogue Scale also showed a highly statistically significant improvement in the test group at 3rd week and 6th week. Statistically significant improvement was observed in AROM in right and left knee joints at 3rd week and 6th week. Walking time was also improved significantly. These parameters were compared with baseline values and the values are represented in (Table 2) with their standard Error of Mean (SEM).

In control group, statistically significant improvement was observed at 3rd week and 6th week in KOOS pain, score, KOOS symptoms score, KOOS ADL, KOOS QOL, KOOS total score, AROM, VAS, walking time, when comparing with base line findings (Table 3).

There were statistically significant improvements found in KOOS pain, score, KOOS symptoms score, KOOS ADL, KOOS sports/rec, KOOS QOL, KOOS total score, AROM, VAS, walking time in test group when compared with control group (Table 4)

Table 2: Outcome measures for the test group before treatment (BT), 3rd week, after treatment (AT).

	BL	3rd week	6th week
KOOS Pain Score	37.8±7.8	58.3±9.8	74.1±9.6
KOOS Symptom Score	42.1±11.2	58.6±9.1	76.15±9.4
KOOS ADL Score	38.55±14.26	57.1±9.8	75.80±8.00
KOOS Sports / Rec. Score	22.9±9.9	40.5±9.0	54.7±13.02
KOOS QOL Score	35.85±11.87	55.5±8.9	76.65±9.29
KOOS Total Score	35.00±10.36	53.2±7.9	71.50±6.10
AROM of Right Knee	120.3±9.8	—	124.9±10.52
AROM of Left Knee	119.5±11.0	—	124.0±11.2
VAS Score	66.0±9.97	—	30.75±7.59
Walking Time	25.9±4.22	—	21.3±3.40
Kellgren–Lawrence (K-L) Radiographic Grading Scale	1.8 ± 0.8	—	1.8 ± 0.8

Values are expressed in means ± SEM

Table 3: Outcome measures for the control group before treatment (BT), 3rd week, after treatment (AT)

	BT	3rd week	AT
KOOS Pain Score	36.5±10.1	45.8±10.4	54.6±11.3
KOOS Symptom Score	44.7±14.0	48.8±12.0	55.6±12.8
KOOS ADL Score	37.3±12.07	46.1±10.9	55.4±12.12
KOOS Sports/Rec. Score	24.5±12.4	29.8±11.1	34.7±12.2
KOOS QOL Score	36.55±13.68	41.5±10.4	50.4±11.44
KOOS Total Score	35.90±12.15	43.2±11.8	50.50±10.14
AROM of Right Knee	121.7±9.9	-	123.6±9.8
AROM of Left Knee	121.30±9.7	-	123.1±9.8
VAS Score	65.5±10.22	-	53.45±13.55
Walking Time	24.9±2.7	-	22.5±2.96
Kellgren–Lawrence (K-L) Radiographic Grading Scale	1.9±0.8	-	1.9±0.8

Values are expressed in means ± SEM

Laboratory Investigations

There was no statistically significant difference in ESR count was noticed before and after treatment in test group (mean value and SEM at baseline 23.1 ± 6.2; after treatment 23.6 ± 5.2) and Control group (mean value and SEM at baseline 23.5 ± 5.03; after treatment 23.3 ± 4.9), P >0.05. There was no change in the number of CRP in both the groups throughout the therapy. X-ray was performed and it showed no significant changes.

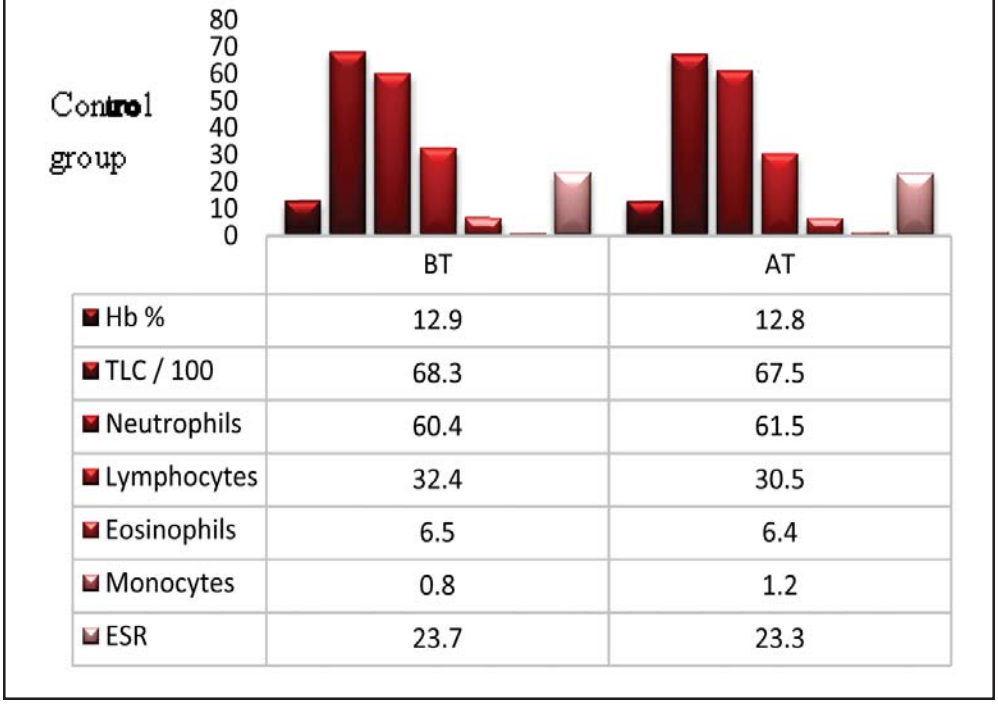
Laboratory investigations were performed before and after the treatment (42 days), which include haematology, liver function test (LFT), kidney function test (KFT).

These laboratory parameters were taken to evaluate the safety of the treatment. Hematological assessment such as Hb%, TLC, Neutrophils, Lymphocytes, Eeosinophils ,Monocytes were not changed significantly when compared both, before and after treatment (Fig. 1&2).

There was no statistically significant change in Liver function test (LFT) Total bilirubin, SGOT, SGPT and Alkaline Phosphatase in the subjects before and after the treatment and between the two groups (Fig 3&4).

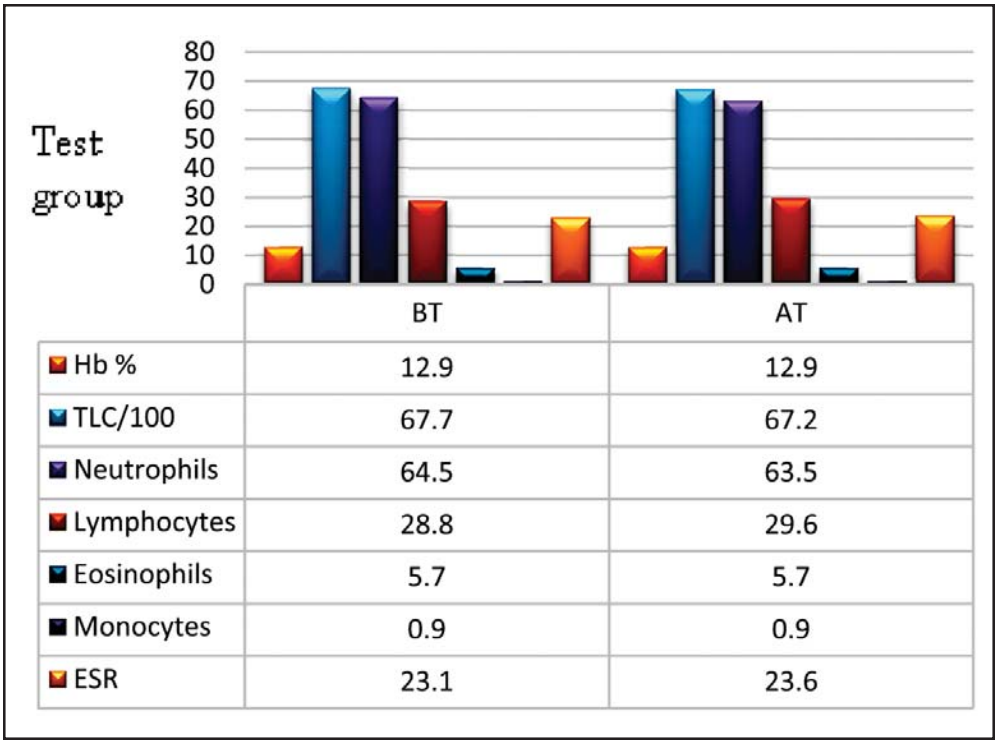
End point	Test group C			Control group A			P value Group A Vs Group C
		% age of Change			% age of Change		
KOOS Pain Score							t =7.0
Baseline (BL)							P<.001
3rd week	37.8±7.8	NC	t = -17.8	36.5± 10.1	NC	t = -11.8	
6th week	58.3±9.8	20.5	p = 0.000	45.8±10.4	9.2	p = 0.000	
	74.1±9.6	36.3		54.6±11.3	18.1		
KOOS Symptom Score							t =8.7
							P<.001
Baseline (BL)	42.1±11.2	NC	t = 14.98	44.7±14.0	NC	t = 8.114	
3rd week	58.6±9.1	16.5	p = 0.000	48.8±12.0	4.1	p = 0.000	
6th week	76.15±9.4	34.1		55.6±12.8	10.9		
KOOS ADL Score							t =8.7
Baseline (BL)							P<.001
3rd week	38.55±14.26	NC	t = 16.157	37.3±12.07	NC	t = 10.708	
6th week	57.1±9.8	18.6	p = 0.000	46.1±10.9	8.8	p = 0.000	
	75.80±8.00	37.3		55.4±12.12	18.1		
KOOS Sports/ Rec. Score							t = 7.8
							P<.001
Baseline (BL)	22.9±9.9	NC	t = -12.6	24.5±12.4	NC	t = - 9.7	
3rd week	40.5±9.0	17.6	p= 0.000	29.8±11.1	5.3	p = 0.000	
6th week	54.7±13.02	31.8		34.7±12.2	10.2		
KOOS QOL Score							t = 7.8
Baseline (BL)							P < 0.001
3rd week	35.85±11.87	NC	t = -16.251	36.55±13.68	NC	t = - 6.012	
6th week	55.5±8.9	19.7	p= 0.000	41.5±10.4	5.0	p = 0.000	
	76.65±9.29	40.8		50.4±11.44	13.9		
KOOS Total Score							t = 13.1
Baseline (BL)							P < 0.001
3rd week	35.00±10.36	NC	t = -27.717	35.90±12.15	NC	t = - 14.701	
6th week	53.2±7.9	18.2	p= 0.000	43.2±11.8	7.3	p= 0.000	
	71.50±6.10	36.5		50.50±10.14	14.6		
VAS Score			t = 16.073			t = 8.923	t = 8.9
Baseline (BL)	66.0±9.97	NC	p = 0.000	65.5±10.22	NC	p = 0.000	P < 0.001
3rd week	—			—			
6th week	30.75±7.59	35.3		53.45±13.55	12.1		

! Not Significant (P > 0.05) *Significant (P < 0.05) **Very Significant (P < 0.001)



	BT	AT
Hb %	12.9	12.8
TLC / 100	68.3	67.5
Neutrophils	60.4	61.5
Lymphocytes	32.4	30.5
Eosinophils	6.5	6.4
Monocytes	0.8	1.2
ESR	23.7	23.3

Figure 1: Effect on CBC (Hb %, TLC, Neutrophils, Lymphocytes, Eosinophils, Monocytes, ESR), before and after treatment in control group



	BT	AT
Hb %	12.9	12.9
TLC/100	67.7	67.2
Neutrophils	64.5	63.5
Lymphocytes	28.8	29.6
Eosinophils	5.7	5.7
Monocytes	0.9	0.9
ESR	23.1	23.6

Figure 2: Effect on CBC (Hb %, TLC, Neutrophils, Lymphocytes, Eosinophils, Monocytes, ESR), before and after treatment in test group

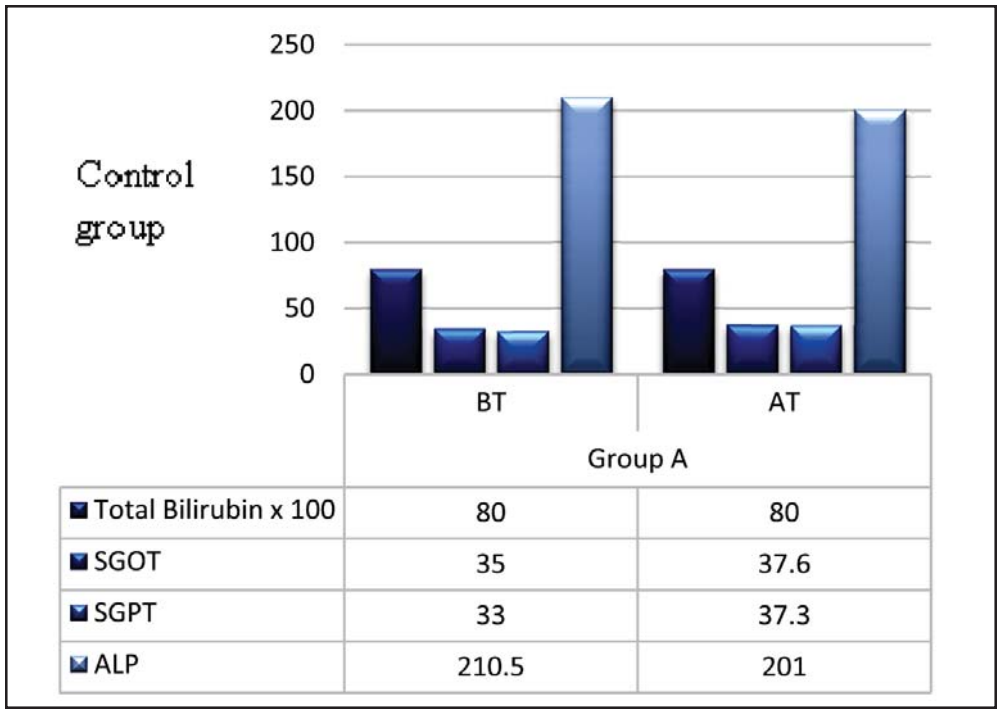


Figure 3: Effect on LFT, serum glutamine oxalo-acetic transaminase (SGOT), serum glutamine pyruvic transaminase (SGPT) and alkaline phosphatase ALP before and after treatment in control group.

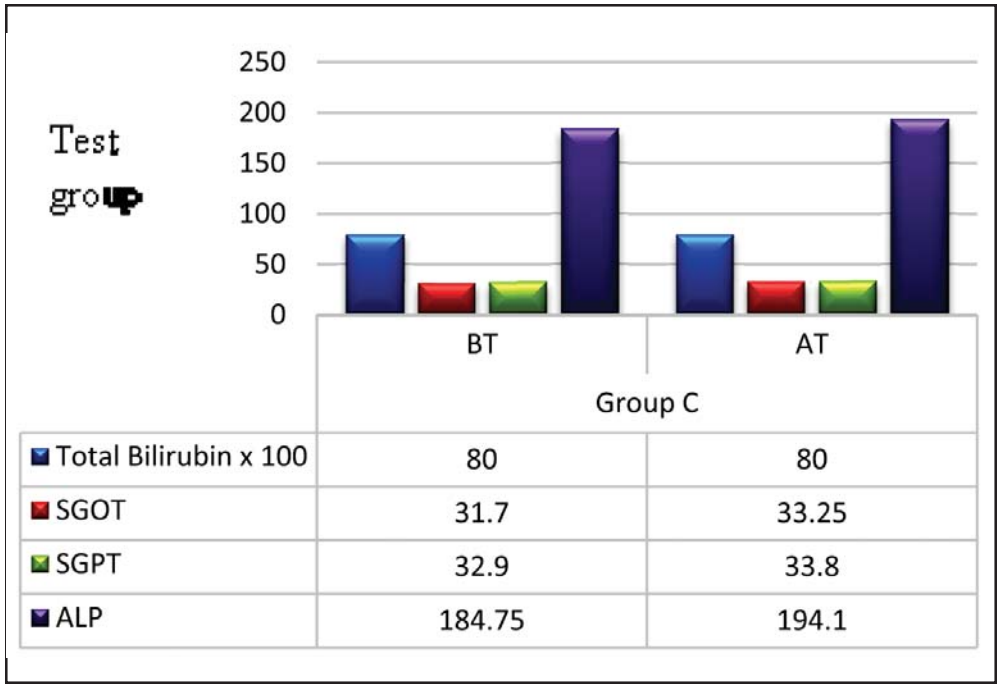


Figure 4: Effect on LFT, serum glutamine oxalo-acetic transaminase (SGOT), serum glutamine pyruvic transaminase (SGPT) and alkaline phosphatase ALP before and after treatment in test group.

In addition there was no statistically significant difference in kidney function test (KFT) such as serum Creatinine Uric Acid and Blood urea, in both groups as well as in between the groups at the end of treatment (Fig 5&6).

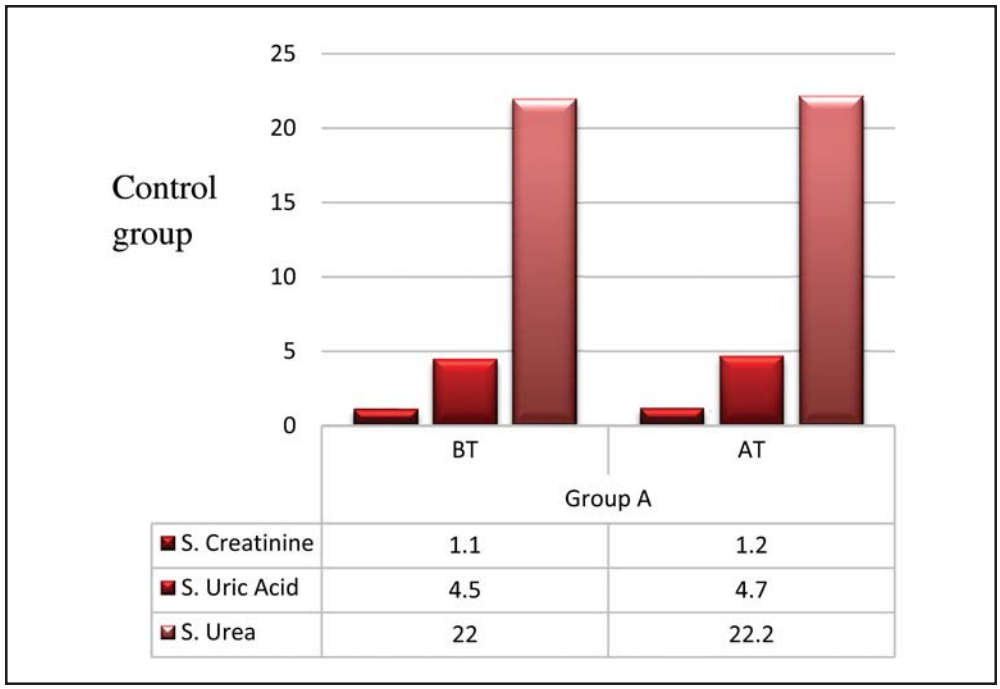


Figure 5: Effect on KFT (s.creatinine serum uric acid, and urea) before and after treatment in control group

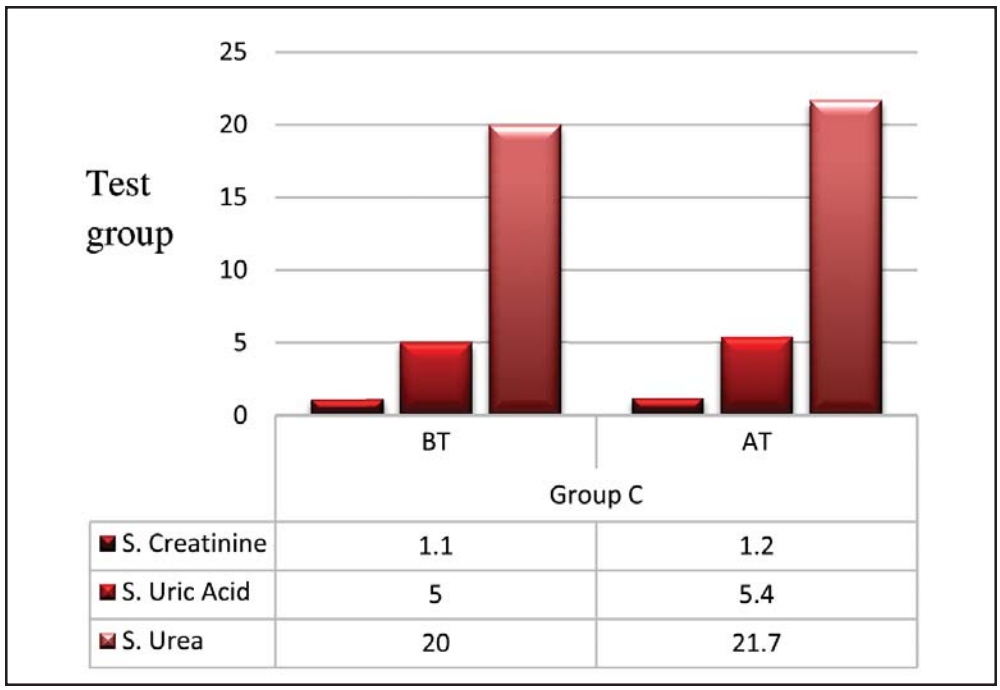


Figure 6: Effect on KFT(serum creatinine, serum uric acid, and urea) before and after treatment test group

Discussion

Osteoarthritis (OA) is a chronic disorder of synovial joints in which there is progressive softening and disintegration of articular cartilage and bone at the joint margins (osteophytes), cyst formation and sclerosis in the subchondral bone, mild synovitis and capsular fibrosis (Kelley, 1992; Issel, 2001; Anonymous, 1986). OA is the most common type of musculoskeletal disorder, and the fourth leading cause of the economic burden on healthcare (Johanne, 2011). It is a threat to the physical, psychological, social and economic well being of human beings. It often deprives people of their freedom and independence. With the advancement in medical science and health awareness schemes, the mortality rate has declined but the prevalence rate is still high, due to unavailability of absolute treatment (Shiffa *et al.*, 2013).

Unfortunately, there is currently no cure for osteoarthritis; the available treatment produces severe adverse effects on the long term use. The major goal of treatment is to reduce cartilage loss and slow the progression of the condition and minimize pain and other symptoms (Brandt K, 1996), in addition to that the treatment should be tolerable when used for longer period with less adverse effects and toxicity. Treatment for OA focuses on relieving pain, improvement of joint mobility and function, and improved health related quality of life and can include pharmacological and non pharmacological interventions including physiotherapy, occupational therapy, weight loss, and exercise (Felson, 2000; Issel, 2001).

According to the concept of Unani system of medicine diseases are either due to humoral discordance or superfluous humors inside the body. The humours which are in disproportion gets collected in various parts of the body and results in abnormal functioning or diseases in that specific part (Ibn-e-Sina, 1932).. Various eminent scholars as Allama nafees, Ibn-e-sinha, Hakeem Akbar Arzanj, Ismail Jurjani have described that the saba-e-faaili (active cause) of waja-ul-mafasil is su-e-mizaj maddi and the most commonly predominating khilt is Balgham (Jurjani, 1878). Observations suggest that waja-ul-mafasil barid has obvious resemblance to osteoarthritis in such a way that the signs and symptoms of Balghami and saudavi type of waja-ul-mafasil (barid) have more similar features with OA (Nayab, 2011; Faris, 2010; Shiffa *et al.*, 2013). Hkm akbar arzani and Ismail jurjani have described that if any patients of waja-ul-mafasil does not responds to any therapy then Mahajam nari should be induced which causes to pull out the causative matter from innermost areas and it is an important therapy for pain relief (Azam, 2007). Considering its vital role and successful use in Unani medicine, the present study was designed and conducted to rationalize this idea scientifically.

In this randomized, controlled trial, patients who were in the test group experienced clinically significant improvement in most of the evaluated parameters. They had decreased perception in most of the evaluated parameters. They had decreased perception in pain and other symptoms of osteoarthritis. Furthermore, they experienced improved functional ability and day to day performance. In osteoarthritis, pain is the earliest and leading symptom for which patient frequently visits a physician. In this study extremely significant improvement was found in KOOS pain score 20.5% at 3rd week 36.3% at 6th week in test group, while in control group there was 9 .2% improvement at 3rd week 18.1% at 6th week when compared with baseline. This shows pain relief more in test group. The same kind of observations were recorded in KOOS symptom score, KOOS ADL score, KOOS Sports/Recreational activity score, KOOS QOL score, KOOS Total score, Pain and other symptoms of osteoarthritis are interconnected with each other. Whenever pain is relieved, than it leads to relieve other symptoms like morning stiffness, swelling, tenderness, etc. Physical function is attributed mainly to the reduction in pain, as it is the chief symptom, which produces other complications.

The improvement in other outcome measures test group showed extremely statistically significant improvement in active range of motion before and after treatment. Similarly walking time also improved significantly in test group. These improvements could also be due to decrease of pain and inflammation.

In test group and control group no significant improvement was observed in ESR and Arthritic profiles when comparing both groups at the end of treatment. Pre and post treatment X-ray were performed and showed on significant change, probably due to short duration of therapy. It is important to mention that the main limitation of this study was the placebo effects of cupping therapy could not be ruled out.

As far as the safety of the therapy was concerned, hematological and biochemical parameters were evaluated before and after the therapy. During the whole therapy period no significant change was seen in Hb %, TLC, Neutrophils, Lymphocytes, Eeosinophils ,Monocytes and ESR, KFT, LFT.

Conclusion

There was statistically significant improvement observed in reduction of pain, other symptoms, and physical functions during treatment and even after treatment. Therefore, Hijaamah (Cupping Therapy) seems to be an effective treatment for reducing pain and other symptoms of knee osteoarthritis and restoring the physical functions, moreover the therapy was found to be safe and well tolerated.

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The authors are thankful to the Aligarh Muslim University, Aligarh for providing necessary facilities to conduct the research and also to Dr. Ahmer Javed Siddiqui, Orthopedics and Dr. Shahnawaz, Physiotherapist, J.N. Medical College & Hospital, AMU, Aligarh for their valuable suggestions, guidance and cooperation throughout the study.

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Physico-chemical Standardization of Kanduri Root (*Coccinia cordifolia* Linn.)

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Abstract

Kanduri (*Coccinia cordifolia* Linn.) (root) is one of the important herbs mentioned in Unani literatures. Hkm Azam Khan (1893AD) has described it for the treatment of renal diseases whereas Hkm Najmul Ghani (2011 AD) has mentioned its efficacy in kasrat-e-baul (polyuria) along with other diseases. In present study, an attempt is being made to work on standardization and quality assurance of Kanduri root. Various parameters have been used to ensure its quality. These parameters include Ash value (total ash, acid insoluble ash, water soluble ash), Extractive values (successive), Solubility in alcohol and water, Loss on drying, pH at 1% & 10%, Bulk density. Qualitative tests have also been used to determine the presence of phytochemicals in the drug studied.

Keywords: Kanduri *(Coccinia cordifolia* Linn.), Standardization, Ash value and Physicochemical.

Introduction

Kanduri (*Coccinia cordifolia* Linn.) belongs to the family Cucurbitaceae, is a perennial creeping herb with long tapering tuberous roots and deep green leaves (Fig. 1 & 2). It grows in a wild state abundantly in Bengal and in most parts of India, Tropical Africa, Australia, Fiji and throughtout the oriental countries (Khatoon *et al*., 2012). It has a smooth green fleshy fruit with an extremely bitter taste, when ripe the fruit becomes scarlet in colour and sweet to the taste and is occasionally eaten as a vegetable (Ghani, 2010). The plant has the reputation in Bengal of having a remarkable effect in reducing the amount of sugar in the urine of patient suffering from Diabetes mellitus(Chopra, 1958; Anonymus, 2001). The plant has also been used extensively in Ayurvedic and Unani practice. It is one of the constituents of many pharmacopoeial preparations. Though the entire plant has medicinal value however, its roots and leaves are more commonly used as theraputic agent in different pathological conditions. However, inspite of being used commonly by the physicians of traditional medicine in Indian subcontinent and other countries, this plant has not been standardized so far. In view of the above, the present study has been undertaken to determine its physicochemical and some of the qualitative standards.

Material and Method

The raw material was collected from Naqwi Park, Aligarh besides Ajmal Khan Tibbiya College Hospital in the month of March and the sample was authenticated

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Fig. 1: Plant of Kanduri (*Coccinia cordifolia*)

Fig. 2: Roots of Kanduri (*C. cordifolia*)

in Pharmacognosy section of department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh. Voucher specimen was preserved in the herbarium of department (Voucher No. SC-0168/15) for future reference

Chemical Parameters: First the organoleptic characters were studied. The dried powder of Kanduri roots was used for chemical analysis. Various physico-chemicals studies including total ash, acid insoluble ash, water soluble ash, alcohol and water soluble matter, moisture content, successive extractive values using soxhlet extraction method, bulk density and pH studies were carried out as per guidelines of WHO (Anonymus, 1998). Qualitative analysis of the drug was conducted to identify the organic chemical constituents present in the drug (Overtone, 1963; Harbrne, 1973).

The Thin Layer Chromatographic analysis was conducted according to the method of Stahl (1969) and Harborne (1973) on precoated silica gel 60F $_{254}$ TLC plates. The plates were visualised in day light, UV Short and UV Long and they were also derivatised using iodine vapour.

Observations and Results

(a) Organoleptic characters: The organoleptic characters of powder of the root of Kanduri are depicted in Table 1.

Table 1: Organoleptic Characters of *Coccinia cordifolia* root

S.No.	Organoleptic characters	
1.	Colour	Light brown
2.	Appearance	Powder
3.	Texture	Coarse
4.	Taste	Astringent
5.	Smell	Agreeable

(b) Physicochemical constants: The analytical values of different physicochemical constants have been described in Table 2.

(c) Qualitative analysis of organic chemical constituents of drug: The phytochemicals present in the drug were identified on the basis of different chemical tests done for various plant constituents (Table 3).

(d) FTAR Analysis: Fluorescence analysis of successive extract was studied under day light as well as Ultra Violet (Short and long wave length) light; results have been summarized in Table-4. FTAR analysis of the powder drug was also done after allowing it to react with various chemical reagents (Table 5).

Table 2: Physicochemical study of powder of Kanduri root

S.No.	Parameters	Percentage (w/w)
1.	Ash value	
	Total ash	9.78
	Acid insoluble ash	1.48
	Water soluble ash	7.95
2	Soluble Part	
	Ethanol soluble	6.10
	Aqueous soluble	20.7
3	Successive Extractive Values	
	Pet. Ether	0.48
	Di-ethyl ether	0.18
	Chloroform	0.33
	Acetone	0.51
	Alcohol	2.82
	Aqueous	9.12
4	Moisture Content	15
5	Loss on Drying	8.5
6	pH Value	
	1% water solution	7.19
	10% water solution	6.49
7	Bulk density	0.66

*Note: Values are average of five experiments.

Table 3: Preliminary screening of major phytochemicals

S.No.	Chemical Constituent	Tests/Reagent	Inference
1	Alkaloids	Dragendrorff's reagent Wagner's reagent Mayer's reagent	+ + –
2	Carbohydrate	Molisch's Test Fehling's Test Benedict Test	+ + +
3	Glycosides	NaOH Test	+
4	Flavonoids	Mg ribbon Dil. Hcl	+
5	Tannins/Phenols	Ferric Chloride Test Liebermann's Test Lead Acetate Test	– – –
6	Proteins	Xanthoprotein Test Biurate Test	– –
7	Starch	Iodine Test	–
8	Saponins	Frothing With $NaHCO_3$	+
9	Steroid/Terpenes	Salkowski Reaction	+
10	Amino Acid	Ninhydrin Solution	–
11	Resin	Acetic Anhydride Test	–

Indications: '_' Absence and '+' presence of constituent.

Table 4: FTAR analysis of Kanduri Extract

S.No.	Extract	Day Light	UV Long	UV Short
1	Pet. Ether	Transparent	Dark Blue	Transparent
2.	Di- Ether	Transparent	Bluish	Light Green
3	Chloroform	Light Green	Light Blue	Transparent
4	Acetone	Grey	Violet	Greenish
5	Alcohol	Yellowish Brown	Black	Muddy Green
6	Aqueous	Dark Brown	Light Green	Greenish Brown

(e) Thin layer chromatographic profile: Thin layer chromatographic analysis of successive extract was carried out using different solvent systems and visualizing agents and R_f values were calculated. The findings have been summarized in Table 6 and Fig. 3 & 4.

Table 5: Fluorescence analysis of Kanduri with different chemical reagent

S.No.	Powder drug + Chemical Reagent	Day light	UV Short	UV Long
1.	Powdered drug + Conc. HNO_3	Brown	Dark Green	Black
2.	Powdered drug + Conc.Hcl	Grey	Dark Green	Black
3.	Powdered drug + Conc.H_2SO_4	Brown	Green	Redish Black
4.	Powdered drug + 2 % Iodine solution	Red	Green	Black
5.	Powdered drug + Galcial Acetic Acid +HNO_3	Brown	Light green	Green
6.	Powdered drug + Galcial Acetic Acid	Pale	Brown	Black
7.	Powdered drug +NaOH (10%)	Light Brown	Green	Light Green
8.	Powdered drug + Dil. HNO_3	Brown	Green	Green
9.	Powdered drug + Dil. H_2SO_4	Brown	Green	Black
10.	Powdered drug +Dil. Hcl	Light Brown	Green	Cherry Red
11.	Powdered drug + Dragendorff's	Greenish. B	Dark Green	Black
12.	Powdered drug + Wagner's Reagent	Grey	Green	Black
13.	Powdered drug + Benedict' Reagent	Whitish Green	Light Grey	Grey
14.	Powdered drug + Fehling Reagent	Brown	Light Green	Green
15.	Powdered drug + KOH(10%) Methno	Dark Brown	Light Green	Brown
16.	Powdered drug + $CuSO_4$ (5%)	Whitish Brown	Green	Cherry Red
17.	Powdered drug +Ninhydrin (2%) in Acetone	Brown	Green	Grey
18.	Powdered drug + Picric Acid	Yellow	Green	Black
19.	Powdered drug + Lead Acetate (5%)	White	Light Green	Dark Brown

Table 6: Thin Layer Chromatography Profile

Treatment	Mobile Phase	No of Spots	R_f Value and colour spots
Petroleum Ether Extract			
Iodine Vapour	Petroleum ether: Di-ethyl ether (2:1)	4	0.33 (yellow Brown,
			0.38 (Mustard Yellow),
			0.55 (Light Yellow),
			0.61 (Light Yellow),
UV Long		4	0.33 (Sky Blue), 0.44 (Blue),
			0.57 (Dark Blue), 0.65 (Dark Blue)
Di-ethyl Ether Extract			
Iodine Vapour	Petroleum ether: Di-ether ether (2:1)	1	0.12 (Yellow)
Chloroform Extract			
Iodine Vapour	Chloroform: Methanol (1:1)	3	0.22 (Yellow. Brown)
			0.62 (Mustard Yellow)
			0.85 (Light Yellow)
UV Short		3	0.22 (Yellow), 0.62 (Bluish),
			0.85 (Light Brown
UV Long		5	0.22 (Dark Blue), 0.30 (Dark Blue)
			0.33 (Blue), 0.62 (Sky Blue)
			0.85 (Sky Blue
Alcoholic Extract			
Day Light	Butanol: Acetic acid: Water (5: 1: 4)	2	0.33 (Dark Brown), 0.85 (Brown)
UV Long		2	0.33 (Sky Blue), 0.85 (Whitish Blue)
UV Short		3	0.30 (Green), 0.53 (Green), 0.84 (Green)
Iodine Vapour		2	0.26 (Yellow), 0.80 (Brown)
Aqueous Extract			
Day Light	Butanol: Acetic acid: Water (5: 1: 4)	1	0.30 (Light Brown)
UV Long		2	0.30 (Sky Blue), 0.92 (Sky Blue)
UV Short		2	0.30 (Green), 0.92 (Greenish Blue)
Iodine Vapour		2	0.30 (Brown), 0.92 (Yellow)

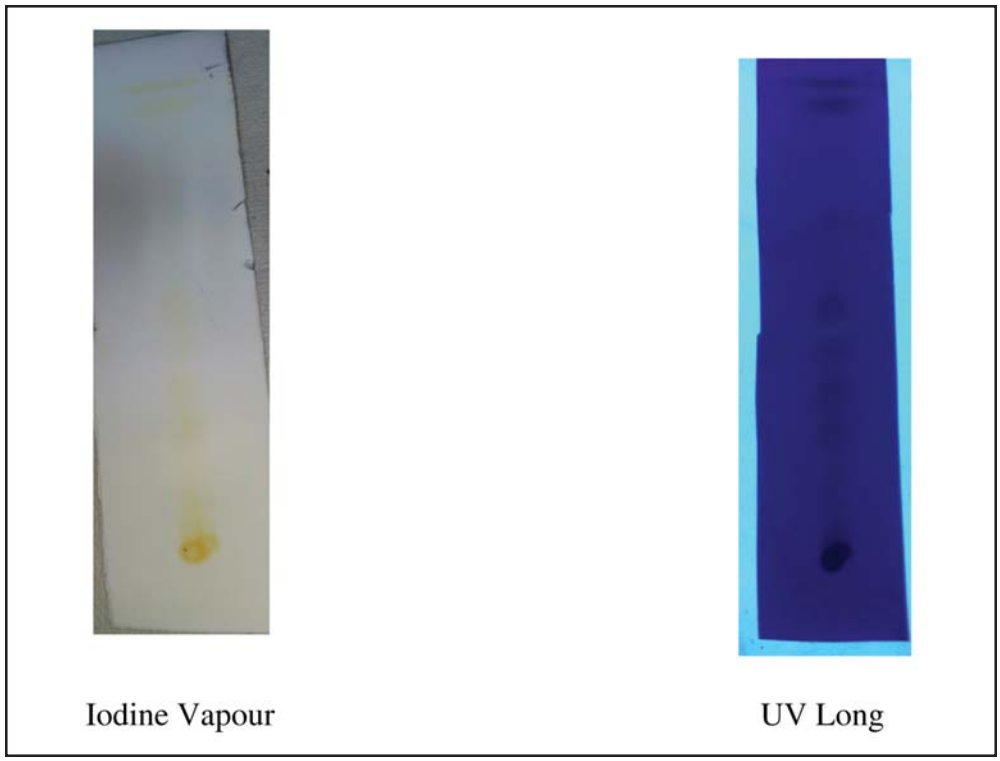

Fig. 3: TLC Profile of Petroleum ether extract of Kanduri Root
Petroleum ether: Diethylether:2:1

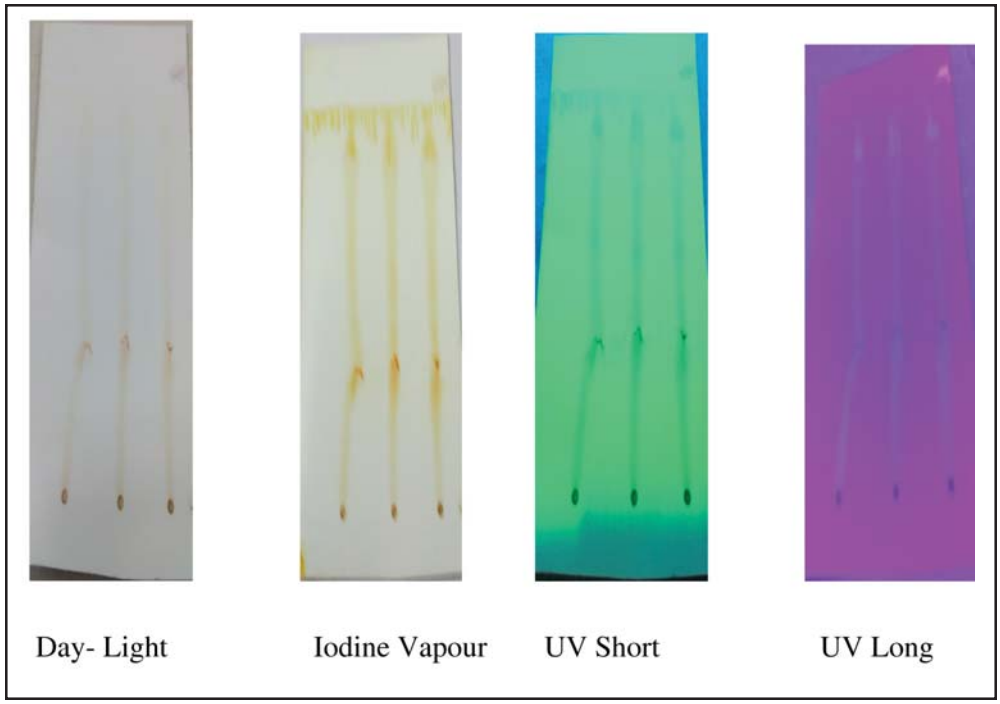

Fig. 4: TLC Profile of Alcohlic extract of Kanduri Root
Butanol: Acetic acid: Water; (5:1:4)

Discussion

Since the efficacy of a drug depends mainly upon its physical and chemical properties therefore, the determination of physicochemical characters is considered mandatory so as to ensure the authenticity of a drug. It also helps in determining the dose response relationship and thereby maximizing the therapeutic utility. Following parameters were used for the physicochemical study of Kanduri.

For establishing the standards of a drug the extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine sample (Jenkins *et al*., 1967).Percentage of solubility is also considered as an index of purity, as alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc.The ash value determination furnishes the basis of judging the identity and cleanliness of a drug and give information related to its adulteration with inorganic matter (Jenkins *et al.,* 1967).The moisture content of the drug is variable because mostly herbal drugs are hygroscopic and excessive moisture content becomes an ideal medium for the growth of different type of micro-organisms like bacteria and fungi they subsequently spoil the purity of drug.The pH provides a useful practical means for the quantitative indication of the acidity and alkalinity of a solution (Anonymous, 1968).Qualitative phytochemical analysis of Kanduri was also carried out for the determination of the presence of alkaloids, flavonoids, glycosides, tannins, phenols, resins, sterols/terpenes, sugars, starch, amino acid, proteins and saponins. The therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs, and the lower percentage of chemical constituents may cause lesser therapeutic value. Thin layer chromatography is one of the important parameters used to detect the adulteration for judging the quality of drugs. The resolution of different kinds of chemical components are separated by using TLC and calculating the R_f values after detecting the spots in order to standardize the drug for its identity, purity and strength. The exhausted or deteriorated drugs may lose the components and the number of spots appeared might be less. Keeping this in mind TLC studies of different extracts obtained in different organic solvents of the test drug have been conducted, and $R_f$values of various spots appeared in different solvents system have been noted.

Physicochemical study helps in characterization of constituents or groups of constituents that frequently lead to establish the structure-activity relationship and the likely mechanism of action of the drug. Physicochemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and

drying conditions. A little deviation from the normal in terms of quality and quantity of the constituents may alter the effect of the drug. Apart from the degradation in the quality of the drugs that occurs due to above conditions, adulteration also contributes to variability. The findings of the study may be used to set the physicochemical standards of a genuine sample of 'Kanduri.

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Therapeutics, Phytochemistry and Pharmacology of an Important Unani Drug Qurtum (*Catharanthus tinctorius* L.) : A Review

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Abstract

Unani drug *Qurtum* is comprised of the seeds of a plant *Carthamus tinctorius* Linn. It is one of the most ancient crops cultivated in Egypt as a dye yielding herb. Now it is cultivated as an oil seed plant and regarded as substitute for sunflower.The seeds are white, somewhat flat, angular, smooth and shining like little conch shells, broad at the base and pointed towards the apex. Fixed oil is obtained from the ripe and dry seeds. The plant has shown diverse biological and pharmacological activities. It has been used in Unani Medicine (*Tibb-e-Unani*) and other Traditional Systems of Medicine from time immemorial. Keeping in view the high medicinal importance of the drug in Unani Medicine, the present review provides available information on traditional uses, phytochemistry and pharmacological properties of the unani drug Qurtum.

Keywords: *Carthamus tinctorius*, Qurtum, Unani Medicine

Introduction

Qurtum is a famous Unani drug used in a number of pathological conditions. Although the entire plant has medicinal value but its seed, oil and flowers have more important and interesting medicinal values. Its different parts are used after little processing as a single drug but mostly it is included as an ingredient in Unani formulations. Botanicalyy known as *Carthamus tinctorius* Linn. (Family: Asteraceae). Qurtum is a slender, glabrous or pubescent, much branched, annual herb (Chatterjee & Pakrashi, 1997), growing to a height of 45-60 cm (tall varieties 85-150 cm) (Anonymous, 1992) (Fig 1). The leaves are broad, lanceolate, spinosely serrate (rarely unarmed) sub erect, oblong, sessile (Kirtikar & Basu, 1987; Khory & Katrak, 1985). Flowering takes place during December to January (Chatterjee & Pakrashi, 1997). The flowers have a bitter taste and a bad odour (Kirtikar & Basu, 1987). Flower heads are orange-red, sometimes white or yellow in colour and globular in shape (Anonymous, 1992). Terminal heads of flowers are 2.5-3.3 cm long. Outer involucral bracts are large, foliaceous ovate-oblong 2.5-3.8 cm long constricted above the base, green, usually spinous, inner ovate-oblong or lanceolate acute (Kirtikar & Basu, 1987). They are orange-red achenes (often deformed) obovoid 4-angled truncate at the top with 4 bosses pappus (Hooker, 1882). Seeds (Fig 2) are white, somewhat flat, angular, smooth and shining like little conch shells, broad at the base and pointed towards the apex; apex is marked with concentric rings. Near the base is a small brownish scar; cotyledons, greyish and oily; odour slight, taste bitter (Khory & Katrak, 1985; Singh, 1974).The oil of *Carthamus tinctorius* is golden or clear straw colour used

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mainly for edible and illuminating purposes and for manufacture of soap (Anonymous, 1950; Nadkarni, 1954). It has characteristic odour and taste. It thickens and becomes rancid on exposure to air. It is slightly soluble in alcohol and freely soluble in ether, chloroform, benzene and petroleum ether (Kokate *et al*., 2004). Whole plant, seed, flower (Chatterjee & Pakrashi, 1997), root (Nadkarni, 1954) and oil are medicinally used (Khare, 2004).

Fig 1. Carthamus tinctorius Plant

Fig 2. Carthamus tinctorius Seeds

The plant is native to Europe and Asia (Anonymous, 1992). The cultivated safflower is considered to have originated either from the saffron thistle (*Carthamus lanata*) or the wild safflower (*Carthamus oxyacantha*) in the two primary centres of origin i.e. the mountainous regions of Ethiopia and Afghanistan; and also from the plains of India and Mayanmar (Burma), which are considered to be its secondary centre of origin. India is the second largest producer of safflower in the world, Mexico producing the maximum safflower (Anonymous, 1992). The plant is cultivated throughout a large part of India (Chopra *et al*., 1956; Hooker, 1882; Kirtikar & Basu, 1987) as an oil seed crop, particularly in Andhra Pradesh, Bihar, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh and West Bengal (Chatterjee & Pakrashi, 1997).

Numerous varieties of safflower are known under cultivation. Nearly 63 types have been recorded. The plant can be broadly classified under two distinct varieties; one with very spinous leaves and the other with spineless or moderately spinous leaves. The spinous varieties are considered particularly valuable for oil production and spineless forms for dye extraction (Anonymous, 1950).

It is recorded that the grave clothes of the ancient Egyptian mummies used to be dyed with a safflower dye. Fragments of the safflower plants and seeds have been found in some of the ancient tombs (Anonymous, 1992). It is one of the most ancient crops cultivated in Egypt as a dye yielding herb. Now it is cultivated as an oil seed plant and regarded as substitute for sunflower. Fixed oil is obtained from the ripe and dry seeds. About 1000 seeds of safflower weigh 20 to 50 gm. The seeds normally contain 35-38 % of fixed oil. The oil is prepared by expression in expellers or with the help of hydraulic presses. The oil is filtered and further purified. The seed meal or round seeds are subjected to cooking by means of open steam, which ensures maximum yield of oil. The filtered and decolourized oil is packed into suitable containers (Kokate *et al*., 2004).

Vernaculars

The plant is known by different vernacular names: *Usfar*, *Qurtum*, *Bazrul Ahris*, *Habbul Asfar*, *Habbul Mu'safar*, *Hariz*, *Mu'safar*, *Turan*, *Ahris*, *Khari* (Arabic); *Kusum*, *Kajirah*, *Kusum phul* (Bengali); *Heboo*, *Hshu*, *Su*, *Suban*, *Supan* (Burma); *Hong Hoa*, *Hong lang Hoa* (Chinese); *Safflower*, *Parrot Seed*, *Bastard Saffron*, *Wild Saffron*, *African Saffron*, *American saffron*, *Dyer's Saffron* (English); *Carthame*, *Faux safran*, *Safranon* (French); *Farber safflor*, *Safflor*, *Gartensafran*, *Falschesafran* (German); *Kusumbi*, *Karada*, *Kabri*, *Kusumbo* (Gujrati); *Kur*, *Kasumba*, *Kusumbar*, *Kusum*, *Barre*, *Karrah* (Hindi); *Carthamus tinctorius*, Linn (Latin); *Chendurakam* (Malyalam); *Galapmachu* (Manipur); *Kadaya*, *Kararhi*, *Kardai*, *Kardi*, *Sadhi* (Marathi); *Khasakdana*, *Kazirah*, *Gule ma'sfar*, *Mua'sfir*,

Bahram, *Bahraman*, *Kafisha*, *Gule Kafisha*, *Tukhme Kafisha*, *Tukhme Kajira* (Persian); *Karkarar*, *Kurtam*, *Kusam*, *Kushumbha*, *Kusumba*, *Ma'safir* (Punjabi); *Saflor* (Russian); *Kamalottara*, *Kusumba*, *Agnishikha*, *Gramyakunkuma*, *Rakta*, *Kamalottama*, *Kukkutashikha*, *Kusumbha*, *Lohita*, *Maharajana*, *Padmottara*, *Papaka*, *Pawadi*, *Pita*, *Vanishikha*, *Vasraranjana* (Sanskirit); *Sendurkam*, *Sendurukkai*, *Kusumbavirai*, *Chendurukam*, *Kusumba*, *Sendurgam*, *Sendurakam*, *Sethurangam* (Tamil); *Agnisikha*, *Kusumbha*, *Kushumba*, *Kusumbalu* (Telgu); *Atarqatoos*, *Faiqas* (Unani); *Karha*, *Qurtum*, *Kusum* (Urdu) (Aawan, 1984; Ibn Nafees, 1891; Anonymous, 1992; Chatterjee & Pakrashi, 1997; Chopra *et al*., 1956; Farooq, 2005; Ghani, 1920; Hakim 1999; Ibn Baitar, 2003; Ibn Sina, 1992; Karim, 1888; Khan, 1313H; Khare, 2004; Khory & Katrak 1985; Kirtikar and Basu, 1987; Nabi, 1893; Singh, 1974).

Mizaj (Temperament)

The Unani physicians described the temperament of *Qurtum* as Hot in second degree and Dry in first degree (Ghani, 1920; Hakim, 1999; Karim, 1888; Khan, 1313H; Nabi, 1893).

Afa'al (Action)

In classical Unani literature, various actions of the plant *Carthamus tinctorius* have been described such as *Mudirr-e-Baul wa Haiz* (Aawan, 1984), *Mohallil* (Husain, 1872; Ibn Nafees, 1891), *Kasir-e-Riyah* (Ibn Baitar, 2003), *Mohallil-e-Riyah*, *Munzij* (Ghani, 1920; Hakim, 1999; Karim, 1888; Nabi, 1893), *Mukhrij-e-Balgham Ghaleez*, *Mukhrij Khilt-e-Sauda*, *Mulaiyan* (Ghani, 1920), *Mushil* (Ghani, 1920; Hakim, 1999; Husain, 1872; Ibn Baitar, 2003; Khan, 1313H), *Mushil-e-Balgham* (Aawan, 1984; Ibn Baitar, 2003; Khan, 1313H; Nabi, 1893), *Mushil-e-Balgham Sokhta* (Ibn Baitar, 2003; Ibn Sina, 1992), *Mushil-e-Kaimus Sokhta Ghaleez* (Ibn Baitar, 2003), *Mushil wa Mukhrij-e-Balgham Raqeeq wa Akhlat-e-Muharriqa* (Karim, 1888), *Mushil wa Mukhrij-e-Balgham Ghaleez wa Akhlat-e-Muharriqa* (Hakim, 1999), *Muqawwi-e-Basr* (Nabi, 1893), *Munaqqi-e-Sadr*, *Musffi-e-Saut* (Aawan, 1984; Ghani, 1920; Hakim, 1999; Ibn Sina, 1992; Karim, 1888; Khan, 1313H; Nabi, 1893), *Muqawwi-e-Bah* (Hakim, 1999; Ibn Sina, 1992; Karim, 1888; Nabi, 1893) along with milk or honey or *anjeer* (Ibn Baitar, 2003), *Muwallid-e-Mani* (Aawan, 1984; Hakim, 1999; Ibn Baitar, 2003; Karim, 1888; Nabi, 1893).

Istemal (Uses)

Qurtum Has been described to be useful in various ailments such as *Istisqa* (Ghani, 1920; Hakim, 1999), *Malikholiya* (given with *Aftimoon*) (Ghani, 1920; Hakim, 1999; Nabi, 1893), *Wiswas*, *Kharish* (Ghani, 1920; Nabi, 1893), all types of *Jarb* (Ibn Baitar, 2003), *Khadr*, *Wajaul Mafasil* (oil is locally applied), *Surfa*,

Zeequn Nafas (Aawan, 1984), *Khafqan*, *Amraz-e-Saudawi* (Hakim, 1999), *Juzam* (Ghani, 1920; Hakim, 1999; Ibn Baitar, 2003), *Qaulanj* (Aawan, 1984;Ghani, 1920; Hakim, 1999; Ibn Baitar, 2003; Ibn Sina, 1992;Nabi, 1893); useful in almost all respiratory diseases (Hakim, 1999; Karim, 1888). Powder of the seed improves the complexion (Ghani, 1920; Karim, 1888) and the whole plant is good for old people (Hakim, 1999).

Pharmacological Actions (As described in Ethnobatanical and Traditional literature)

The drug *Carthamus tinctorius* is described in detail in ethnobotanical and scientific literature. Some pharmacological actions and therapeutic uses are as follows:

The flowers of the plant act as analgesic, circulatory stimulant and mensturation regulator (Evans, 2002). They are also used as emmenogogue, sedative, stimulant (Kirtikar and Basu, 1987), diaphoretic and laxative (Chatterjee & Pakrashi, 1997; Chopra *et al*., 1956). The flowers have also been described to be diuretic, hypnotic, expectorant and tonic to liver (Kirtikar and Basu, 1987); and cure the jaundice (Nadkarni, 1954; Chopra *et al*., 1956).

In Chinese medicine, the flowers are given to stimulate menstruation and to relieve abdominal pain. The flowers are also used to cleanse and heal the wound and sores. Chinese researchers indicate that the flowers and oil can reduce coronary artery disease and lowers the cholesterol levels (Khare, 2004). The seeds are purgative (Nadkarni, 1954), diuretic, tonic (Chopra *et al*., 1956) and antirheumatic (Chatterjee & Pakrashi, 1997). They are antihypertensive (Farooq, 2005); laxative (Dymock, 1891) and nephroprotective (Huang, 1999). Oil from seed is sweetish; good in all disease; tonic, purgative, carminative, aphrodisiac, bechic and cures pain in liver and joints (Kirtikar & Basu, 1987). Patients with hypertension and heart ailments use the refined oil in cooking, as it is rich in polyunsaturated fatty acids (Khare, 2004). The root is diuretic (Anonymous, 1950; Farooq, 2005).

Therapeutic Uses

The whole plant is valuable remedy for itch, paralytic limbs, rheumatism and intractable ulcers (Chatterjee & Pakrashi, 1997). Plant boiled in sesamum oil is a valuable remedy for itch. This medicated oil is locally applied to rheumatic and painful joints, paralytic limbs and intractable ulcers. Hot infusion of dried flowers is given as a diaphoretic in jaundice, nasal catarrh and muscular rheumatism (Nadkarni, 1954). An infusion of flowers is given to children and infants in measles, fevers and eruptive skin affections (Khare, 2004). Flowers cure

inflammation, boils, ring worm, scabies, leucoderma, piles, and bronchitis, and improve the complexion (Kirtikar & Basu, 1987). They are also used in abdominal pain (Anonymous, 1996); dysmenorrhoea (Caius, 2003) and fever (Bhattacharjee, 2004; Bhattacharjee and De, 2005; Caius, 2003; Anonymous, 1996). Tender leaves and stems, from the 4^{th} to the 6^{th} week of sowing, are eaten boiled as a vegetable (Nadkarni, 1954). The seeds are used to cure the pain in chest and throat, catarrh, leucoderma and scabies (Kirtikar & Basu, 1987). They are also used in rheumatism (Chopra *et al*., 1956; Nadkarni, 1954); hypercholesteremia (Farooq, 2005) and lupus erythematosus (Huang, 1999). The oil from the seed is used for healing sore and in rheumatism (Chopra *et al*., 1956). It is most valuable edible oil used in cookery. It is also used in the manufacture of soap and oil paints (Nadkarni, 1954).The edible oil is used in the manufacture of oleomargarine, as a dietary supplement in hypercholesteremia and also in the treatment of atherosclerosis. Due to its high linoleic acid content, it is consumed for preparation of vegetable ghee. Industrially, it is used for preparation of soft-soap varnishes, linoleum and water proofing material (Kokate *et al*., 2004).

Phyto-chemistry

The flowers contain red colouring principle carthamin or carthamite($C_{14}H_{16}O_7$) insoluble in water 0.3-0.6%, a yellow colouring matter soluble in water26.1-36.01%, extractive matter 3.6-5.6%, albumin 1.5-8.0%, wax 0.6-1.5%, cellulose 38.4-56.0%, silica 1.0-8.4%, alumina and oxide of iron 0.4-1.6%, manganese 0.1- 0.5% (Dymock 1891).Carthamin and neocarthamin from yellow and ivory white varieties of plant, kaempferol-3-rhamnoglucoside and kaempferol glycoside from ivory white flowershave been isolated (Rastogi & Mehrotra, 1990). Three acyl-serotonins isolated from oil-free safflower and identified as N-feruloyl-serotonin, N-p-coumaroyl-serotonin and N-p-coumaroyl-serotonin-*â*-D-glucopyranoside. A steroid cellobiosidehas been isolated from flowers (Rastogi & Mehrotra, 1991).Safflor yellow B isolated from petals. Nonacosane, *â*-sitosterol, palmitic, myristic and lauric acids isolated from flowers (Rastogi & Mehrotra, 1993). Luteolin and its 7-O-glucoside, and glucoside of*â*-sitosterol were isolated from flowers (Rastogi & Mehrotra, 1995).Safflor yellow A and B, safflomin A and C, isocarthamin, isocarthamidin, hydroxysafflor yellow A, and tinctormine have been reported from the flower petals of *Carthamus tinctorius*, as well as several new flavonoids and phenolic compounds. Four compounds including a new flavonoid glucoside were also isolated from 95% ethanol extract of dried petals. They are 6-hydroxykaempferol 3-O-glucoside, 6-hydroxykaempferol 7-O-glucoside, kaempferol 3-O-rutinoside and quercetin 3-O-glucoside (Li & Che, 1998).The flower petals reported to contain C-glycosylquinocholcone. They also contain the flavonoids, 6-hydroxykaempherol, and its 3 glucoside 3,6 diglucoside,

3,6,7-triglucoside and 3-rutinoside-6-glucoside (Anonymous 2000).The flowercontains 1-O-hexadecanolenin, trans-3-tridecene-5,7,9,11-tetrayne-1,2-diol, trans-trans-3,11-tridecadiene-5,7,9-triyne-1,2-diol, coumaric acid, daucosterol and apigenin(Liu *et al*., 2005). Two new acetylenic glucoside, 4', 6'-acetonide-8Z-decaene-4,6-diyne-1-O-beta-D-glucopyranoside named carthamoside A1 and 4,6-decadiyne-1-O-beta-D-glucopyranoside named carthamoside A2, along with one known acetylenic glucoside, 8Z-decaene-4,6-diyne-1-O-beta-D-glucopyranoside, were isolated from the air dried flower (Zhou *et al*., 2006). Two new spermidine compounds, namely safflospermidine A (1) and safflospermidine B (2), together with two known compounds, N(1),N(5),N(10)-(Z)-tri-p-coumaroylspermidine (3) and N(1),N(5),N(10)-(E)-tri-p-coumaroylspermidine (4), were isolated from the florets of *Carthamus tinctorius* (Jiang *et al*., 2008). From the dried petalsof *Carthamus tinctorius*, a new flavonoid, (2R)-4',5-dihydroxyl-6,7-di-O-beta-D-glucopyranosyl flavanone and a new aromatic glucoside, methyl-3-(4-O-beta-D-glucopyranosylphenyl) propionate were isolated along with four known compounds (25)-4', 5-dihydroxyl-6, 7-di-O-beta-D-glucopyranosyl flavanone (1), 6-hydroxykaempferol-3, 6-di-O-beta-D-glucopyranoside (2), 4-O-beta-D-glucosyl-trans-p-coumaric acid (3), and 4-O-beta-D-glucosyl-cis-p-coumaric acid (4) (Zhou *et al*., 2008).A study reveals that ten chemical constituents from the flowers were isolated and identified as 7,8-dimethylpyrazino [2,3-g] quinazolin-2, 4-(1H, 3H) -dione (1), adenosine (2), adenine (3), uridine (4), thymine (5), uracil (6), roseoside (7), 4'-O-dihydrophaseic acid-beta-D-glucopyranoside methylester (8), 4-O-beta-D-glucopyranosyloxy-benzoic acid (9) and p-hydroxybenzoic acid (10) (Jiang *et al*., 2008).Three new aromatic glucosides, 2,3-dimethoxy-5-methylphenyl-1-O-beta-d-glucopyranoside (1), 2,6-dimethoxy-4-methylphenyl-1-O-beta-d-glucopyranoside (2), and ethyl-3-(4-O-beta-d-glucopyranosyl-3-methoxyphenyl)propionate (3), named as carthamosides B1, B2, and B3, respectively, along with three known aromatic glucosides, methyl-3-(4-O-beta-D-glucopyranosyl-3-methoxyphenyl)propionate (4), ethylsyringin (5), and methylsyringin (6), have been isolated from the air-dried flower of *Carthamus tinctorius* (Zhou *et al*., 2008).

A total of eight flavonoids (1-8), including a novel quercetin-7-O-(6''-O-acetyl)-beta-D-glucopyranoside (6) and seven known flavonoids, luteolin (1), quercetin (2), luteolin 7-O-beta-D-glucopyranoside (3), luteolin-7-O-(6''-O-acetyl)-beta-D-glucopyranoside (4) quercetin 7-O-beta-D-glucopyranoside (5), acacetin 7-O-beta-D-glucuronide (7) and apigenin-6-C-beta-D-glucopyrano syl-8-C-beta-D-glucopyranoside (8), have been isolated from the leaves of the *Carthamus tinctorius* (Lee *et al*., 2002).A new triterpenoid saponin was obtained from the ethanolic fraction of the leaves (Yadav & Navneeta, 2007).

Seeds contain a clear straw coloured fixed oil (Nadkarni, 1954).A glucopyranoside

of tracheloside, *â*-sitosterol, campesterol, glucose, maltose and raffinose isolated from seeds; seed cake contained protein (37.53%) and carbohydrates (57.98%). Three new serotonins – N-feruloylserotonin, N – (p-coumaroyl) serotonin and N – (p-coumaroyl) serotonin-mono-*â*-D-glucopyranoside isolated from seeds along with 2-hydroxyarctin, matairesinol mono- *â*-D-glucopyranoside and acacetin (Rastogi & Mehrotra, 1993).New indole alkaloid serotobenine – isolated from seeds along with N-feruloyltryptamine and N- (p-coumaroyl) tryptamine (Rastogi & Mehrotra, 1995).Seven antioxidative serotonin derivatives were isolated from safflower oil cake. Their structures were established as N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]ferulamide (1), N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-p-coumaramide (2), N,N'-[2,2'-(5,5'-dihydroxy-4,4'-bi-1H-indol-3,3'-yl)diethyl]- di-p-coumaramide (3), N-[2-[3'-[2-(p-coumaramido)ethyl]-5,5'-dihydroxy- 4,4'-bi-1H-indol-3-yl]ethyl]ferulamide (4), and N,N'-[2,2'-(5,5'-dihydroxy-4,4'-bi-1H-indol-3,3'-yl)diethyl]- diferulamide (5), N-[2-[5-(beta-D-glucosyloxy)-1H-indol-3-yl)ethyl]- p-coumaramide (6), and N-[2-[5-(beta-D-glucosyloxy)-1H-indol-3-yl)ethyl]ferulamide (7) (Zhang *et al*., 1997). From the aqueous ethanol extract of seeds of *Carthamus tinctorius*, a new acacetin diglycoside has been isolated and identified as acacetin 7-O-beta-D-apiofuranosyl-(1"'—>6" instead of 6')-O-beta-D-glucopyranoside together with previously isolated kaempferol 7-O-beta-D-glucopyranoside, acacetin 7-O-alpha-L-rhamnopyranoside and acacetin (Ahmed *et al*., 2000). Safflower oil contains glycerides of palmitic (6.5%), stearic (3%), arachidic (0.296), oleic (13%), linoleic (76-79%) and linolenic acids (90.15%). The polyunsaturated fatty acid content of the oil is highest (75%) and is said to be responsible to control cholesterol level in the blood, and thereby, reduces incidence of heart attacks (Kokate *et al*., 2004).

A new bioactive triterpenoid saponin 3beta-O-[beta-D-xylopyranosyl(1 -> 3)-O-beta-D-galactopyranosyl]-lup-12-ene-28 oic acid-28-O-alpha-L-rhamnopyranosyl ester compound (A), was isolated from the methanolic fraction of the roots of *Carthamus tinctorius* (Yadava & Chakravarti, 2008).

Pharmacological Studies

A number of studies have been carried out on*Carthamus tinctorius* in recent years showing that it possesses diverse pharmacological effects. Some of the important pharmacological effects are as follows:

Anticoagulant

Thrombolytic and anticoagulant activity of *Carthamus tinctorius* in carrageenan induced mice model has been reported. The fermented extracts demonstrated significant thrombolytic and anticoagulant effect (He *et al*., 2005).

Anti-estrogenic

Anti-estrogenic activity of the lignan glycoside, tracheloside, isolated from seeds of *Carthamus tinctorius* was investigated against cultured Ishikawa cells by employing a bioassay-linked HPLC-ELSD method. Tracheloside significantly decreased the activity of alkaline phosphatase (AP), an estrogen-inducible marker enzyme, a level of inhibition comparable to that of tamoxifen (Yoo *et al*., 2006).

Antihypertensive

A study reported that safflower yellow, a mixture of chalconoid compounds extracted from *Carthamus tinctorius* increased blood pressure, plasma rennin and angiotensin II level in experimental group (Liu *et al*., 1992).

Antiinflammatory

The effects of dried safflower petals aqueous extracts and Carthamus yellow, the main constituent of safflower, on lipopolysaccharide-induced inflammation were investigated. The results suggest that they provide anti-inflammatory response (Wang *et al*., 2011). In an experimental study the possible molecular mechanism by which methanol extracts of *Carthamus tinctorius* produced anti-inflammatory action has been explored. The extract induces heme oxygenase-1 expression so that it reduces inflammation by suppression of inducible nitric oxide synthase and cyclooxygenase-2 expression in cells activated with lipopolysaccharide (Jun *et al*., 2011).

A new bioactive triterpenoid saponin, isolated from the methanolic fraction of the root of *Carthamus tinctorius*, showed anti-inflammatory activity (Yadava & Chakravarti, 2008). It has also been reported that N-(p-coumaroyl) serotonin isolated from safflower oil cake inhibits the production of pro inflammatory cytokines by endotoxin (LPS)- stimulated human monocytes. The results indicate that serotonin and its derivatives inhibit the production of pro inflammatory cytokines through multiple mechanisms (Takii *et al*., 2003).

Antimicrobial

Methanolic extract from the leaves of *Carthamus tinctorius* subjected to screen anthelmintic, antibacterial and antiviral activities, was found to possess significan effect. The anthelmintic activity of extract was performed on Indian earthworm. It exhibited significant reduction in time of paralysis and death of worms. The antibacterial activity was carried out on different pathogens however *Pseudomonas auerogenosa* was found to be more sensitive. The antiviral activity of the extract was studied successfully against tobacco viruses (Paramesh *et al*., 2009).

Antioxidant

It has been reported that the flavonoids isolated from the leaves of the *Carthamus tinctorius* have significant anti-oxidant activities against 2-deoxyribose degradation and lipid per-oxidation in rat liver microsomes (Lee *et al*., 2002). Safflower yellow from *Carthamus tinctorius* has also been reported to possess anti-oxidant property. It has hydroxyl radical scavenging effect and decreases the rate of lipid peroxidation in mouse liver suspension (Jin *et al*., 2004). *In vitro* antioxidant activity of the extract of *Carthamus tinctorius* was also evaluated and it has been reported that flavonoids were the main components of extract and were active in scavenging all three radicals in a dose-dependent manner (Han *et al*., 2010). The antioxidant effect of its aqueous extract was found effective in ox-LDL induced injury in rat cardiac microvascular endothelial cell as it decrease the oxygen derived free radicals. The mechanism has been related with scavenging of free radicals, enhancing its clearance and enhancing endogenous antioxidant activity (Ye *et al*., 2008). The serotonin derivatives isolated from safflower oil cake been found to have relatively strong antioxidative activity (Zhang *et al*., 1997). The potential protective effects of *Carthamus tinctorius* flower extract against reactive oxygen species induced osteoblast dysfunction were investigated. The results demonstrate that it can act as a biological antioxidant in a cell culture experimental model and protect osteoblasts from oxidative stress-induced toxicity (Choi *et al*., 2010). Hiramatsu *et al*. (2009) have reported that petal extract of *Carthamus tinctorius* has free radical scavenging activity and neuroprotective effect and carthamin is one of the major active components. Kinobeon A, isolated from cultured cells of safflower has been shown to be a useful cytoprotective agent as it has demonstrated to prevent oxidative stresses (Kanehira *et al*., 2003).

Atherosclerosis

The effect of defatted safflower seed extract and its phenolic constituents, serotonin derivatives, were studied on atherosclerosis. The findings demonstrate that serotonin derivatives of ethanol-ethyl acetate extract of safflower seeds are absorbed into circulation and attenuate atherosclerotic lesion development possibly because of the inhibition of oxidized low-density lipoprotein (LDL) formation through their strong antioxidative activity (Koyama *et al*., 2006).

Blood

The carthamin yellow has been reported to significantly decrease the whole blood viscosity, plasma viscosity, and erythrocyte aggregation index, which were found increased in blood stasis model. Hematocrit and platelet aggregation were reduced, while prothrombin time delayed with the increasing dose (Li *et al*., 2009).

In a rat model of left heart failure after myocardial infarction injection of *Carthamus tinctorius* was given for activating blood circulation. It showed certain inhibitory effect of left ventricular remodeling (Wang *et al*., 2002).

Bone

Kim *et al.* (2002) reported that safflower seeds have a protective effect on bone loss caused by estrogen deficiency, without substantial effect on the uterus. E2 (17b-estradiol) treatment almost completely prevented bone loss as well as marrow adiposity. However, safflower seeds partially prevented ovariectomy-induced bone loss and slightly reduced marrow adiposity. Monfared and Salati (2013) on the other hand have reported detrimental effects on the ovarian histomorphology and female reproductive hormones. The effect of methanolic extract of safflower seeds, containing high mineral content, such as calcium, potassium and phosphorous, were evaluated on bone formation and is likely appears to be mediated by insulin-like growth factor I at the early stage of treatment (Lee *et al*., 2009). It has also been reported to be useful for the treatment of diseases associated with elevated bone loss (Yuk *et al*., 2002).

Diuretic and Nephroprotective

Hydroalcoholic extract of seeds of *Carthamus tinctorius* have been reported to possess protective and curative effects against gentamicin induced acute renal injury along with diuretic effect, in albino rats (Wasim *et al*., 2011).

Enzyme Inhibiting

Hung *et al* (2007) has demonstrated inhibiting activity of the enzymes of alpha-amylase and protein tyrosine phosphatase IB by using the ethyl acetate extract of *Carthamus tinctorius*. It supports the ethnomedicinal use of the drug in diabetes.

Food Additive

Nobakht *et al*., (2000) have studied its flowers for teratogenic and cytotoxic effect of flowers of *Carthamus tinctorius*, which is used as a coloring and flavoring agent in food items. They have concluded that the use of flowers as a food additive should be reconsidered.

Hepatoprotective

Hydroxyl safflor yellow A has been shown to possess hepatoprotective effect aginst carbon tetrachloride induced liver fibrosis. Its promising role as an

antifibrotic agent in chronic liver disease has also been predicted (Zhang *et al*., 2011).

Immune Function

Suppressive effect of safflower yellow on immune functionwas was carried out and it has been reported that it decreases both nonspecific and specific immune functions (Lu *et al*., 1991).

Melanin

Roh *et al*., (2004) reported the inhibitory effect of active compounds isolated from safflower seeds for melanogenesis. It was found that N-feruloylserotonin and N-(p-coumaroyl) serotoninstrongly inhibited the melanin production in comparison with a known melanogenesis inhibitor, arbutin.

Memory

Protective effect of Nicotiflorin, a natural flavonoid extracted from coronal of *Carthamus tinctorius*, was evaluated on cerebral multi-infarct dementia in rats. The result suggested that it has protective effects on reducing memory dysfunction, energy metabolism failure and oxidative stress in multi-infarct dementia rat model (Huang *et al*., 2007).

Myocardial ischemia

In a study, effect of a purified extract of *Carthamus tinctorius* on myocardial ischemia was investigated using both *in vivo* and *in vitro* models. The result revealed that pretreatment with the extract could protect the heart from ischemia injury by limiting infarct size and improving cardiac function (Han *et al*., 2009).

The protective effects of N-(p-Coumaroyl) serotonin (C) and N-feruroylserotonin (F), present in safflower oil,were investigated in perfused guinea-pig Langendorff hearts subjected to ischemia and reperfusion.The findings suggest that the antioxidant effects of both derivatives isolated from safflower play an important role in ischemia-reperfusion hearts in close relation with nitric oxide (Hotta *et al*., 2002). The protective effects of *Carthamus tinctorius* injection on isoprenaline-induced acute myocardial ischemia in rats has been reported by Wan *et al*., (2011). Further it has also been reported that aqueous extracts of *Carthamus tinctorius* reduce myocardial infarct size and leakage of myocardial enzyme, and increase the level of 6-keto-PGF1alpha, so as to inhibit platelet aggregation and prevent thrombosis, the result of which is to reduce myocardial ischemic reperfusion injury (Liu *et al*., 2011).

Neuroprotective

The hydroxyl safflor yellow A, a soluble constituent extracted from *Carthamus tinctorius*, was administered to rats after the onset of cerebral ischaemia. It exerted significant neuroprotective effects on rats with focal cerebral ischaemic injury as expressed by neurological deficit scores and reduced the infarct area as compared with saline group (Zhu *et al*., 2005).

The protective effect of hydroxyl safflor yellow A was investigated on focal cerebral ischemia in rats. In *in vitro* studies, it significantly inhibited neuron damage induced by exposure to glutamate and NaCN in cultured fetal cortical cells (Zhu *et al*., 2005).

The therapeutic effects of hydroxylsafflor yellow A on focal cerebral ischemic injury in rats and its related mechanisms have been investigated. It appears to be a good potential agent to treat focal cerebral ischemia, and the underlying mechanisms exerted by HSYA might be involved in its inhibitory effects on thrombosis formation and platelet aggregation (Zhu *et al*., 2005). Zhu *et al*. (2003) have further reported neuroprotective effect of hydroxysafflor yellow A on cerebral ischemic injury in both *in vivo* and *in vitro* studies suggesting that it might act as a potential neuroprotective agent useful in the treatment in focal cerebral ischemia In another study hydroxysafflor yellow A (5 mg/kg, i.p.) was shown to improve the brain injury induced by lymphostatic encephalopathy and significantly alleviated the neurological deficits (Pan *et al*., 2012). It has also been reported to it protect the cortical neurons, at least partially, from inhibiting the expression NR2B-containing NMDA receptors and by regulating Bcl-2 family (Yang *et al*., 2010).

Osteoporosis

The effects of safflower seed oil on osteoporosis induced-ovariectomized rats were investigated. The result suggested that the safflower seeds have possible roles in the improvement of osteoporosis induced-ovariectomized rats (Alam *et al*., 2006).

Pharmacokinetic

Studies were conducted to characterize the pharmacokinetics and excretions of hydroxysafflor yellow A in rats and dogs after administration by intravenous injection or infusion. Plasma, urine, feces and bile concentrations of HSYA were measured. The results indicated that HSYA was rapidly excreted as unchanged drug in the urine (Chu *et al*., 2006).

The pharmacokinetic characteristics of Hydroxysafflor yellow A in healthy Chinese female volunteers was investigated. The findings suggested that its

pharmacokinetic properties are based on first-order kinetics over the dose range tested (Yang *et al*., 2009).

The distributive character of safflor yellow A in mice was investigated. After IV injection of safflor yellow A in mice, the AUC of safflor yellow A was highest in plasma, followed by kidney, liver, lung, heart, spleen. But it was not found in the brain (Liu *et al*., 2004).

Spermatogenesis

The effect of aqueous extract of *Carthamus tinctorius* on mouse spermatogenesis was evaluated and testicular histopathology, morphometric analysis and spermatogenesis assessments were performed. The findings suggested that it has toxic effects on mouse testicular tissue (Mirhoseini *et al*., 2012).

Spinal Cord

The potential protective effect of Hydroxysafflor yellow A in spinal cord ischemia/ reperfusion injury was investigated. The findings suggested that it may protect spinal cords from ischemia / reperfusion injury by alleviating oxidative stress and reducing neuronal apoptosis in rabbits (Shan *et al*., 2010).

Stone

The effect of *Carthamus tinctorius* on calcium oxalate formation in ethylene glycol fed rats was investigated. Safflower administration appeared to inhibit the deposition of CaOx crystal in ethylene glycol fed rats therefore it may be effective in preventing the stone disease (Lin *et al*., 2012).

Uterus

The experimental results indicate that the decoction of *Carthamus tinctorius* has stimulating action on the uterus of mouse (*in vitro*). The stimulating action has been found related to stimulating H_1-receptor and alpha-adrenergic receptor of uterus (Shi *et al*., 1995).

α-glucosidase inhibitor

In a study α-glucosidase inhibitor activity of serotonin derivatives (e.g. N-p-coumaroyl serotonin and N-feruloyl serotonin), isolated from safflower seed (*Carthamus tinctorius*), has been evaluated (Takahashi & Miyazawa, 2012).

Conclusion

Qurtum (*Carthamus tinctorius*) has been in use since times immemorial to treat wide range of indications. It has been subjected to quite extensive phytochemical,

experimental and clinical investigations. Experimental studies have demonstrated its anticoagulant, antistress, antihypertensive, anti-inflammatory, antimicrobial, antioxidant, antiatherosclerotic, diuretic, nephroprotective, enzyme inhibiting, food additive, hepatoprotective, immune function, melanogenesis, cardioprotective, neuroprotective, antiosteoporotic, spermatogenesis and α-glucosidase inhibitor effects. The scientific studies have proved most of the claims of traditional medicines. However, further, detailed clinical research appears worthwhile to explore the full therapeutic potential of this plant in order to establish it as a standard drug.

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Physico-chemical Standardization of Safoofe Deedan – A Unani Anthelmintic Powder

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Abstract

Present study has been designed to study *Safoofe Deedan* on certain physicochemical parameters in order to determine its quality standards. The method mentioned in National Formulary of Unani Medicine (NFUM) Part II was followed for the preparation of *Safoofe Deedan*. Physicochemical parameters such as organoleptic properties, powder characterization, extractive value, ash value, loss of weight on drying at 105°C pH value and TLC were investigated.

Physicochemical and powder characterization standards were set in. Non successive extractive values were found to be 4.02±0.1129, 6.13±0.200, 15.42±0.3645 and 11.00±0.44090 in petroleum ether, chloroform, ethyl alcohol and water, respectively. Total ash, water soluble ash and acid insoluble ash were determined to be 5.80±0.0288, 2.21±0.1424 and 1.39±0.0781. Loss of weight on drying was 2.033±0.03712 and the pH at 1% and 10% solution was recorded to be 6.74±0.04842 and 6.04±0.0318, respectively. R_f value was calculated from TLC profile which has been shown in Table 6. These findings may be used to determine the quality of *Safoofe Deedan*.

Keywords: Physicochemical, Standardization, Unani, *Safoofe Deedan*, Anthelmintic.

Introduction

Safoofe Deedan is an important drug of Unani medicine. It has been discussed to possess anti-helmintic effect and useful in all three types of intestinal worms, as it either kills them or facilitate their removal from the gut (Anonymous, 2011; Kantoori, 1889). Safoof (Powder) as a dosage form has certain advantages as it has been attributed to have flexibility of compounding, chemical stability, rapid dispersion of ingredients etc. However, there are certain disadvantages that have been associated with powdered drugs (Connor *et al*., 2005). These include unpleasant tasting, hygroscopic and deliquescent nature and shorter shelf life etc. Therefore, the powdered drugs should be standardized more carefully because their kinetic and dynamic profile may alter quickly because of their peculiar nature. Despite the fact that *Safoofe Deedan* is a pharmacopoeial preparation and is in use since decades, it has not been standardized on physicochemical parameters. Therefore, present study was undertaken to set its physicochemical standard so as to establish its quality. The pharmacological activity and dose response relationship can only be ascertained only if the quality of the drug is ensured.

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Materials and Methods

Collection of Drugs and its identification

The ingredients of *Safoofe Deedan* viz. Afsanteen Roomi, Baobarang, Turbud Safaid, Gule Surkh and Sate Ajwain (Table 1) were procured from the Unani Pharmacy of NIUM and A.B. General Store, Avenue Road, Bangalore, and identified by the experts of Unani Medicine and Botany.

Method of preparation of *Safoofe Deedan*

The method mentioned in National Formulary of Unani Medicine (NFUM) Part II was followed for the preparation of *Safoof Deedan* (Anonymous, 2007). All the ingredients except *Sate Ajwain* were first dried in shade and then powdered separately in a mixer grinder and passed through 80 no. sieve. *Sate Ajwain* was powdered manually by *kharal* and passed through 80 no. mesh. All these powdered drugs were mixed together in a mixer grinder and properly stored in air tight container (Fig. 1).

Table 1: Ingredients of *Safoofe Deedan*

S.No.	Unani Name	Botanical Name	Quantity
1.	Afsanteen Roomi	*Artemisia absinthium Linn.*	200 g.
2.	Baobarang	*Embelia ribes Burm f.*	200 g.
3.	Turbud Safai	*Ipomoea turpethum* R.Br	200 g. Hollow Root
4.	Gule Surkh	*Rosa damascena* Mill	200 g. petals
5.	Sate Ajwain	*Trachyspermum ammi* (L.) Sprague	10 g.

Fig. 1: *Safoofe Deedan*

Physiochemical parameters

Organoleptic properties: Appearance, Colour, Smell, Taste were evaluated (Anonymous, 2006).

Powder characterization

Bulk density: The volume of the packing was determined by taking a known weight of powder of *Safoofe Deedan* and carefully poured into a long measuring cylinder then the volume corresponding to top level of the sample in the cylinder was noted and the bulk density was calculated using specific formula. (Bulk Density = Mass / Bulk Volume (Anonymous, 2014)).

Tapped density: Powder of *Safoofe Deedan* was carefully poured into a long measuring cylinder and subjected to 500, 750 and 1250 tapping's until constant tapped volume was not obtained, the volume corresponding to top level of the sample was noted and bulk density was calculated by dividing the mass by tapped volume (Anonymous, 2014).

Compressibility index: This method is also used to evaluate the flowability of the powder sample and the rate at which it packs down. For Carr's index same process was fallowed as in Tapped density and was calculated by the following equation (Anonymous, 2014; Manjula *et al.,* 2012).

$$\text{Carr's index (\%)} = \frac{(\text{Unsettled apparent volume} - \text{Final tapped volume}) \times 100}{\text{Unsettled apparent volume}}$$

Hausner's ratio: It is well known that particle size influences flowability. The fine particles below 100 ìm tend to be more cohesive and therefore less free-flowing, whereas larger denser particles tend to be free flowing. Hence the Hausner's ratio and compressibility index are both measure to evaluate the flowability of the powder substances. Hausner's ratio is related to inter particle friction and as such can be used to predict the powder flow properties. For Hausner's ratio same process was followed as in Tapped density and it was calculated by the following equation (Anonymous, 2014; Manjula *et al.,* 2012).

$$\text{Hausner's ratio} = Vo/Vf$$

Vo = Unsettled apparent volume V_f = final tapped volume

Angle of repose: Angle of repose was calculated by fixed funnel and free standing conc. method. On a flat horizontal surface a funnel was clamped with its tip 2 cm above a graph paper. The powders were poured through the funnel carefully until the cone formed by powder just reached the tip of the funnel. The mean diameter of the powder cones were noted and angle of repose was calculated by using following formula (Manjula *et al.,* 2012 and Musa *et al*., 2011).

Tan Ø =2h / D [h = Height of powder (from graph paper to tip of funnel), D = Mean diameter of the powder]

Non successive extractive value: The powder was extracted by Soxhlet apparatus separately in different solvents (petroleum ether, chloroform, ethyl alcohol and water). 10 g. powdered drug was taken and subjected to separate extraction with each solvent. The extracts were filtered using filter paper (Whatman No. 1) and evaporated on water bath. Extractive values were determined with reference to total drug taken (w/w) (Agrawal and Paridhavi, 2007)

Ash value

Total ash: 5 g. accurately weighted powdered drug was taken in a tarred silica dish and incinerated in Muffle furnace (Optics technology Sr.no. 3163) at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighted and the percentage was calculated with reference to ground drug (Anonymous, YNM).

Acid insoluble ash: Ash obtained from above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. Insoluble matter was collected on an ashless filter paper (Whatman 41) and washed with hot water, and ignited in Muffle furnace at a temperature not exceeding 450°C to constant weight. Residue was allowed to cool in desiccator for 30 minutes and weighed without delay. The percentage of acid insoluble ash was calculated with reference to the air dried drug (Anonymous, 2009).

Water soluble ash: Ash obtained from above method was boiled for 5 minutes with 25 ml of water. Insoluble matter was collected on an ashless filter paper and washed with hot water. It was then ignited for 15 minutes at a temperature not exceeding 450°C in Muffle furness and weighed. Weight of insoluble matter was subtracted from weight of ash, difference in weight represented water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug (Anonymous, YNM).

Loss of weight on drying at 105°C: In tarred evaporating dish, about 10 g. of drug was taken and dried in oven (Labline mod. no. HO 6.7) at 105°C for 5 hours and weighed. Drying and weighing was continued at one hour interval until difference between two successive weighing corresponded to not more than 0.25%. Two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference, until constant weight was reached. The % loss of weight was calculated with reference to original weight of the drug (Anonymous, 2009).

pH value

pH value of 1% solution: Accurately weighed 1 g. of *Safoofe Deedan* powder was taken and dissolved in accurately measured 100 ml of water, pH of filtrate was measured with standard glass electrode (Anonymous, 2006).

pH value of 10% solution: Accurately weighed 1 g. of powder of *Safoofe Deedan* was dissolved in accurately measured 10 ml of water, pH of filtrate was determined with pH meter (Eutech instrument Sr.no. 1544421) (Anonymous, 2006).

Thin layer chromatography: TLC pre-coated plates of silica gel 60 F 254 (layer thickness 0.25 mm) on aluminium sheets was used. TLC test was carried out on these pre coated plates for pet. ether, chloroform and ethanol extract of *Safoofe Deedan*. Two different mobile phases used were Chloroform: Methanol (9:1), Toluene: Ethyle acetate: Formic acid (5:4:1) for each extract. The plates were examined under U V light (254nm) to detect the spots. After detecting spots Rf value was calculated by the following formula (Anonymous, 2009)**:**

Rf value = Distance travelled by the spot / Distance travelled by mobile phase

Results

Organoleptic properties: Appearance: Fine powder, Colour: Brown, Smell: Pleasant, Taste: Bitter Powder characterization: The mean values of Bulk Density, Tapped Density, Compressibility index and Hausner´s ratio of powder of *Safoofe Deedan* were found to be 0.2604±0.0015, 0.4550±0.0015, 23.873±0.081 and 1.308±0.0041, respectively (Table 2). Angle of repose was found to be 39.69±0.356 (Table 2).

The mean percentage of the non successive extractive values was found to be 4.02±0.1129, 6.13±0.200, 15.42±0.3645 and 11.00±0.44090 in petroleum ether, chloroform, ethyl alcohol and water respectively (Table 3). The mean percentage of the values of total ash, water soluble ash and acid insoluble ash were found

Table 2: Powder characterization

Sr.No.	Parameters	Mean± SEM Value
1.	Bulk Density (gm/ml)	0.2604±0.0015
2.	Tapped Density (gm/ml)	0.4550±0.0015
3.	Compressibility index (%)	23.873±0.081
4.	Hausner´s ratio	1.308±0.0041
5.	Angle of repose	39.69±0.356

to be 5.80±0.0288, 2.21±0.1424 and 1.39±0.0781 respectively (Table 4). The mean % age value of Loss of weight on drying was found to be 2.033±0.03712 (Table 3). The mean value of pH was determined at 1% and 10% solution and was found to be 6.74±0.04842 and 6.04±0.0318, respectively (Table 4).

TLC Study

Mobile phase: Benzene: Chloroform (4:1) 5 spots in chloroform (Rf values 0.033, 0.050, 0.080, 0.118,0.542); 5 spots in petroleum ether (Rf values 0.050, 0.067, 0.107, 0.135, 0.559); and 3 spots in ethanol (Rf values 0.118, 0.237, 0.542) (Table 5) (Fig. 2)

Table 3: Extractive values

Sr.No.	Solvents	Non-successive Extractive values (%) (Mean± SEM)
1.	Petroleum ether	4.02±0.1129
2.	Chloroform	6.13±0.200
3.	Ethyl alcohol	15.42±0.3645
4.	Water	11.00±0.44090

Table 4: Physicochemical Parameters

Sr.No.	Physicochemical Parameters	Mean± SEM
1.	Total ash (%)	5.80±0.0288
2.	Water soluble ash (%)	2.21±0.1424
3.	Acid insoluble ash (%)	1.39±0.0781
4.	Loss of weight on drying (105^{o}) (%)	2.033±0.03712
5.	pH value at 1% 10%	 6.74±0.04842 6.04±0.0318

Table 5: TLC Mobile phase = Benzene: Chloroform (4:1)

Sr.No	Extract	Treatment	No. of spot	Rf value
1.	Chloroform	Iodine vapours	5	0.033, 0.050, 0.080, 0.118, 0.542
2.	Petroleum ether	Iodine vapours	5	0.050, 0.067, 0.107, 0.135, 0.559
3.	Ethanol	Iodine vapours	3	0.118, 0.237, 0.542

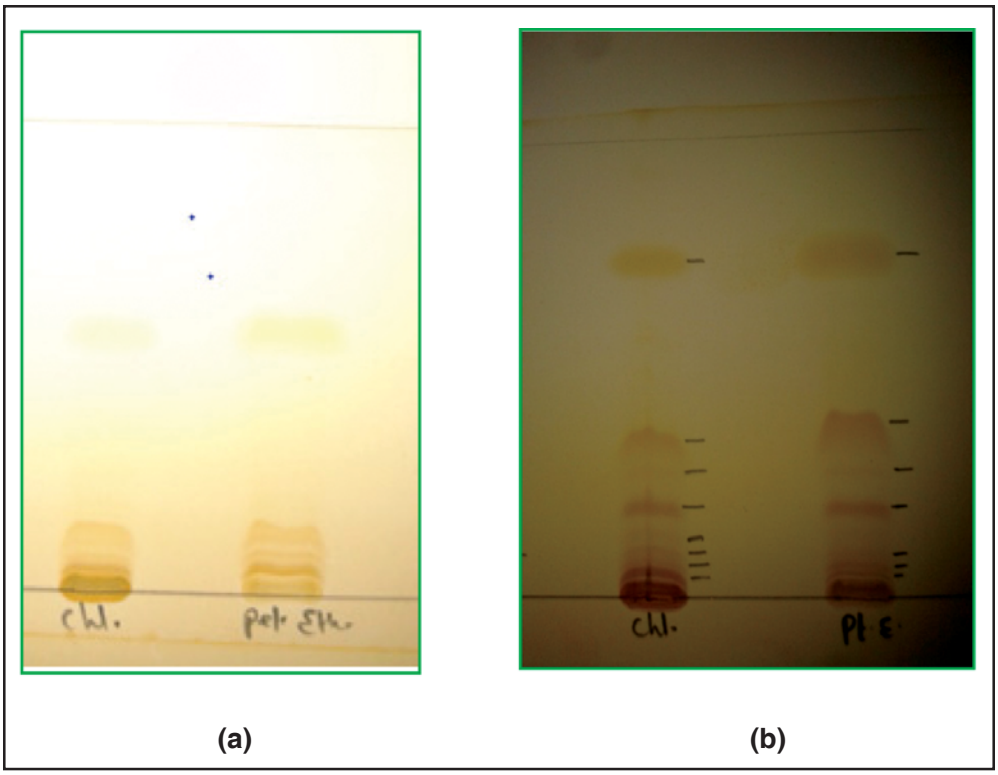

Fig. 2: (a) TLC for chloroform and pet. ether extract in mobile phase Benzene: Chloroform (4:1),
(b) TLC for chloroform and pet. ether extract in mobile phase Toluene: ethyle acetate (9:1)

Mobile phase- Toluene : Ethyl acetate (9:1) 8 spots in chloroform Rf vlues 0.050, 0.066, 0.10, 0.133, 0.20, 0.266, 0.333, 0.733; 7 spots in petroleum ether Rf values 0.050, 0.080, 0.10, 0.183, 0.283, 0.383, 0.733 and 5 spots in ethanol Rf values 0.050, 0.116, 0.150, 0.30, 0.35 (Table 6) (Fig. 3).

Table 6: TLC Mobile phase = Toluene: Ethyl acetate (9:1)

Sr.No.	Extract	Treatment	No. of spot	Rf value
1.	Chloroform	Iodine vapours	8	0.050, 0.066, 0.10, 0.133, 0.20, 0.266, 0.333, 0.733
2.	Petroleum ether	Iodine vapours	7	0.050, 0.080, 0.10, 0.183, 0.283, 0.383, 0.735
3.	Ethanol	Iodine vapours	5	0.050, 0.116, 0.150, 0.30, 0.35

Discussion

Organoleptic features such as appearance, colour, smell and taste plays an important role in quick identification of the drug and these characteristics are peculiar with each drug and provide a qualitative index of identity and quality. The features as observed in respect of *Safoofe Deedan* will also be helpful to determine its identity and quality. Extractive value of a drug in specific solvent is an index of purity of a drug and plays an important role to find out adulteration, if any. The amount of drug soluble in a particular solvent is an index of its purity (Jenkins *et al.,* 2008). Ash value is a significant parameter for finding of adulteration and impurities. Loss of weight on drying indicates the amount of water and volatile substances present in a particular drug. A drug becomes ideal medium for growth of different types of bacteria and fungi if it has moisture. These bacteria and fungi affect the purity, quality and efficacy of drug. pH determines the absorbability of oral dosage forms as with increase and decrease in pH level the ability of drug to get absorbed is altered (Goodman and Gilman, 2001). Altered number of spots and Rf value in a particular mobile phase is an index of purity and quality of a drug and plays an important role to find out adulteration in the drug. The data generated in respect of physicochemical standardization such as bulk density, tapped density, compressibility index and Hausner´s ratio, angle of repose, loss of weight on drying, pH, total ash, water soluble and acid insoluble, extractive values, TLC may be used as standard for future reference to ensure the quality standards of *Safoofe Deedan*.

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Comparative Physico-chemical and Phyto-chemical Study of Different Samples of a Unani Pharmacopoeial Preparation Itrifal Ustukhuddus

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Abstract

In the present study, three pharmacopoeial compound formulations of Itrifal Ustukhuddus have been selected for physicochemical and phytochemical study. Itrifal Ustukhuddus is a reputed poly-herbal preparation of Unani system of medicine. It is commonly used in the treatment of chronic sinusitis and related conditions. The parameters studied for quality assurance of Itrifal Ustukhuddus included physicochemical parameters and qualitative and quantitative analysis of various phytochemicals. The TLC profile of the test drug was also prepared. Three samples of Itrifal Ustukhuddus prepared by three different pharmaceutical units were taken up for the study with an aim to compare the physicochemical and phytochemical parameters in order to check the quality of different samples in in market. It was concluded that all three samples were of pharmacopoeial standard.

Keywords: Itrifal Ustukhuddus, Standardization, Physicochemical, Phytochemical

Introduction

For thousands of years, natural products have been used in traditional medicine all over the world. It is believed that plant derived drugs are safe and more dependable and have little side effects than the costly synthetic drugs. The medicinal value of a crude drug depends on the presence of one or more chemical constituents of physiological importance. They may be glycosides, alkaloids, resins, enzymes etc. The plant drugs have been accepted due to their safety, efficacy, cultural acceptability and lesser side effects (Kamboj, 2000).

Unani medicine uses hundreds of polyherbal and other compound preparations both pharmacopoeial and non-pharmacopoeial. One important polyherbal preparation Itrifal Ustukhuddus is commonly used by Unani physicians to manage especially the sinusitis. A number of pharmaceutical companies prepare this pharmacopoeial preparation. Although, a number of single and compound drugs are standardized on routine basis but unfortunately, the different samples of the same drug prepared by different manufacturing units are usually not undertaken to ensure the quality and to establish their bio-equivalance.

In view of the above, the present study was designed to study the three samples of Itrifal Ustukhuddus prepared by Dawakhana Tibbiya College, A.M.U., Aligarh, Sadar Dawakhana, Delhi and Indian Medicine Pharmaceutical Corporation Limited (IMPCL), Almora, on physicochemical parameters to establish their quality

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standards. The findings will help to know the status of the supply of pharmacopoeial drugs by the drug industry and will also help to know whether the guidelines set by the Govt. are being followed or not in terms of using genuine crude drugs and the methodology recommended for the manufacturing.

Materials and Methods

Market samples of Itrifal Ustukhuddus manufactured by Dawakhana Tibbiya College, AMU, Aligarh (Batch No. 01, Mfg. date 09/2014, Exp. Date 08/2017), Sadar Laboratories, Delhi (Batch No. 117, Mfg. Date 04/2014, Exp. Date 03/2017) and Indian Medicine Pharmaceutical Corporation Limited, Almora (Batch No. UTI 05, Mfg. Date 08/2014, Exp. Date 07/2017) were purchased from Local market of Aligarh. (These samples henceforth will be known as DKTCS, SLS and IMPCLS, respectively).

The Physicochemical parameters included the organoleptic characters of all three test drugs, alcohol and water soluble matter, specific gravity, moisture content, ash values, loss of weight on drying and pH values (Afaq *et al*., 1994; Jenkins *et al*., 1967; Anonymous, 2009). The phytochemical analysis included determination of successive extractive values of the test drug in different organic solvents using soxhlet apparatus, qualitative and quantitative estimation of the chemical constituents present in the drug sample and thin layer chromatography (Afaq *et al.,* 1994, Anonymous, 1968; 1970; 2009; 1982). The Physico-chemical and Phytochemical standardization of all three samples of Itrifal Ustukhuddus was undertaken in Ayush Section, Delhi Test House (A Unani and Ayurvedic Medicines Testing Laboratory), Azadpur, Delhi, India.

(i) Physico-Chemical Analysis

Organoleptic characters of all three samples such as appearance, physical state, colour, smell and taste were observed.

Specific Gravity

The specific gravity of all three samples was determined at 25°C by using a specific gravity bottle.

Extractive Values

The extractive values of the all test drugs in different organic solvents viz. petroleum ether, diethyl ether, chloroform, alcohol and distilled water were determined by the soxhlet apparatus. The heat was applied for six hours on a water bath for each solvent except water, which was heated directly on a heating mantle. The extracts were filtered and after evaporation of the solvents; the

extractive values were determined with reference to the weight of crude drug. The procedures were repeated five times and the mean value was calculated.

Water and Alcohol Soluble Contents

5 gm of all samples of Itrifal Ustukhuddus were taken separately into 250 ml glass stoppard conical flask. 100 ml of distilled water were added and kept for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Samples were filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat bottom dish, and dried at 105°C to constant weight. The percentage of water soluble matter was calculated with reference to the drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water.

Moisture Content

The toluene distillation method was used for the determination of moisture content. 10 gm of each drug was taken in a flask and 75 ml of toluene was added to it. Distillation was carried out for 6 hours and the process was repeated for five times. The volume of water collected in receiver tube (graduated in ml) was noted and the percentage of moisture calculated with reference to the weight of the air dried drug taken for the process.

Ash Values

Total Ash

2 gm of each sample was incinerated in a silica crucible of a constant weight at a temperature not exceeding 450°C in a muffle furnace until carbon free ash obtained, cooled and weighed and the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible with ash. The percentage of total ash was calculated with reference to the weight of drug taken.

Acid Insoluble Ash

The total ash of each sample was boiled with 25 ml of 5N hydrochloric acid for 5 min. The insoluble matter was collected on ash less filter paper (Whatman No. 41), washed with hot water and ignited in crucible at a temperature not exceeding 450°C and weighed after cooling in desiccator. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken.

Water Soluble Ash

The obtained ash of each sample was boiled with 25 ml of distilled water for 5 min. The insoluble matter was collected in an ashless filter paper, (Whatman

No. 41) washed with hot water and ignited in crucible, at a temperature not exceeding 450° C, the weight of insoluble ash was subtracted from the weight of total ash, giving the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the drug taken.

Loss of Weight on Drying

5 gm of each sample was taken into a flat petridish and spread uniformly into a thin layer. It was heated at a regulated temperature of 105° C, cooled in a desiccator and weighed. The process was repeated many times till two consecutive weights were found constant. The percentage of loss in weight was calculated with respect to initial weight.

pH Value

Determination of pH of each sample was carried out by a digital pH meter (model no. DB 1011, Make Decibel) equipped with a combined electrode. The instrument was standardized by using buffer solution of 4.0, 7.0, and 9.20 to ascertain the accuracy of the instrument prior to the experiment. The pH value of 1% solution and 10% of powder drug solution was measured.

(ii) Quantitative estimation of sugar, protein and crude fibre content

The quantitative estimation of total and reducing sugar of each sample was carried out as per the method described in Unani Pharmacopoeia of India (Anonymous, 2009). The quantitative estimation of protein of each sample was carried out as per the method described in Pharmacopoeia of India (Anonymous, 2014). The quantitative estimation of crude fibre content of each sample was also carried out as per the method described in IS: 10226, 1982.

(iii) Phytochemical Evaluation

Test for Alkaloids

A drop of Dragendorff's reagent was added in the sample taken in a test tube. The brown precipitate shows the presence of alkaloids. 1 ml aqueous extract of the sample was taken in a test tube and a drop of Mayer's reagent was added. The white precipitate indicated the presence of alkaloids in the test solution.

Test for Flavonoids

Magnesium ribbon was added to the ethanolic extract of the material followed by drop wise addition of conc. Hcl. Colour change from orange to red is a confirmatory test for flavonoids (Fransworth, 1966).

Test for Glycosides

The test solution is to be filtered and sugar is removed by fermentation with baker's yeast. The acid is removed by precipitation with magnesium oxide or barium hydroxide. The remaining ethanolic extract contains the glycosides which are subsequently detected by the following methods.

- The hydrolysis of the solution is to be done with concentrated sulphuric acid and after the hydrolysis sugar is determined with the help of Fehling's solutions.
- The Molisch's test is done for sugar using α-napthol and concentrated sulphuric acid.

Test for Tannins

Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, shows the presence of tannin.

Test for Starch

0.015 gm of Iodine and 0.015 gm of Potassium Iodide was added in 5 ml of distilled water; 2 ml of this solution formed was added to 2 ml of aqueous test solution, the presence of blue colour indicates the presence of starch.

Test for Phenol

5–8 drops of 1% aqueous solution of Lead acetate was added to aqueous or ethanolic test solution. The presence of yellow colour precipitate indicates the presence of phenols (Brewster and Mc Even, 1971).

Test for Steroid/Terpenes

Salkowski reaction: In the test solution of chloroform 2 ml sulphuric acid (concentrated) was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicates the presence of the steroids/terpenes.

Test for Amino Acids

The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on water bath for few minutes it gives a blue to red-violet colour that indicates the presence of amino acids (Brewster and Mc Even, 1971).

Test for Resins

The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that rapidly changed to violet indicates the presence of the resins.

(iv) Chromatographic Studies

Thin Layer Chromatography (TLC)

It was carried out on TLC pre-coated aluminum plates with silica gel 60 of F_{254} (layer thickness 0.25 mm) (E Merck) of alcoholic and methanolic extract. Taking Toluene: Ethyl acetate: Formic acid in ratio (2: 5: 1.5) as the mobile phases. The R_f values of the spots were calculated by the following formula (Anonymous, 1968):

$$R_f \text{ Value} = \frac{\text{Distance traveled by the spot}}{\text{Distance traveled by solvent system}}$$

Results and Discussion

(i) Physico-chemical Studies

The colour of each test samples was dark brown in colour, semisolid preparation with specific odour and sweetish bitter in taste.

Physico-chemical study is important, because it helps in characterization of constituent or group of constituents that frequently lead to establish the structure-activity relationship and likely mechanism of action of the drug. Phytochemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and drying condition. Thus, keeping in view the above considerations, both the physico-chemical & Phytochemical studies were carried out and the findings are given in table 1 & 2, respectively.

Specific Gravity

The specific gravity of Dawakhana sample (DKTCS), Sadar Laboratories sample (SLS) and IMPCL sample (IMPCLS) was determined at 25°C by using a specific gravity bottle and was found 1.320±0.01, 1.312±0.02 and 1.314±0.02 respectively.

Extractive Value

The extractive value is a parameter for detecting the adulteration in any drug. The amount of the extract that the drug yields in a solvent is often an approximate

measure of the amount of certain constituents that the drug contains. Therefore, for establishing the standards of any drug these extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one (Jenkins *et al.*, 1967) . The mean percentages of extractive values of each sample of Itrifal Ustukhuddus in different organic solvents are given in Table 1.

Water and Alcohol Soluble Matter

Percentage of solubility is also considered an index of purity, as alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc. The Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The mean percentage of alcohol and water soluble matters of each samples of Itrifal Ustukhuddus are given in Table 1.

Moisture Content

The moisture content of the drugs is variable because mostly herbal drugs are hygroscopic and excessive moisture content becomes an ideal medium for the growth of different types of micro-organisms such as bacteria and fungi. They subsequently spoil the purity of drug. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The percentage of moisture content by Toluene distillation method of each sample is given in Table 1.

Ash Values

The ash value is useful in determining authenticity and purity of drugs. Ash value is the residue that remains after complete incineration of the drug, which consists chiefly of silica, partly derived from the constituents of the cells and their walls and partly from foreign mineral matters, mainly soil. Ash value plays an important role in ascertaining the standard of a drug, because the sand, earthy matters are generally added for increasing the weight of the drug resulting in higher ash percentage. Therefore, the ash value determination serves as the basis of judging the identity and cleanliness of a drug and give information related to its adulteration in inorganic matter (Jenkins *et al.*, 1967). The mean of percentage of each samples are given in Table 1.

Table 1: Physico-chemical analysis of Itrifal Ustukhuddus

S. No.	Physicochemical Parameter	DKTCS Mean±S.E.M.	SLS Mean±S.E.M.	IMPCLS Mean±S.E.M.
1	Specific gravity	1.320 ± 0.01	1.312 ± 0.02	1.314±0.02
2	Moisture content (%)	14.80 ± 0.02	13.73 ± 0.03	10.43 ± 0.02
3	Loss of weight on drying at 105°C (%)	24.53 ± 0.02	24.28±0.04	19.36±0.02
4	Ash value in (%)			
	Total Ash	1.10 ± 0.00	1.50 ± 0.00	1.16 ± 0.00
	Acid Insoluble Ash	0.23 ± 0.01	0.49 ± 0.00	0.06 ± 0.00
	Water Soluble Ash	0.44 ± 0.00	0.35 ± 0.00	0.32 ± 0.00
5	pH value			
	pH at 1% aqueous solution	3.81 ± 0.00	3.89 ± 0.00	5.88 ± 0.00
	pH at 10% aqueous solution	3.72 ± 0.00	3.86 ± 0.00	5.70 ± 0.00
6	Solubility (%)			
	Alcohol Soluble extractive	62.65 ± 0.88	45.25 ± 0.40	23.10 ± 0.20
	Water Soluble extractive	58.56 ± 1.20	55.60 ± 0.20	64.80 ± 0.10
7	Extractive values in different organic solvent (%)			
	Petroleum Ether	1.27 ± 0.05	0.60 ± 0.02	0.40 ± 0.02
	Diethyl Ether	0.11 ± 0.01	0.09 ± 0.02	0.07 ± 0.02
	Chloroform	0.64 ± 0.05	0.20 ± 0.04	0.14 ± 0.04
	Ethanol	45.88 ± 0.85	16.60 ± 0.45	10.60 ± 0.04
	Aqueous	20.70 ± 0.95	47.97 ± 0.20	38.47 ± 0.20
8	Sugar Contents (%)			
	Total Sugar	61.59	60.75	62.03
	Reducing Sugar	47.02	41.02	52.90
	Non-reducing sugar	14.57	19.73	9.13
9	Protein (%)	1.27±0.02	1.38±0.04	1.12±0.02
10	Crude fibre content (%)	0.20±0.04	0.22±0.02	0.16±0.02

Loss of Weight on Drying at 105°C

Percentage of loss of weight on drying at 105° C indicates towards the loss of volatile substance along with the water, which is determined by subtracting the moisture contents of the drug from the loss of weight in drying. So the percentage of loss of weight determined for each samples of Itrifal Ustukhuddus are given in Table 1.

pH of 1% and 10% Solution

pH value of the drug is also an important parameter to determine its quality and standard. Further, it also helps in determining the pharmaco-dynamic and pharmaco-kinetic character of a drug (Gilman *et al*., 2001). The mean of pH value of 1% and 10% solution are given in Table 1.

(ii) Quantitative analysis for sugar, proteins and crude fibre content

Quantitative estimation of each sample of Itrifal Ustukhuddus was carried out for total and reducing sugar. The quantitative determination of protein and crude fibre content was also carried out in each test sample; the results are given in table 1.

(iii) Qualitative phytochemical analysis for various chemical constituents

Qualitative phyto-chemical analysis of each samples of Itrifal Ustukhuddus was also carried out for the determination of the presence of alkaloids, flavonoids, glycosides, tannins, phenols, starch, steroids/terpenes, amino acids and resins. The results are given in table 2. The biological activity of medicinal plants and crude drugs depends mainly on the physiologically active constituents present in the drug. The presence of a number of constituents in the test drugs indicated that their medicinal value is mainly indicated to these chemical constituents.

Table 2: Qualitative analysis of the phyto-constituents

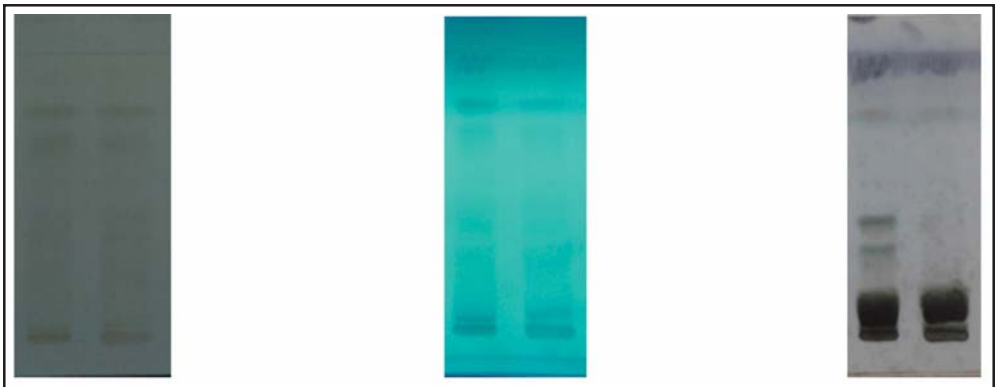
S. No.	Chemical Constituent	Tests/Reagent	DKTCS	SLS	IMPCLS
1	Alkaloids	Dragendorff's reagent Mayer's reagent	–	–	–
2	Flavonoids	Mg ribbon and Dil. HCl	–	–	–
3	Glycosides	NaOH Test	+	+	+
4	Tannins	Ferric Chloride Test	+	+	+
5	Starch	Iodine Test	–	–	–
6	Phenols	Lead Acetate Test	+	+	+
7	Steroid/Terpenes	Salkowski Reaction	+	+	+
8	Amino Acids	Ninhydrin Solution	+	+	+
9	Resin	Acetic Anhydride test	–	–	–

(iv) Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is one of the important parameters used for detecting the adulteration and judging the quality of the drug. The resolution of different kinds of chemical components are separated by using TLC and calculating the R_f values after detecting the spots. If the drug is adulterated, there might be appearance of the other components present as adulterants; in turn the number of spots may increase. On the other hand, the extracted or deteriorated drugs may lose the components and the number of spots appeared might be less. The findings summarized in Table 3 and Fig. 1,2 3 indicated the Rf value of all three samples are almost similar. It atleast partially indicated that genuine samples of crude drug were used to prepare in compound drugs (Table 3 Figure 1, 2 & 3).

Table 3: Thin layer chromatography of Itrifal Ustukhuddus

Extract	Solvent System	Visible in	DKTCS		SLS		IMPCLS	
			No.of Spots	Rf value	No.of Spots	Rf value	No.of Spots	Rf value
Alcoholic	Toluene: Ethyl acetate: Formic acid (2: 5:1.5)	Daylight UV Spray (by Ani-saldehyde Sulphuric acid)	3 4 4	0.06, 0.7, 0.80.3, 0.4, 0.7, 0.8 0.1, 0.3, 0.4, 0.8	2 4 4	0.7, 0.8 0.27, 0.3, 0.7, 0.8 0.06, 0.26, 0.37, 0.8	3 4 4	0.06, 0.7, 0.8, 0.3, 0.41, 0.71, 0.8, 0.06, 0.3, 0.41, 0.78
Metha-nolic	Toluene: Ethyl acetate: Formic acid (2: 5:1.5)	Daylight UV Spray (by Ani-saldehyde Sulphuric acid)	2 3 2	0.7, 0.8 0.3, 0.7, 0.8, 0.1, 0.8	2 3 2	0.7, 0.8 0.3, 0.7, 0.8, 0.1, 0.8	2 3 2	0.7, 0.8 0.05, 0.71, 0.8, 0.07, 0.8



(A) (M) Day light | (A) (M) UV Short | (A) (M) After derivatisation

Fig. 1: TLC of Alcoholic (A) and Methanolic (M) Extract of Itrifal Ustukhuddus (DKTCS)

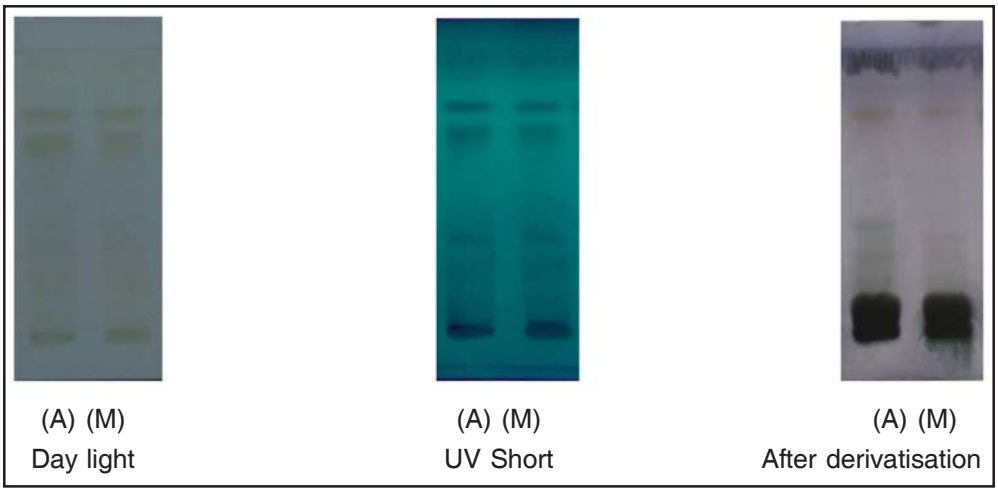


Fig. 2: TLC of Alcoholic (A) and Methanolic (M) Extract of Itrifal Ustukhuddus (SLS)

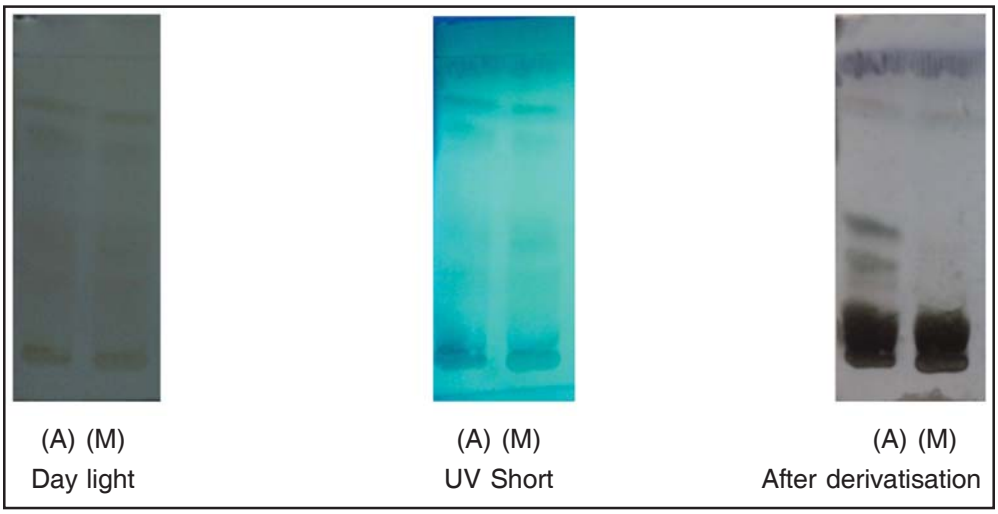


Fig.3: TLC of Alcoholic (A) and Methanolic (M) Extract of Itrifal Ustukhuddus (IMPCLS)

Conclusion

It can be concluded that the market samples of Itrifal Ustokhuddus represented by three major pharmaceutical companies are genuine as they satisfy by and large, the pharmacopoeial standards set by the Unani Pharmacopoeia of India and other legal documents.

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Physico-chemical and Phyto-chemical Standardization of a Unani Drug Banafshah (*Viola odorata* Linn.)

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Abstract

In view of the growing demand of Ayurvedic and Unani drugs in domestic and global market, there is a need to ensure their quality, efficacy and safety through scientitific evaluation and laying down pharmacopoeial standards. In the present work, Banafshah (*Viola odorata* L.) has been standardized for its physico-chemical and phyto-chemical parameters as per WHO pharmacopoieal guidelines. The parameters evaluated includes: ash values: total ash, acid insoluble ash, water soluble ash, sulphated ash; moisture content, loss on drying; pH value at 1% solution and at 10% aqueous solution; melting range; solubility: water soluble extractive and alcohol soluble extractive; bulk density; crude fibre content and total alkaloid content. On phytochemical analysis it was found that Banafshah contains alkaloids, carbohydrates, flavonoids, glycosides, phenols and proteins. Besides this, determination of organoleptic characters of powder drug, extractive values in different organic solvents using soxhlet extractor, thin layer chromatography and fluorescence analysis of successive extracts of powder drug had been done. The study will help in laying down pharmacopoeial standards to determine the quality and purity of Unani drug *Viola odorata* Linn. for wider use in the manufacture of genuine herbal medicines.

Keywords: *Viola odorata* Linn., Physico-chemical, Phyto-chemical, Standardization.

Introduction

Herbal remedies derived from plants represent a substantial proportion of the global drug market and in this respect internationally recognized guidelines for their quality assessment are necessary. WHO has therefore stressed the need to ensure quality control of medicinal plant products for global consumption by using modern techniques and applying suitable standards (Iyengar, 2002).

Unani system of medicine is entirely based on the drugs of natural source and majority of the drugs are of herbal origin. And like any other system of medicine the efficacy of Unani system also depends on the efficacy and purity of drugs used. With the tremendous increase in the global use of medicinal plants, several concerns regarding the efficacy and safety of the herbal medicines have also been raised (Latif and Rehman, 2014). Hence it has become priority to standardize the Ayurvedic and Unani drugs to have uniform efficacy and safety measures so as to ensure regular supply of authentic medicinal plants and raw drugs. Present work is based on this rationale and deals with the pharmacopoeial standardization of a Unani drug Banafshah, *Viola odorata* L. in an attempt to

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ensure its identity, purity and genuineness to manufacture quality herbal medicines.

Viola odorata L. (Family-Violaceae) has been in use since ancient times by Greeks. Baitar (1985) has also mentioned about its medicinal uses. Native doctors consider the purple flowered variety to be the best; they use the flower separately and also the entire plant (Dymock, 1890). The herb is well known in India for its medicinal virtues and has been in use since ancient times. It is used for several diseases in Ayurvedic and Unani medicines.

Morphology and distribution

It is a glabrous or pubescent herb, rarely more than 15 cm. in height, arising from a rootstock, found in Kashmir and the temperate Western Himalaya at an altitude of 1500-1800m, above 5000 ft (Anonymous, 1976; Chopra *et al*., 1958; Ghani, 1921; Hooker, 1875; Nadkarni, 2000) in north temperate zone Nepal, Mishmi, and Khasi hills, China (Bhattacharjee and De, 2005; Dymock, 1890), temperate climates, Europe, North America (Khory and Katrak, 1985). *V. odorata* from Kashmir is considered to be of finest in quality (Anonymous, 1976).

Therapeutic effect

It is especially valued as a diuretic and expectorant, as a purgative in bilious infections; it is seldom given alone, but is prescribed along with other drugs, which also have an aperiant action such as tamarind, myrobalan. 'Banafshah' is recommended generally in those diseases where cooling treatment is thought to be indicated by the Unani physicians (Anonymous, 1976; Dymock, 1890; Khory and Katrak, 1985; Ibne Sina, 1887). Its leaves are said to relieve pain possibly due to cancerous growths, particularly in the mouth and throat (Anonymous, 1976). The fresh flowering herb is used in the homeopathy for the treatment of the diseases of skin and eyes, and for relief from pain in the ear. In folk medicine, it is used as a blood purifier. In large doses, the leaves as well as the roots are used as cathartic. The seeds are purgative and diuretic, they contain salicylic acid (Anonymous, 1976).

Substitutes / Adulterants

Being so much of therapeutic importance, the drug *Viola odorata* L. is substituted with many adulterants. The commercial drug available in the Indian markets is generally highly adulterated with other *Viola* spp. These include *V. biflora*, *V. canescens*, *V. cinerea*, *V. pilosa*, *V. sylvestris* (Anonymous, 1976). In northern India *Viola cineria* Bioss. and *Viola serpenes* Wall. are used as substitute for *Viola odorata,* and are called as Banafshah (Dymock, 1890; Trease and Evans, 2009).

Therefore, present study was done to lay down standards on physico-chemical and phyto-chemical profile for *Viola odorata* as per WHO guidelines laid down for standardizing herbal drugs.

Material and Method

Collection of plant material

Whole herb of *Viola odorata* was procured from Kashmir and was identified by the Pharmacognosy Section, Department of Ilmul Advia, Aligarh Muslim University, Aligarh. The studied sample is preserved in the Herbarium of the Department, for future reference (Voucher No. SC-0099/09-V). Herb so obtained was dried at optimum temperature and further crushed and sieved to coarse powder mechanically and stored in air tight container for study (Fig-1).

Physico-chemical analysis

The analysis included the determination of ash value, melting point, moisture content, pH value at 1% and 10% solution, solubility, bulk density, loss on drying (Afaq *et al.*, 1994; Anonymous, 1968; 1970).

Fig. 1: Crude drug sample of *Viola odorata* Linn.

Phytochemical analysis

The analysis included the determination of the extractive values in different organic solvents, qualitative analysis of the chemical constituents present in the drug sample (Anonymous, 1987; Brewster and Ewen, 1971). Fluorescence analysis of the powdered drugs and successive extracts (FTAR Analysis), crude fibre content, alkaloid estimation (Farnsworth, 1966; Jenkins *et al*., 1967, Peach and Tracey, 1955).

IR spectroscopic study

For this, alcoholic extract of the drug was obtained by refluxing powdered drug (5.0 g) with absolute alcohol (50 ml) for 5 hrs and removing the solvent under reduced pressure. The IR spectrum of alcoholic extract was determined in KBr pellets with Perkin Elmer 1600 FTIR spectrometer (Peach and Tracey, 1955).

Thin layer chromatography

TLC analysis was conducted using different organic solvent systems in percolated silica gel 60F254 TLC plates. Thin Layer Chromatography of the extract of the test drug was carried out by spotted TLC plates were exposed to Iodine vapours in Iodine chamber and then heated at 105^0 C in oven for 10 minutes; plates were visualized in day light and UV short and long wavelength. The R_f value of spots was determined by the given formulae (Afaq *et al*., 1994; Anonymous, 1968; 1970).

$$R_f \text{ value} = \frac{\text{Distance travelled by the Spot}}{\text{Distance travelled by the Solvent}}$$

Observations and Results

Organoleptic characters: The powder of the dried herb of *V. odorata* was dark green with characteristic odourless and slightly taste, summarized in table-1.

Physico-chemical constants: Different physico-chemical constants were determined three times and then average values depicted in table-2.

Table 1: Organoleptic characters of powder of *Viola odorata* Linn.

S.No.	Parameter	Appearance
1.	Colour	Dark Green
2	Smell	Odourless
3.	Taste	Slightly bitter

Table 2: Physico-chemical analysis of *Viola odorata* Linn.

S.No.	Physicochemical Parameter	Results Mean±S.E.M. (S.D.)
1.	Moisture Content	
	Loss of Weight on Drying	12.28 ± 0.01 (0.02)
	Toulene Distillation Method	12.60 ± 0.01 (0.02)
2.	Ash Value (in %)	
	Total Ash	11.24 ± 0.01 (0.02)
	Acid Insoluble Ash	3.15 ± 0.00 (0.01)
	Water Soluble Ash	2.35 ± 0.07 (0.19)
	Sulphated Ash	0.59 ± 0.02 (0.05)
3	pH Values (in %)	
	pH at 1%	7.05 ± 0.01 (0.02)
	pH at 10%	6.02 ± 0.01 (0.02)
4	Bulk Density (in gm/ml)	0.54 ± 0.01 (0.02)
5	Melting Range	102-120^{0}C
6.	Solubility (in %)	
	Alcohol Soluble extractive	18.49 ± 0.02 (0.04)
	Water Soluble extractive	26.72 ± 0.02 (0.04)

Phyto-chemical analysis: The phyto-chemicals present in the drug were qualitatively analysed by different chemical tests and results are given in table-3.

Qualitative analysis of the Phyto-chemicals: Qualitative analysis of the phyto-chemical reveals the presence of alkaloids, carbohydrates, proteins, amino acids, phenols, sterols, glycosides, flavonoids, tannins, resins, sterols/ terpenes and volatile oil presented in table-4.

Table 3: Phyto-chemical analysis of *Viola odorata* Linn.

S.No.	Physicochemical Parameter	Results Mean ± S.E.M. (S.D.)
1.	Crude Fibre Content	7.33 ± 0.01 (0.02)
2.	Total Alkaloid Estimation	6.04 ± 0.08 (0.01)
3.	Extractive values in different organic solvent	
	Petroleum ether (60-80^0)	1.69 ± 0.02 (0.05)
	Diethyl Ether	0.85 ± 0.02 (0.03)
	Chloroform	0.76 ± 0.01 (0.03)
	Alcohol	9.53 ± 0.32 (0.56)
	Aqueous	11.88 ± 0.28 (0.49)

Table 4: Qualitative analysis of the phytochemicals of *Viola odorata* Linn.

S.No.	Chemical Constituents	Test Reagents	Results
1.	Alkaloids	Dragendorff's Reagent	+ve
		Wagner's reagent	+ve
		Mayer's reagent	+ve
2.	Carbohydrates	Molish Test	+ve
		Fehling Test	+ve
		Benedict Test	+ve
3.	Flavonoids	Mg Ribbon and dil. Hcl	+ve
4.	Glycosides	NaOH Test	+ve
5.	Tannins/Phenols	Ferric Chloride Test	+ve
		Liebermann's test	+ve
		Lead Acetate test	+ve
6.	Proteins	Xanthoproteic test	-ve
		Biuret test	+ve
7.	Starch	Iodine Test	-ve
8.	Saponins	Frothing with $NaHCO_3$	+ve
9.	Steroids/Terpenes	Salkowski Reaction	+ve
10.	Amino acids	Ninhydrin Solution	+ve
11.	Resins	Acetic anhydride test	+ve

Indications: ' -ve 'Absence and '+ve' Presence of constituents

Florescence analysis: Florescence analysis under UV light is sometime very characteristic for a drug. As many drugs and the constituents present in the drug emit specific colour when they are exposed to ultraviolet radiations because the radiant energy excites the solution which emits that particular colour known as fluorescence. Hence the fluorescence analysis of the successive extracts and the powdered drug of Banafshah treated with different chemical reagent was done and different change in the colour so appeared was observed and noted. The details are presented in table-5 & 6.

IR spectral study of the drug: Novel IR spectral study of the alcoholic extract of the drug was done by running the alcoholic extract in the IR range (3500-490 cm^{-1}) of the electro-magnetic spectra and major characteristic peaks were noted (Table 7).

Table 5: Fluorescence analysis of *Viola odorata* Linn.

S.No.	Powdered drug	Day Light	UV Short	UV Long
1.	P. drug + Con. HNO_3	Light Orange	Light Green	Green
2.	P. drug + Con. Hcl	Dark Green	Light Green	Light Green
3.	P. drug +Con. H_2SO_4	Dark Brown	Black	Black
4.	P. drug + NaOH Sol. (10%)	Dark Green	Dark Green	Black
5.	P. drug + Glacial Acetic acid	Green	Green	Black
6.	P. drug +dil. HNO_3	Green	Dark Green	Black
7.	P. drug + dil. H_2SO_4	Dark Green	Dark Green	Black
8.	P. drug + dil. Hcl	Dark Green	Green	Black
9.	P. drug +Wagner's reagent	Dark Green	Brownish Green	Dark Green
10.	P. drug + Benedict's reagent	Dark Green	Bright Green	Dark Green
11.	P. drug + Fehling Reagent	Very Dark Green	Dark Green	Dark Blue
12.	P. drug + Picric acid	Light Green	Light Green	Green
13.	P. drug + Lead Acetate (5%)	Dark Green	Light Green	Black
14.	P. drug +$CuSO_4$ (5%)	Light Green	Dark Green	Black
15.	P. drug + KOH (10%) methanolic	Very Light Yellow	Green	Dark Green
16.	P. drug + Glacial Acetic acid+ HNO_3	Green	Green	Dark Green
17.	P. drug +10%NaOH + $Conc^n$ HNO_3	Brown	Dark Green	Very dark Green
18.	P. drug + Dragendorff reagent	Brownish Green	Dark Green	Black
19.	P. drug + Ninhydrin (2%) in acetone	Dark Green	Dark Green	Black
20.	P. drug + Iodine sol. (5%) in alcohol	Gold Brown	Brownish Green	Black

P. drug = Powdered Drug

Table 6: Fluorescence analysis of the successive extracts of *Viola odorata* Linn.

Extracts	Day Light	UV Short	UV Long
Petroleum ether	Brown	Light Green	Dark Brown
Diethyl ether	Dark Green	Dark Brown	Black
Chloroform	Black	Green	Dark Black
Alcohol	Brown	Green	Greenish Brown
Aqueous	Brown	Dark Green	Black

Table 7: IR Spectral study of *Viola odorata* Linn.

Test Drug	IR , υ (cm^{-1})
Banafshah (*V.odorata* Linn.)	3463.19, 2930.35, 2365.70

Thin layer chromatographic profile: Thin layer chromatographic analysis of the various extracts of *V. odorata* was carried out using different solvent systems methanol: acetic acid (45: 8: 4) as solvent system. R_f values were calculated after the development of chromatogram. The R_f values in the given solvent are used to characterize the drugs identity and purity. The results obtained are given in fig. 2; table-8.

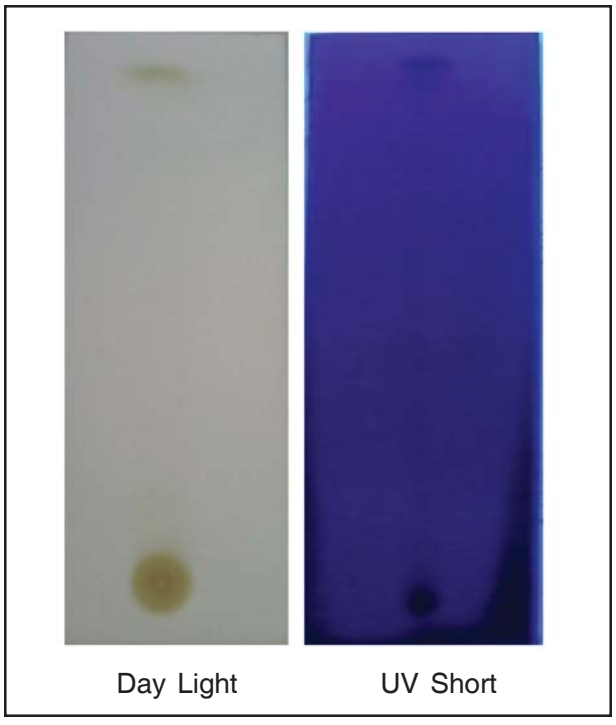

Fig. 2: TLC Banafshah- Petroleum ether extract

Table 8: Thin layer chromatography of *Viola odorata* Linn.

Extract	Solvent System	Treatment	Visualizing Agent	No. of Spots	R_f value
Petroleum ether	Benzene: Chloroform (8:2)	I_2 Vapour	Day Light UV Long UV Short	3 3 1	0.06, 0.10, 0.20 0.06, 0.10, 0.20 0.10(G)
	Petroleum ether: ether (8:2)	"	Day Light UV Long UV Short	4 3 1	0.07,0.15, 0.53, 0.61, 0.07, 0.53, 0.61 0.53 (D.G)
Chloroform	Benzene: Chloroform (4:1)	I_2 Vapour	Day Light UV Long UV Short	1 2 1	0.08 0.13 0.13(L.G)
	Chloroform: Methanol (3:7)	"	Day Light UV Long UV Short	1 4 5	0.41 0.33. 0.5, 0.75, 0.83 0.50 (G), 0.54 (D.G), 0.63(L.G), 0.83(G), 0.90(D.G)
Alcohol	Toulene: Ethyl acetate: Benzene: Acetic acid (4:1:2:2 drops)	I_2 Vapour	Day Light UV Long UV Short	6 6 5	0.23, 0.30,0.35,0.38,0.49, 0.52 0.23,0.30,0.35,0.38,0.49,0.52 0.30(L.Br.),0.35(Br.),0.38(Br.), 0.49(G),0.52(L.G)
	Benzene: Ethyl acetate: Di ethyl ether	"	Day Light UV Long UV Short	1 1 1	0.54, 0.63 0.54 0.54(D.Br.)

D: Dark L: Light Br.: Brown Bl: Blue G: Green
Y: Yellow O: Orange B: Black Fl.: Fluorescent

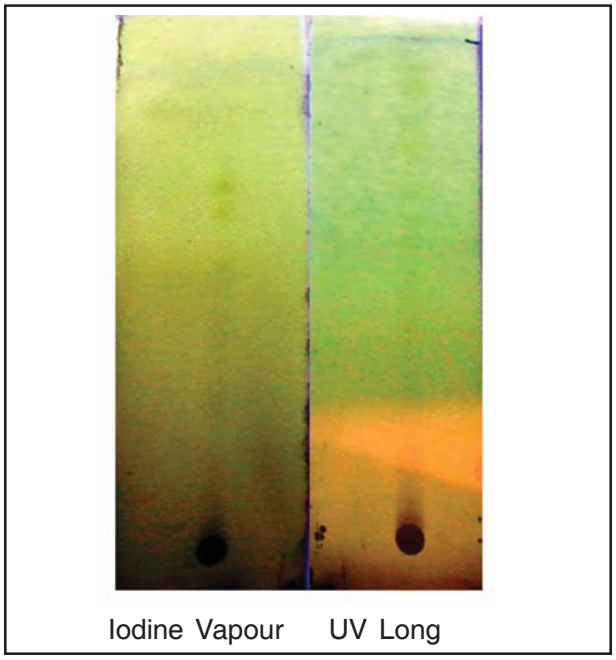

Fig. 3: TLC Banafshah-Chloroform extract

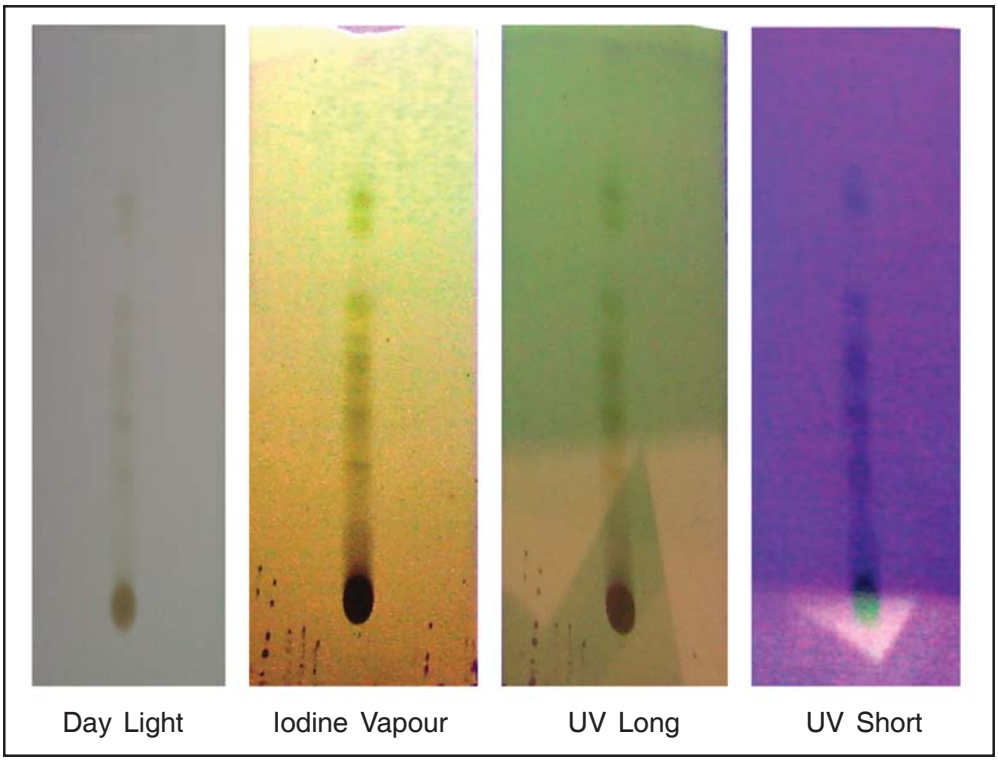

Fig. 4: TLC Banafshah- Ethanolic extract

Conclusion

The physico-chemical evaluation of the powder drug reveals the standard parameters for the quality and purity of herbal drugs and also gives information regarding the authenticity of crude drug. The data generated in the present study for Banafshah (*Viola odorata* L.) will be helpful in future for determining the quality and purity of this drug so as to ensure its therapeutic efficacy.

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Indigenous Uses of Medicinal Plants of Keonjhar Forests, Odisha, India

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Abstract

Based on field surveys during 2014-15, the present paper deals with the field observations on the traditional phytotherapy of indigenous people of the Keonjahr forest division of Keonjhar district, Odisha. A total of 64 folk medicinal plants species belonging to 60 genera and 31 families were collected from the study area and identified. First-hand information on medicinal uses was gathered from knowledgeable tribals, rural and traditional healers ('*Vaidyas*') through semi structured questionnaire. The inhabitants of the area investigated mostly rely on medicinal plants for the treatment of different types of ailments such as cuts, wounds, itching, eczema, burn sensation, boils, scabies, indigestion, stomachache, joint pain, headache, kidney stone, diabetes, jaundice, malaria etc. It is re-stressed that pharmacological and phytochemical investigations may be undertaken on all these reported folk medicinal plants to validate the claims. The information provided may also help in the discovery of new drugs of plant origin.

Keywords: Folk medicinal plants, Keonjhar forests, Odisha.

Introduction

Herbal system of medicine has been practiced since historical times and traces its roots to ancient civilizations (Martin, 1995). Plants contain a large number of pharmacologically active ingredients and each herb possesses its own unique combination and properties. According to World Health Organization about 25% of modern medicines are developed from plants sources used traditionally; and in this context, this traditional knowledge of plants has led to the discovery of 75% of herbal drugs (Malla *et al.*, 2015; Mian-Ying *et al.*, 2002). Therefore, traditional knowledge of medicinal plants in the tribal people is unique source for exploring bioactive compounds of therapeutic importance in phytochemical research (Malla *et al.*, 2015; Newman, 2008; Sharma and Mujundar, 2003).

Odisha is rich with diversity of ethno-botanical species and valuable herbal medicinal knowledge (Sen and Behera, 2015). Keonjhar, the northern district of the Odisha state, lies between 21°63' N latitudes and 85°60' E longitude and spread over an area of 8,240 km^2. About half area of the district (4043 km^2) is covered by tropical moist deciduous type forests which possess good amount of diversity of medicinal plants. The district is the homeland of various tribal communities which constitutes 43.88% of its total population, out of which about 86.36% tribal communities are living in the rural areas of different isolated hill pockets (as per 2001 census). The principal tribes of area are Bathudi, Bhuyan,

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Bhumij, Gond, Ho, Juang, Kharwar, Kisan, Kolha, Kora, Munda, Oraon, Santal, Saora, Sabar and Sounti. Due to poverty and lack of primary health care centers (PHC's) in many areas, they depend on the herbal products to cure various ailments. Generally, the people of this area still have a strong belief in the efficacy of herbal medicines and possess a good amount of knowledge regarding the medicinal plants. Though, traditional knowledge among indigenous people exist orally in most parts of Odisha without any manuscript, therefore in the emerging threats of modernization, industrialization and lack of interest of local youth to learn the traditional knowledge from the old herbal healers, it is constantly eroding due to lack of proper recording (Gadgil, 1996; Utarsh et al., 1999). Therefore, it has becomes imperative to document the valuable indigenous knowledge of these plants before it is lost. Consequently the present study is an effort with the ultimate aim of exploring the phytodiversity and their utilization pattern in the study area.

A review of literature, however, indicates that the forests of Keonjhar were earlier investigated in 1980 collecting some 277 medicinal plants species. Of these, 79 were reported to be used in folk medicines of the study area comprising 34 recipes for treating various diseases and conditions (Singh and Dhar, 1993; Singh and Khan, 1989).

Material and Method

Field surveys were carried out during December, 2014 to January, 2015 to collect ethno-medicinal plants from the study area. Some 57 villages of BJP, Patana, Ghatgaon and Keonjhar forest ranges of the district were explored to collect the botanical specimens and folk information on medicinal plants. The studied villages were located in interior pockets surrounded by hills and forests. The information on traditional knowledge of medicinal plants species were collected by interacting and discussions with the local traditional healers '*Vaidyas*', elderly knowledgeable people, and various tribal communities through semi-structured interviews. The medicinal plants specimens collected during the field trip were mostly known to the local informants.

For collecting, preserving and identifying the plant specimens standard procedures were adopted (Jain and Rao, 1977). The terminologies followed for describing and identifying the plants are in conformity with Harris and Harris (1994), Jain and Rao (1977) and Womersley (1981). International Code of Botanical Nomenclature, Ambasta (1986), Bennet (1987) and several other floras have been followed for correctly naming the plants. Plant specimens were identified with the help of flora of Odisha (Saxena and Brahmam, 1996), Botany of Bihar & Orissa (Haines, 1921-25) and other regional floras. Botanical

specimens are deposited in the Herbarium of the Survey of Medicinal Plants Unit of Regional Research Institute of Unani Medicine, Bhadrak for future reference.

Observations

The present study identifies and documents some 64 plants species which are well known for medicinal value by virtue of their tribal and rural traditional practices. Medicinal plants species discussed are provided with botanical names, their family, local names, locality with collection number, part(s) used, medical efficacy claimed, and mode of administration in respect to different diseases:

Abrus precatorius L. (Fabaceae); Rati/Runjo; Newgaon-10127; Fruit; to improve eye site and itching; Fruits extraction is used to improve eye site. Leaves paste is used for itching.

Acacia auriculaeformis A. Cumm. (Mimosaceae); Akashi/Jangli jalebi; Biokhuntia-10056; Leaf, and stem; Headache and bloody dysentery; Extraction of leaves used for headache and bloody dysentery. The stem is used for cleaning teeth.

Acacia nilotica (L.) Del. syn. *A. arabica* Willd. (Mimosaceae); Babool; Goripokhari-10137; Bark; Diarrhea; Powder of bark is taken orally to cure diarrhea.

Achyranthes aspera L. (Amaranthaceae); Apamarang; Dhudh Kundh-10042; Leaf and root; Diarrhea and cut/wound; Extraction of fresh leaves (5-6 ml) is orally given for diarrhea and externally it is also applied on cuts and wounds for healing. Root is used for cleaning teeth.

Aegle marmelos Corr. (Rutaceae); Bel Ptra; Godo Chompe-10045; Leaf; Diabetes; Leaves extraction is used for diabetes.

Aerva lanata (L.) Juss. *ex* Schults. (Amaranthaceae); Paunsia; Junga-10191; Whole plant; Wound and kidney stone; Extraction of whole plant is used for healing on wounds. Decoction of whole plant is used for kidney stone.

Alangium salvifolium (L.f.) Wang (Alangiaceae); Ankulo; Kontiyapada-10150; Root; Diabetes; Roots are dried in shade and made into powder. One tablespoon of powder is taken for diabetes.

Albizzia lebbeck (L.) Benth. (Mimosaceae); Siris; Goripokhari-10136; Bark; Boils; Bark paste is used to cure boils.

Aloe barbadensis Mill. syn. *A. vera* (L.) Burm.f. (Liliaceae); Ghritkumari, Batkumari; Ban Mohuldih-10108; Whole plant; Diabetes; Decoction of whole plant is used for diabetes.

Alternanthera pungens Kunth. (Amaranthaceae); Gonthi Gass; Koipur-10088; Whole plant; Malaria and itching; Decoction of whole plant is used for malaria and itching.

Amaranthus spinosus L. (Amaranthaceae); Kanta Mariso; Godo Chompe-10044; Leaf; Indigestion; Leaves decoction is used for indigestion.

Andrographis paniculata (Burm.f.) Wall. ex Nees (Acanthaceae); Bhuni/Bhuinimo; Mundura-10059; Leaf; Menstrual cycle problem; Extraction of fresh leaves is given for menstrual cycle problem.

Argemone mexicana L. (Papaveraceae); Agar; Dhumuria-10119; Whole plant; Skin diseases; Past of whole plant is used for skin diseases.

Asparagus racemosus Willd. (Liliaceae); Satmuli; Tandijoda-10073; Whole plant; Jaundice; Extract of plant is used for jaundice.

Atylosia scarabaeoides Benth. (Fabaceae); Ban kulthi; Janghira-10185; Seed; Kidney stone; Seeds are boiled in three cup of water and when one cup left, decoction is consumed for kidney stone.

Azadirachta indica A. Juss. (Meliaceae); Maha neem; Baraduta-10080; Leaf and stem; Fever; Leaves extraction is used for fever. Stem is used for cleaning teeth.

Bambusa bambos Druce syn. *B. arundinacea* Willd. (Poaceae); Banso; Molipasi-10102; Leaf and root; Skin disease (eczema); Leaves and root paste is used for skin disease such as eczema.

Bauhinia purpurea L. (Caesalpiniaceae); Dev Kanchan; Koipur-10084; Leaf and bark; Leucorrhea and digestion; Decoction of bark is used to cure leucorrhea. Young leaves consumed in cooked form to improve digestion.

Bryophyllum calycinum Salisb. syn. *Kalanchoe pinnata* Pers. (Crassulaceae); Amarpoi; Nippo-10075; Leaf and root; Jaundice and Headache; Leaves decoction is used for jaundice and root paste is used for headache.

Cassia fistula L. (Caesalpiniaceae); Sunari; Baipada Dhar-10096; Seed; Gastric problem; Seed powder is used for gastric problem.

Cassia tora L. (Caesalpiniaceae); Chakunda; Madhavpur-10111; Seed; Itching; Seeds paste is used for itching.

Celosia argentea L. var. *argentea* Weight. (Amaranthaceae); Longa; Biokhuntia-10057; Leaf; Itching; The paste of fresh leaves is used for itching.

Centella asiatica (L.) Urban (Apiaceae); Thalkudi; Talpada-10070; Leaf; Joint pain and eczema; Paste of young leaves is used for joint pain and eczema.

Chloroxylon swietiana DC. (Rutaceae); Bheru; Mundura-10061; Leaf; Indigestion; Leaves powder is used for indigestion.

Chromolaena odorata (L.) King. & Robins. (Asteraceae); Poksunga; Bansapal-10040; Leaf; Cutswound; Extraction of young leaves is used on cuts and wounds for healing.

Cleistanthus collinus (Roxb.) Benth. *ex* Hook.f. (Euphorbiaceae); Korda/Sidi; Mangal Pur-10133; Root; Heel cracks; Root paste is applied on heels to cure cracks.

Costus speciosus (Koenig.) Smith. (Zingiberaceae); Ban-maka; Hatinota-10125; Root; Constipation; Dry roots are grinded into powder and one table spoon is consumed for constipation in the morning.

Crotalaria pallida Ait. syn. *C. stricta* DC. (Fabaceae); Nirmishi; Poipani-10142; Leaf; Cut/wound; Leaves paste is used on cuts and wounds.

Croton bonplandianus Baill. syn. *C. sparsiflorus* Morong (Euphorbiaceae); Ban Maricho; Poipani-10141; Leaf; Scabies; Leaves paste is applied to cure scabies.

Cuscuta reflexa Roxb. (Cuscutaceae); Banpoi; Gonasika-10054; Whole plant and stem; Joint pain and urinary tract infections; Paste of whole plant is used for joint pain and decoction of stem is used to cure urinary tract infections.

Datura fastuosa L. syn. *D. metel* L. (Solanaceae); Kala datura; Dhumuria-10120; Leaf; Swelling; Extraction of fresh leaves juice is used for swelling,

Dendrophthoe falcata (L.f.) Etting syn. *Loranthus longifolius* Desr. (Loranthaceae); Malang; Godo Chompe-10046; Bark; Menstrual cycle problem; The decoction of bark is used to regulate the menstrual cycle.

Eclipta alba (L.) Hassk. syn. *E. prostrata* (L.) L. (Asteraceae); Bhringraaj; Buxibaringao-10202; Root; Constipation; Roots powder is used for constipation.

Elephantopus scaber L. (Asteraceae); Mayur Chudi; Bansapal-10038; Root; Cut/wound; Roots paste is used to cure cuts and wounds.

Eranthemum roseum (Vahl) R.Br. (Acanthaceae); Daskrinda; Ban Mohuldih-10105; Root; Burn sensation; Roots past is used to reduce burning sensation.

Holarrhena pubescens (Buch.-Ham.) Wall. *ex*. G. Don. syn. *H. antidysentrica* Wall. (Apocynaceae); Kurchi; Kontiyapada-10149; Bark; Fever; Decoction of bark is used to cure fever.

Jatropha gossypifolia L. (Euphorbiaceae); Gabo; Kuntapada:10140; Leaf; Cut/wound; Paste of leaves is applied on cuts/wounds.

Justicia adhatoda L. syn. *Adhatoda zeylanica* Medic. (Acanthaceae); Basongo; Baraduta-10079; Leaf; Fever; Leaves are boiled in three glass of water and when one glass left decoction is given to cure fever.

Macaranga peltata (Roxb.) Muell.–Arg. Syn. *Macaranga indica* Wight. (Euphorbiaceae); Pohari; Talpada-10068; Bark; Kidney stone; Bark is used for kidney stone.

Madhuca indica J. F. Gmel syn. *Bassia latifolia* Roxb. (Sapotaceae); Mahua; Mundura-10066; Bark; Diarrhea; Decoction (5-10 ml) of bark is used for diarrhea.

Michelia champaca L. (Magnoliaceae); Champaka; Koipur-10087; Bark; Fever; Decoction of bark is used for fever.

Mucuna prurita Hook. (Fabaceae); Bi-danko; Maidankel-10114; Root; Bodyache; Roots paste is applied for body ache.

Murraya koenigii (L.) Spreng. (Rutaceae); Bhursunga; Poipani-10144; Leaf; Indigestion; Leaves are consumed with normal diet for indigestion.

Nyctanthes arbor-tristis L. (Oleaceae); Singarhar; Hatinota-10123; Leaf; Fever; Leaves powder is used to cure fever.

Phyllanthus emblica L. syn. *Emblica officinalis* Gaertn. (Euphorbiaceae); Dhatri; Kandiposi-10097; Leaf; Stomachache; Juice of leaves is used to cure for stomachache.

Pongamia pinnata (L.) Pierre syn. *P. glabra* Vent. (Fabaceae); Karanjo; Bansapal-10037; Seed oil and root; Skin disease, joint pain and to kill lice; Seed oil is used for skin disease, joint pain and to kill lice. Roots are used for cleaning teeth.

Pterocarpus santalinus L. (Fabaceae); Rakat Chandan; Headache and skin problem (boils, skin eruption, infection); Beguna Khamana: 10129; Wood; Wood paste is applied on forehead to reduce headache. Paste of wood also applied on skin problem such as boils, skin eruption and infection.

Rauvolfia serpentina (L.) Benth. ex Kurz. (Apocynaceae); Patal Garud; Maidankel-10113; Root; Snake bite; Root extraction is used for snake bite.

Rauvolfia tetraphylla L. (Apocynaceae); Patal Garudo; Hatinota-10126; Root and Fruit; Snakebite and indigestion; Roots are used for snakebite. Fruits extraction is used for indigestion.

Santalum album L. (Santalaceae); Chandan; Mangal Pur-10131; Wood and leaf; Headache and skin problem (allergy); Wood paste is applied on forehead to relief from headache. Leaves paste is used for allergy.

Semecarpus anacardium L.f. (Anacardiaceae); Bhalia; Purunia-10146; Seed; Heel cracks; Oil extracted from burned seeds is used to heal cracks of heel.

Solanum nigrum L. (Solanaceae); Putu Kundi; Dhudh Kundh-10043; Leaf; Jaundice; Juice (5-10 ml) of the leaves is used for jaundice till cure.

Solanum surattense Burm.f. syn. *S. xanthocarpum* Schrad. *ex* Wendl., *S. virginianum* L. (Solanaceae), Akranti; Gonasika-10053; Fruit; Wound; Fruits extraction is applied on wounds for quick healing.

Sphaeranthus indicus L. (Asteraceae); Bhui Kadam; Mangal Pur: 10130; Leaf; Cut/wound; Leaves paste is applied on cuts and wounds for healing.

Stereospermum tetragonum DC. (Bignoniaceae); Patudi; Kolimati-10183; Fruit; Joint pain; Fruits made into paste with mustered oil and hot paste is applied for joint pain.

Strychnosnux-vomica L. (Strychnaceae); Kochila; Dhamuni-10197; Seed; Fever and stomachache; Seed powder of plant is mixed with seed powder of *Piper nigrum* L. (Kali mirch), rhizome of *Zingiber officinale* Roscoe. (Saonth), seeds of *Trachyspermum ammi* (L.) sprague (Ajwain), fruits of *Myristica fragrans* Houtt. (Jayphal), seeds of *Helecteres isora* L. (Murod phalli), bark of *Cinnamomum zeylanicum* Blume (Dal Chini), fruits of *Ficus religiosa* L. (Pepal phal), fruits of *Piper Longum* L (Long), ghee honey and rock salt. All are mixed and made into tablets. Tablets are given for fever and stomachache pain.

Syzygium cumini (L.) Skeels (Myrtaceae); Jamun; Purunia-10147; Seed; Diabetes; Seed powered (10-15 gm) is used to cure diabetes.

Tephrosia purpurea (L.) Pers. (Fabaceae); Kulthia; Poipani-10143; Whole plant; Acidity; Plant extraction is used to cure acidity.

Terminalia bellirica (Gaertn.) Roxb. (Combretaceae); Baheda; Janghira-10186; Fruit; Diarrhoea and dysentery; Fruits powder is used for diarrhea and dysentery.

Terminalia tomentosa (Roxb. ex DC.) Wight. & Arn. syn. *T. alata* Heyne ex Roth. (Combretaceae); Aasan; Talpada-10067; Bark and leaf; Urinary infection and headache; Decoction of bark is given orally for urinary infection and paste of leaves is applied for headache.

Vanda roxburghii R. Br. syn *Vanda tessellata* (Roxb.) Hook. *ex* G. Don. (Orchidaceae); Madang; Mundura-10062; Leaf; Fever; Paste of leaves is applied on forehead during fever to slow down the temperature.

Viscum articulatum Burm.f. (Loranthaceae); Madang; Dhamuni-10199; Whole Plant; Arthritis; Dried plant is used as poultice to cure arthritis.

Vitex negundo L. (Verbenaceae); Begonia; Mundura-10065; Leaf and stem; Headache and Joint pain; Paste of leaves is used for headache and joint pain. Stems are used as toothbrush.

Zizyphus mauritiana Lamk. (Rhamnaceae); Ber; Jamidalia-10152; Leaf; Cut/wound; Leaves extraction is applied on cut and wound for quick healing.

Results and Discussion

The present study has revealed the traditional folk medicinal uses of 64 plants species belonging to 60 genera and 31 families (Fig. 1). Top ten families are Fabaceae with 7 species followed by Amaranthaceae, Euphorbiaceae (5 sps. each), Asteraceae (4 sps.), Acanthaceae Apocynaceae Caesalpiniaceae

Figure 1: Some ethnomedicinal plants of the study area.

Mimosaceae Rutaceae and Solanaceae (3 sps. each) (Fig. 2). Rest of the families are represented by one or two species.

Folk medicinal species collected are used to treat 30 different types of ailments. Most of the species are used for dermatological problems such as cuts, wounds, itching, eczema, burn sensation, skin eruption, boils, scabies, heel cracks followed by gastrointestinal problems (indigestion, bloody dysentery, gastric problem, stomachache, constipation, acidity, dysentery); muscular/skeletal problem (joint pain, headache, arthritis); fever; renal complaint (urinary tract infection, kidney stone); endocrine disorder (diabetes); reproductive disorders (leucorrhoea, menstrual cycle problem); dental problem; liver complaint (jaundice); poisonous bite; malaria and eye problem (Fig. 3). Different plants parts used for making herbal preparations to cure these ailments are leaves, roots, stem, bark, whole plant, seeds, fruit, wood, and stem (Fig. 4). Mostly the local inhabitants use freshly collected plant parts to prepare the formulation but sometimes seeds, barks and other useful plant parts are collected, dried and stored in homes for future use. They have been employing all these plants in the form of paste, powder, decoction, extraction, juice, oil and also in cooked form. Of the 64 plants uses, 70% of the applications are internal and 30% external. Majority of the external uses are for dermatological problems, muscular/skeletal problems and dental problems; internal uses are for conditions affecting the gastrointestinal complaints, renal complaints, liver complaints, endocrine disorders etc. The highly interesting findings for dermatological problems and gastrointestinal problems require further confirmation and research, while the efficacy of the various other indigenous uses will need to be subjected to scientific

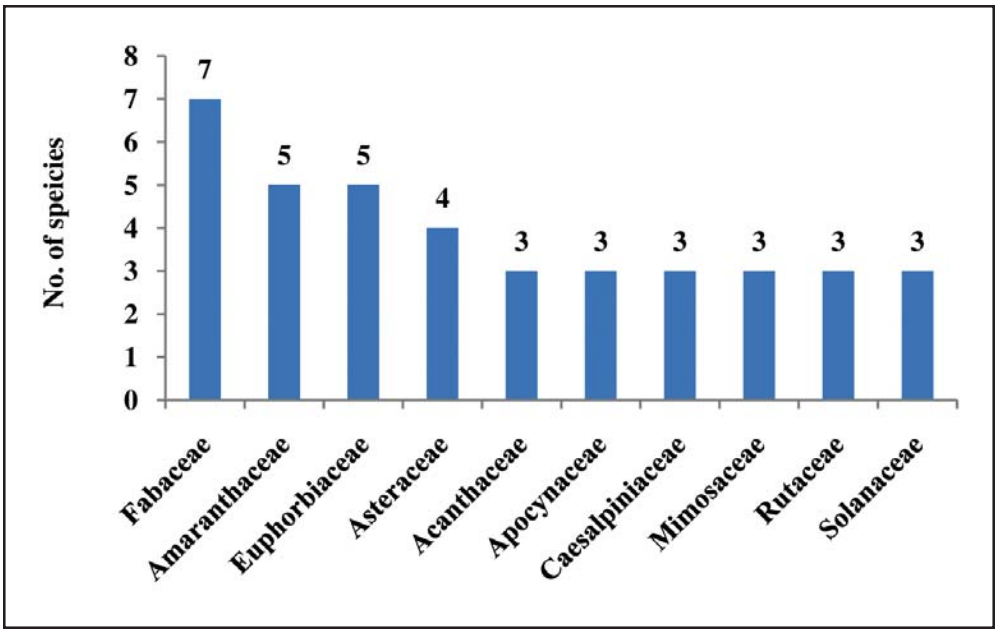

Figure 2: Top ten families with species number in the study area

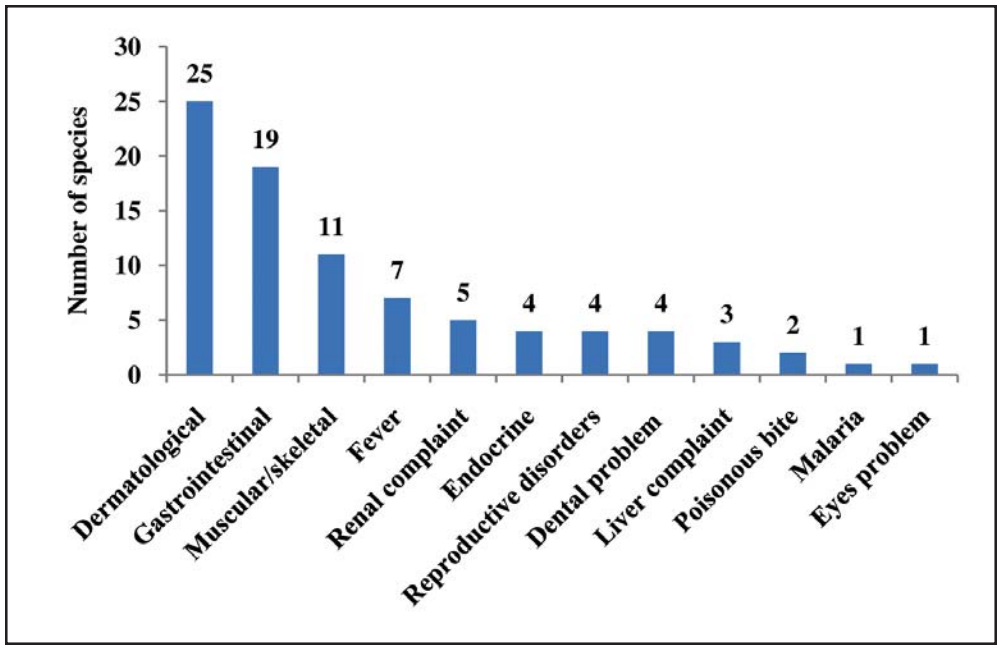

Figure 3: Graph showing categories of ailments cured by different plant species in the study area

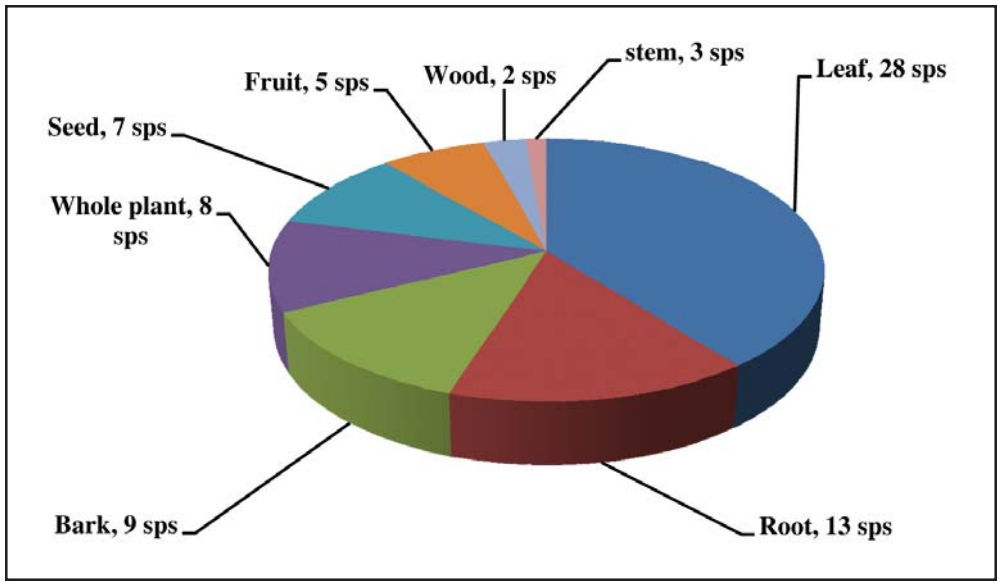

Figure 4: Pie diagram showing different plant part used for curing various ailments in the study area

validation. Information on folk medicinal uses of plants collected from the study area are compared with the existing literature on folk medicines (Ali *et al.*, 2010; Aminuddin *et al.*, 2013; Aminuddin and Girach, 1996; Anonymous, 2001; Behera *et al.*, 2008; Behera *et al.*, 2006; Dhal *et al.*, 2014. Girach *et al.*, 2011; Jain, 1991, Kandari *et al.*, 2012; Kirtikar and Basu, 1935; Mallik *et al.*, 2012; Mukesh

et al., 2011, 2012, 2014a,b; Patra *et al*., 2014; Raut *et al*., 2009, 2013; Sahu *et al*., 2010, 2013a, b; Sen and Behera, 2015; Singh and Dhar, 1993). It has been revealed that majority of the folk claims reported here are either less known or imperfectly known although their mode of administration, ingredients used and plant parts were different. Therefore, present study represents contemporary uses of medicinal plants for the area investigated. It is suggested that detailed phytochemical, pharmacological and clinical researches should be undertaken on all these folk medicinal plants in the context of claims reported. This may help in discovering new therapeutic agents of natural origin, hitherto, unknown to science.

It has also been observed that over exploitation of some species, destructive way of collection, vulnerability due to anthropogenic pressure are some of the major threats to these medicinal plants; therefore, a multi-disciplinary approach must be considered which includes ecological, biological, socio-cultural and economical aspects of these valuable species.

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Standardization of Habb-e-Ustukhuddus: A Classical Unani Formulation

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Abstract

Habb-e-Ustukhuddus is a safe and effective compound Unani formulation with high therapeutic value in hemiplegia (Falij), facial paralysis (Laqwa) and paralysis (Istirkha). SOP of the drug has been developed by preparing the drug at laboratory scale with the standard ingredients and by following the prescribed procedure. The formulation was studied on the basis of pharmacopoeial parameters such as organoleptic, microscopic, physico-chemical analysis, TLC/HPTLC, aflatoxin, heavy metals and level of microbial and pesticide contamination to prove its quality, safety and efficacy.

Keywords: SOP, TLC, HPTLC, UV Spectroscopy, Heavy metal

Introduction

The World Health Organization has reported that more than half of the population in developing countries rely on traditional medicines for their primary health care needs. For last few years developed countries have also been showing interest in traditional herbal medicines. This tremendous growth of herbal medicine consumption leads to concern over its safety issues. There is an urgent need to ensure the quality of these medicines to expand its acceptability worldwide. The Unani system of medicine is very popular traditional system and has wider reach among people. In order to enhance its reliability, several Unani Formulations for numerous diseases have been standardized so far and the exercise is constantly continued. Present work is based on this rationale.

Habb-e-Ustukhuddus is a Unani poly-herbal formulation, categorized as Habb (Anonymous, 2006). The drug is reputed for its demulcent action and is used in ailments of hemiplegia, facial paralysis, tremor, paralysis neurasthenia and epilepsy. It is tonic to the body as well as visceral organs, reduces flabbiness of the muscles and Munaqqi-e-Dimagh (clears toxic humours from brain) (Anonymous, 2003; Khan, 1933; Kabiruddin, 1929).

The drug was prepared at laboratory-scale at D.S.R.I., Ghaziabad. According to the formulation composition of the drug, Habb-e-Ustukhuddus is comprised of 12 ingredients of plant origin (Table I) as described in NFUM Part-IV.

In order to develop SOP and pharmacopoeial standards, the drug was subjected to microscopical and physico-chemical analysis. The present study deals with the preparation, microscopical characters, physico-chemical parameters, TLC & HPTLC profile, U.V. spectroscopic study and heavy metal estimations.

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Table I: Formulation Composition

S.No.	Ingredients	Botanical/English Name	Part used
1.	Turbud	*Operculina turpethum* (L.) S. Manso	Root
2.	Sibr	*Aloe vera* (L.) Burm.f.	Leaf extract
3.	Post-e-Halela Zard	*Terminalia chebula* (Gaertu) Retz.	Fruit Pericarp
4.	Post-e-Halela Kabuli	*Terminalia chebula* (Gaertu) Retz.	Fruit Pericarp
5.	Ustukhuddus	*Lavendula stoechas* L.	Flower
6.	Ghariqoon Safaid	*Agaricus alba* L.	Fruit body
7.	Bisfayez	*Polypodium vulgare* L.	Rhizome
8.	Aftimoon	*Cuscuta reflexa* Roxb.	Whole Plant
9.	Shahm-e-Hanzal	*Citrullus colocynthis* Sehrad	Fruit Pulp
10.	Qaranful	*Syzygium aromaticum* (L.) Merr & Perry	Floral bud
11.	Nana	*Mentha viridis* L.	Aerial Part
12.	Roughan-e-Badam	*Prunus amygdalus* Batsch.	Oil

Material and Methods

All the ingredients were procured from local raw drug dealer and identified botanically (Wallis 1967; Trease & Evans 1972) through pharmacognostical methods. Three batches of Habb-e-Ustukhuddus were prepared at DSRI, Ghaziabad as per the formulation composition given in NFUM, Part IV (Anonymous 2006). All the ingredients were of pharmacopoeial quality and free from physical impurities and dried under the shade to remove moisture, if any.

Except Sibr (*Aloe vera*) and Roughan-e-Badam, all the ingredients were crushed separately in an iron mortar to obtain coarse powders which were further processed in a grinder to obtain the fine forms. Sibr (*Aloe-vera*) was soaked in water for about 24 hours to make Rabeta (Adhesive). The powdered ingredients were added to Rabeta and mixed thoroughly to make lubdi mass. Roughan-e-Badam was then added to lubdi mass by continuous mixing. This lubdi mass was used to prepare Huboob by mechanical process. The huboob was dried under shade and stored in a tightly closed glass container free from moisture.

Microscopy

5 g of the powdered drug was taken and stirred gently with hot water in a beaker. The supernatant was discarded and the residue was washed with the distilled water. A little residue was stained with Iodine solution and mounted in 50% glycerine. Some of the residue was heated in chloral hydrate solution and mounted in 50% glycerine and a little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerine (Johansen, 1940; Wallis, 1967).

Chemical Analysis

Physico-chemical parameters of Habb-e-Ustukhuddus was analysed by standard methods as per the WHO guidelines (Anonymous, 1998) such as removal of foreign matters, solubility in water, alcohol and petroleum ether (60-80^{o}), total ash, acid insoluble ash and water soluble ash, loss on drying at 105^{o}C, pH values of 1% and 10% aqueous solution (Anonymous, 1987), volatile oil estimation, microbial load, aflatoxins, pesticide residue (Anonymous, 2000) and heavy metal estimation (Sahito *et al.,* 2001).

Preparation of extract for TLC/HPTLC

Samples of all the three batches of the formulation were extracted with chloroform and alcohol. The extracts were concentrated and made up to 10ml in a volumetric flask separately. These solutions were used for the TLC/HPTLC finger print analysis by employing CAMAG Linomat IV sample applicator on aluminium TLC plate pre-coated with silica gel 60 F_{254} (E. Merck). The chromatograms were developed using the solvent system toluene: ethyl acetate in the ratio 8:2 & 1:1 respectively for chloroform and alcohol extracts. The plates were dried at room temperature and observed the spots at UV-254 and UV-366. Further the plates were dipped in 1% vanillin-sulphuric acid reagent and heated at 105^{0} C till coloured spots appeared. (Wagner *et al.*, 1984; Sethi, 1996; Stahl, 1996)

Preparation of extract for U.V. Spectroscopic studies

1g of the drug was extracted with 100 ml. Petroleum ether (60-80^{o}) by refluxing for 15 minutes on water bath and filtered. The solution was made up to 100 ml. in volumetric flask. This solution was used for U.V. analysis and pure petroleum ether (60-80^{o}) was used as blank solution. (Willard *et al.,* 1965)

Observations

Habb-e-Ustukhuddus is a brown colour pill, hard in texture with spicy smell and bitter in taste. The drug did not show any filth, fungus or objectionable matter while the sample was spread in a petridish.

Microscopy shows presence of following plant tissues

Cork cells are rectangular radially flattened, thin walled parenchymatous cells, vessels and fibres (Sham-e-Hanzal); brown polygonal epidermal cells, starchy parenchymatous cells and very large resin cells. Various rosettes like raphids (Turbud); barrel shaped cells their inner tangential and radial wall very thick, mesophyll tissue shrunken collapsed (Aftimoon); epidermal cells in surface view with uniformly thick walled cells, several of them divided by a thin septa and fragments of cris-cross fibre (Post-e-Halela Zard); spherical smooth pollen grains fragments of calyx tube with prominent nerves (Ustukhuddus); sclereids of various sizes, collenchyma and raphides (Post-e-Halela Kabuli); non septet fungal hyphae (Ghariqoon); isodiamatric shaped cells long trachieds with scalariform thickening, pigmented parenchymatous cells (Bisfayej); pollen grains and scaleranchymatous pericycle (Qaranfal); nonglandular trichomes diacystic stomata on fragments of leaf (Nana).

Results and Discussion

Chemical Analysis

The physico-chemical data of the drug are shown in Table II. The water soluble extractives (23.93-24.25%) show the absence of any inorganic constituents. The moisture content in the drug is very low as the loss in weight on drying at 105^0 C occurs (5.02-5.38%). The low value of acid insoluble ash indicates that the drug is free from siliceous matter. The results of microbial studies are within the permissible limits while total fungal count is nil (Table-III). The results of aflatoxin

Table II: Physico-Chemical Paramaters

S.No.	Parameters	Results
1.	Alcohol Soluble matter %	10.92 - 11.58
2.	Water Soluble matter %	23.93 - 24.25
3.	Pet ether Soluble matter %	7.66 – 7.78
4.	Loss in weight on drying at 105ºC	5.02 - 5.38
5.	Total Ash %	7.38 – 7.88
6.	Water Soluble Ash %	4.11 – 4.29
7.	Acid Insoluble Ash %	1.42 - 1.80
8.	pH of 1% aqueous solution	4.08 - 4.24
9.	pH of 10% aqueous solution	4.20 - 4.40
10.	Volatile oil %	*Traces*

(Table-IV) and pesticide residue (Table-V) studies show that the drug is free from aflatoxin as well as pesticide residue. The content of heavy metal is below detectable limits (Table-VI).

Table III: Microbial Load

S.No.	Parameter Analyzed	Results	Permissible limit as per WHO
1.	Total Bacterial load	$3x10^3$ cfn/g	10^5 CFU/gm
2.	Total fungal count	< 10 cfu/gm	10^3 CFU/gm
3.	Enter obacteriaceae	Absent	Nil
4.	Escherichia coli	Absent	Nil
5.	Salmonella cpp.	Absent	Nil
6.	Staphoilococcus aureus	Absent	Nil

Table IV: Aflatoxin level

S.No.	Parameter Analyzed	Results	Detection limits
1.	B-1	Not detected	0.50 ppm
2.	B-2	Not detected	0.10 ppm
3.	G-1	Not detected	0.50 ppm
4.	G-2	Not detected	0.10 ppm

Table V: Pesticide Residue

S.No.	Parameter Analyzed	Results	Limit
1.	Chlorpyriphos	Not detected	0.20 mg/Kg
2.	DDT	Not detected	1.00 mg/Kg
3.	Endosulfan	Not detected	3.00 mg/Kg
4.	Malathon	Not detected	1.00 mg/Kg
5.	Parathion	Not detected	0.50 mg/Kg

Table VI: Heavy Metals

S.No.	Heavy Metal Analyzed	Results	Permissible limit as per WHO
1.	Arsenic	Not detected	3.00 ppm
2.	Cadmium	Not detected	0.30 ppm
3.	Mercury	0.0079	01.00 ppm
4.	Lead	Not detected	10.00 ppm

Table VII: TLC Results

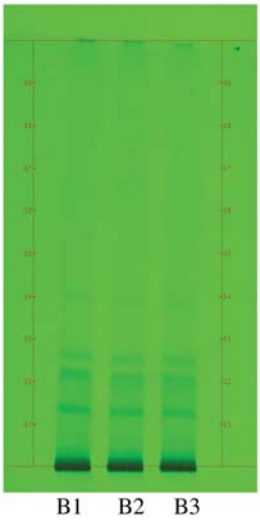
S.No.	Extract	Solvent System	Developing reagent	Rf Values with colour		
				UV254nm	UV366nm	After derivatisation
1.	Chloroform	Toluene: Ethyl acetate (8:2)	Vanillin – Sulphuric acid	0.40 Black	0.77 Red	0.91 Grey
				0.26 Black	0.70 Red	0.72 Grey
				0.22 Black	0.65 Red	0.48 Grey
				0.14 Black	0.62 Blue	0.37 Violet
					0.59 Red	0.26 Green
					0.54 Violet	0.22 Violet
					0.44 Violet	0.14 Violet
					0.40 Brown	
					0.32 Brown	
					0.24 Blue	
					0.17 Violet	
2.	Alcohol	Toluene: Ethyl acetate (1:1)		0.76 Black	0.95 Red	0.92 Grey
				0.66 Black	0.87 Fluorescent blue	0.85 Grey
				0.58 Black	0.76 Violet	0.74 Violet
				0.39 Black	0.69 Blue	0.66 Violet
				0.34 Black	0.63 Blue	0.58 Violet
				0.13 Black	0.56 Green	0.52 Grey
					0.46 Violet	0.48 Violet
					0.31 Green	0.28 Violet
					0.22 Brown	0.16 Green
					0.13 Red	0.13 Violet

HPTLC profile

TLC of all the three batches of Habb-e-Ustukhuddus were observed under UV 254nm, UV 366nm and after derivatization (Table-VII). Chromatogram of chloroform extract shows 04 spots under UV 254nm (Fig. 1), 11 spots under UV 366nm (Fig. 2) and 07 spots after derivatization (Fig. 3). The finger print of chloroform extract shows 10 peaks out of which peaks at R_f 0.15, 0.22, 0.25, 0.29 were major peaks whereas peaks at R_f 0.01, 0.04, 0.07, 0.12, 0.45 and 0.69 are relatively smaller peaks (Fig. 4). HPTLC Chromatogram of chloroform extract is shown in Fig. 5.

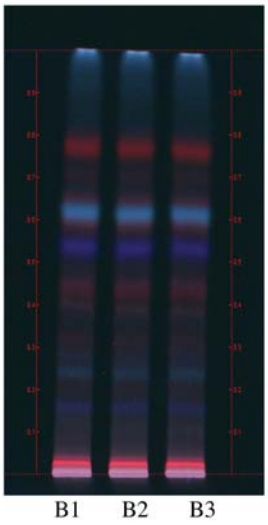
TLC of Chloroform extract of Habb-e-Ustukhuddus

Fig. 1



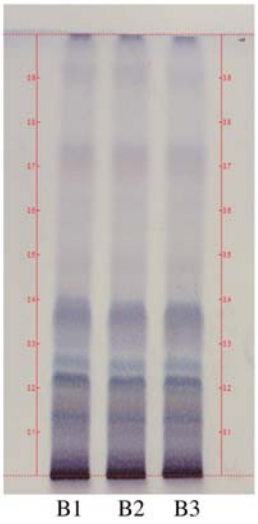
UV254nm

Fig. 2



UV366nm

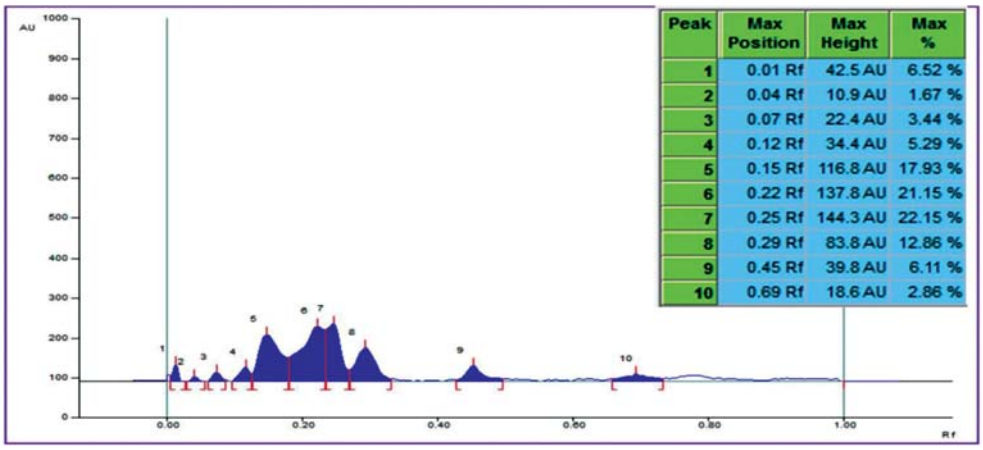
Fig. 3



After derivatization

*B1 – Batch1, B2 – Batch2, B3 – Batch3

Fig. 4

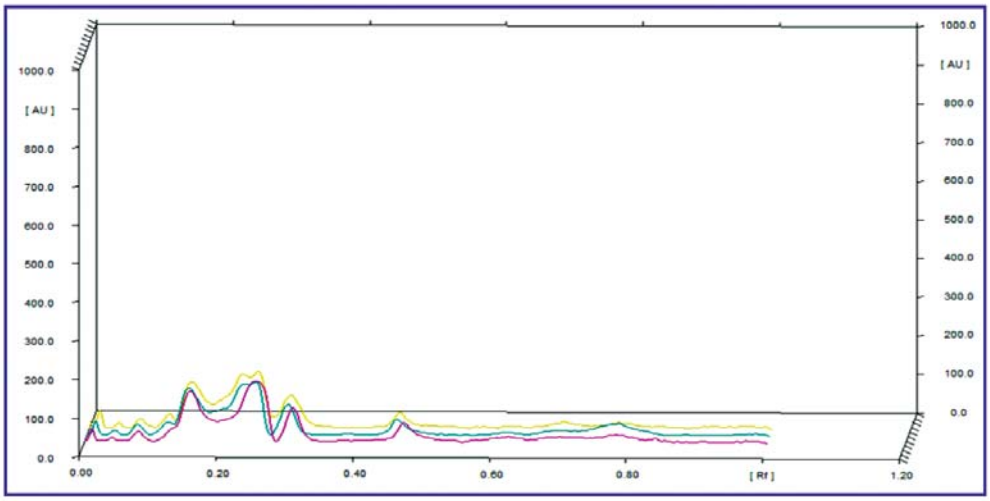


Peak	Max Position	Max Height	Max %
1	0.01 Rf	42.5 AU	6.52 %
2	0.04 Rf	10.9 AU	1.67 %
3	0.07 Rf	22.4 AU	3.44 %
4	0.12 Rf	34.4 AU	5.29 %
5	0.15 Rf	116.8 AU	17.93 %
6	0.22 Rf	137.8 AU	21.15 %
7	0.25 Rf	144.3 AU	22.15 %
8	0.29 Rf	83.8 AU	12.86 %
9	0.45 Rf	39.8 AU	6.11 %
10	0.69 Rf	18.6 AU	2.86 %

HPTLC Finger printing of Chloroform extract

Similarly TLC of alcohol extract shows 06 spots under UV 254nm (Fig. 6), 10 spots under UV 366nm (Fig. 7) and 10 spots after derivatization (Fig. 8)). The finger print of Alcohol extract shows 11 peaks out of which peaks at R_f 0.01, 0.13, 0.69, 0.90 were major peaks whereas peaks at R_f 0.17, 0.34, 0.41, 0.49, 0.59, 0.63 and 0.78 are relatively smaller peaks (Fig. 9). HPTLC Chromatogram of Alcohol extract is shown in Fig. 10.

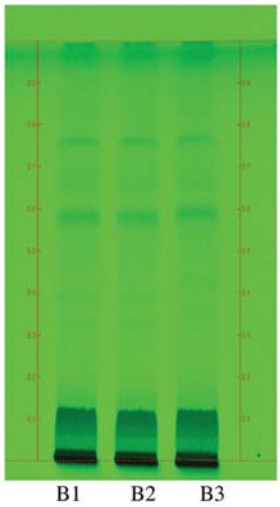
Fig. 5



HPTLC Chromatogram of Chloroform extract

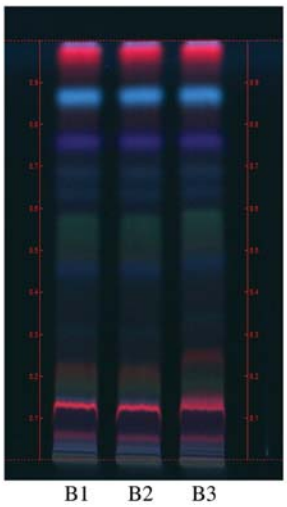
TLC of Alcohol extract of Habb-e-Ustukhuddus

Fig. 6



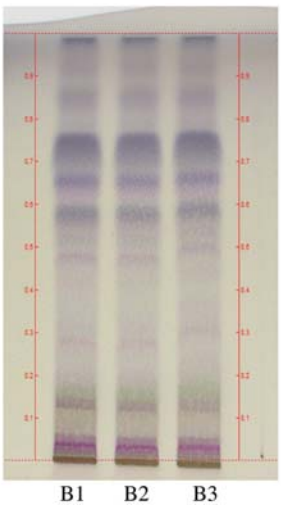
UV254nm

Fig. 7



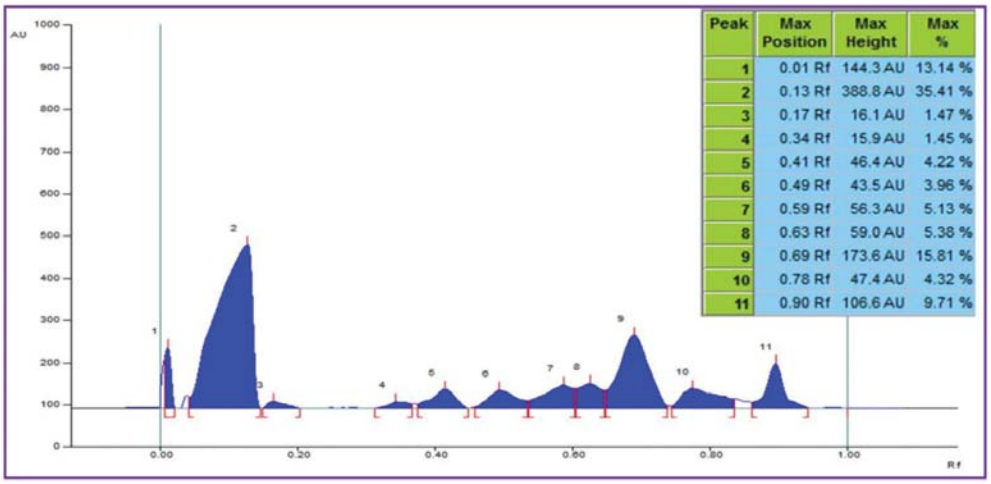
UV366nm

Fig. 8



After derivatization

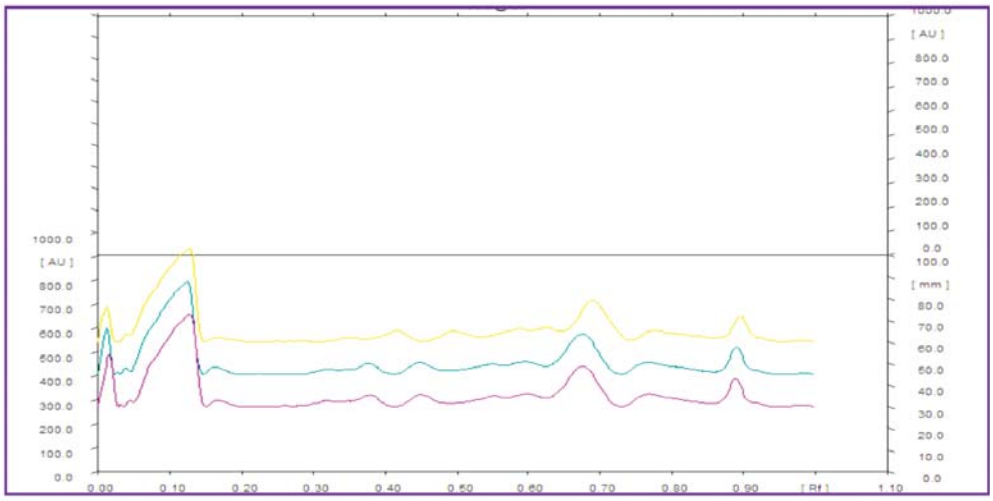
Fig. 9



Peak	Max Position	Max Height	Max %
1	0.01 Rf	144.3 AU	13.14 %
2	0.13 Rf	388.8 AU	35.41 %
3	0.17 Rf	16.1 AU	1.47 %
4	0.34 Rf	15.9 AU	1.45 %
5	0.41 Rf	46.4 AU	4.22 %
6	0.49 Rf	43.5 AU	3.96 %
7	0.59 Rf	56.3 AU	5.13 %
8	0.63 Rf	59.0 AU	5.38 %
9	0.69 Rf	173.6 AU	15.81 %
10	0.78 Rf	47.4 AU	4.32 %
11	0.90 Rf	106.6 AU	9.71 %

HPTLC Finger Printing of Alcohol extract

Fig. 10



HPTLC Chromatogram of Alcohol extract

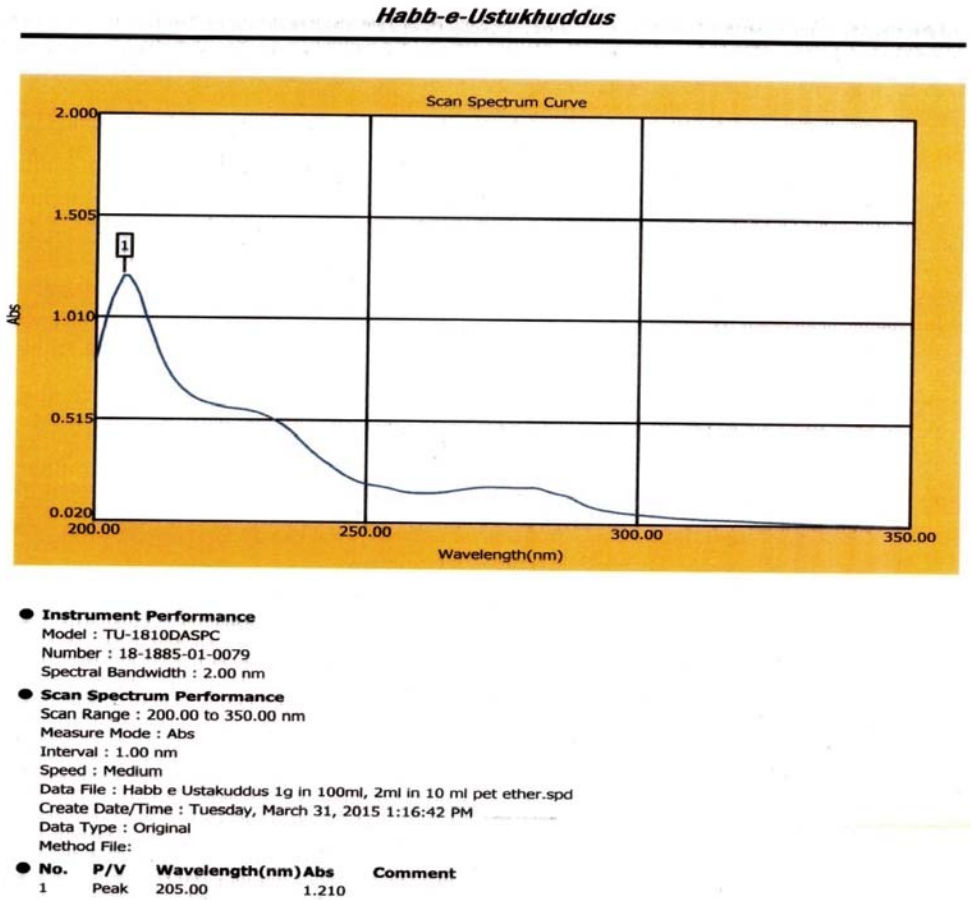
The HPTLC densitometry chromatograms of chloroform and alcohol extract of all the three batches were found to be similar when scanned at 254nm. It indicates batch to batch consistency of the compound formulation.

UV Spectoscopic Studies

The UV spectrum of Habb-e-Ustukhuddus has a single peak at 205nm with an absorbance of 1.210 %. Appearance of the single sharp peak without any noise fortifies the purity of the compound formulation (Fig. 11).

UV Spectrum of Habb-e-Ustukhuddus

Fig. 11



Conclusion

It is very difficult to identify the single drugs once they are powdered and mixed together for preparing compound formulation. The present study, therefore, holds high significance as the microscopic features, various Physico-chemical parameters, HPTLC profile, UV spectrum etc. provide criteria for easy identification of Habb-e-Ustukhuddus and ensure the quality and efficacy of the drug.

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X-ray Diffraction (XRD) Analysis of *Gile armani* (Armenian bole)

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Abstract

Gile Armani (Armenian bole) is a mineral origin drug used in *Unani* system of medicine as astringent, desiccative and antiseptic. Its origin in Unani classical text is said to be Armenia. Different clay / minerals are sold under the name of *Gile Armani*. Keeping in consideration the controversy over its identification this study was carried out. Three different samples of *Gile Armani* were collected from crude drug market of different cities. X-ray diffraction (XRD) for crystallographic study was undertaken with powder method of diffraction. A thorough review was undertaken from various classical as well as contemporary literature for its identity and it was compared with the XRD analysis. Intensity of the peaks in XRD pattern showed that all three samples were crystalline. Sample No.1 and sample No. 3 consisted of similar constituents i.e. $Al_2Si_2O_5$ $(OH)_4$ - Kaolinite, CaCo3 and Fe_2O_3- hematite with no evidence of silica (quartz alpha). Sample No. 2 consisted of Fe_2O_3-Hematite; Silica (SiO_2)-Quartz alpha; $CaCo_3$ and TiO_2-Titanium Oxide, Anatase with no evidence of Kaolinite. The common view from literature that it is usually prepared by mixing pipe-clay or common chalk with oxide of iron or red ochre seems in consonance with XRD analysis findings in sample No.1 and 3; Sample No. 2 resembled Red Ochre. The findings suggested that among the various samples available in the market, the one that resembles with Red Ochre appears to be genuine drug.

Keywords: *Gile Armani*, Armenian bole, X-RAY diffraction, Clay, *Geru*

Introduction

Gile-Armani (*Amenian bole*) is a mineral origin drug used in Unani system of medicine. Various clay / mineral material are sold under the name of *Gile Armani* (GA). External features of the different market samples are very similar but are slightly differing in colour and shape. Keeping in consideration the controversy over its identification, this study was carried out.

Literature regarding its identity reveals that it is blackish red coloured clay having slender pleasant odour and insipid taste. It is soft, greasy and sticks on tongue. It is described to be brought from country *Armenia and Iran* (Kabiruddin, 2007). The clay which is found in *Armenia* is considered to be a better clay (Rafeequddin, 1985). Important pharmacological actions and uses of GA, are *Qabiz* (Astringent), *Mugharri* (Mucilaginous), *Mujaffif* (Desiccative), *Habis-i-ishal* (Anti Diarrhoeal), Habis-*i-nazf al-dam* (Anti haemorrhagic) / *Habis-i-Dam* (Haemostyptic), Mudammil *Qarhae Ama* and *Qarhae Raham* (heals intestinal and uterine ulcers), *Dafe-i-taffun* (Antiseptic), etc. (Kabiruddin, 2007;

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Rafeequddin, 1985; Ghani, YNM; Hakeem, 2002). It is one of the ingredients of various important Unani formulations such as *Qurse Tabasheer* etc. (Kabeeruddin, 1935). Ibn Baitar by reference of *Jalinoos* mentioned that it is obtained from Armenia which is situated near country of *Balad* and *Qabad* (Ibn Baitar, 1999). *Armenian bole* was commonly used in the formulations of Abulcasis (*Abul Qasam Zahrawi*) (Duffin *et al*., 2013). It is also called as *Rubrica Synopica* (due to its presence in the city of Synope). The name *Rubrica synopica* was given by *Dioscorides* as this was taken usually to Synope to be sold in market. It was also named as Roman earth , Cyprus earth or *Terra sigillata rubra* also called as *sealed earth* (shaped into the coin and stamped) (Duffin *et al*., 2013; Foulk and Pickering, 1935).

Nadkarni (2009) mentioned that "Gile Armani is a calcareous mineral often made into small cakes and stamped with certain impression. It occurs in powder or irregular pieces of reddish brown or variegated colours, it is soft and somewhat heavy. On section it is granular and sprinkled with white particles, and the cut portion resembles a piece of rhubarb". He further describes its property that "When exposed to the air, it absorbs moisture very rapidly, If thrown into the water it readily crumbles into atoms, when put in the mouth it sticks firmly to the tongue" He further stated that "It is usually prepared by mixing pipe-clay or common chalk with oxide of iron or red ochre" But on the contrary, Unani texts mention that it is brought from Armenia or Iran. Since long time, there is controversy on identification of this drug. It was up to the extent that Ibn Sina in the introduction of *Gile Armani* in *Alqanoon* mentioned a substitute drug resembling the action of *Gile Armani* (Ibn Sina, 980-1037A.D.). Keeping all these factors in consideration different market samples of *Gile Armani* were studied with the help of XRD (X-Ray diffraction) in an attempt to resolve the controversy.

Materials and Methods

X-Ray Diffraction study

Different samples of *Gile Armani* (Armenian Bole) were collected from crude drug market of Bangalore and other cities in India. Three differently appearing samples processed from Bangalore, Delhi and Malegaon (MS.) were subjected, for identification and determination of constituents by X-Ray diffraction (XRD) method for crystallographic study. XRD was conducted at the Department of Material Engineering, Indian Institute of Sciences, Bangalore.

Material and sample preparation

Powder method of diffraction was adopted in this study. Fine powder of the three different samples was prepared and passed through 300 mesh sieve for its

investigation. One gram of each sample was taken for the study (Fig.1, Fig.2, Fig. 3).

Method: It was carried out by using X-ray diffractometer (PAN analytical, X' pert pro, X-ray source CU k α (α=1.5418 A°), operating voltage 40 KV/30 MA). For all sample 3 strong peak were chosen at different angles (2θ) from 37.97 to 77.25. X-Ray diffraction studies conducted on different sample of *Gile Armani* were confirmed by comparing d-identified values with d- standard peak values. The 2-theta value and intensity of the peak (counts) were represented on X and Y-axis respectively, and higher peak (count) value indicated higher crystallanity of the phase.

Results

Findings of XRD for 3 market procured samples of *Gile Armani*

- Intensity of the peaks in XRD pattern showed that all three samples were crystalline but out of three samples, Sample no. 2 had better crystallinity as compared to sample 1 and sample 3 (Fig. 4, Fig. 5, Fig. 6, Fig. 7).

Fig. 1: Sample No. 1

Fig. 2: Sample No. 2

Fig. 3: Sample No. 3

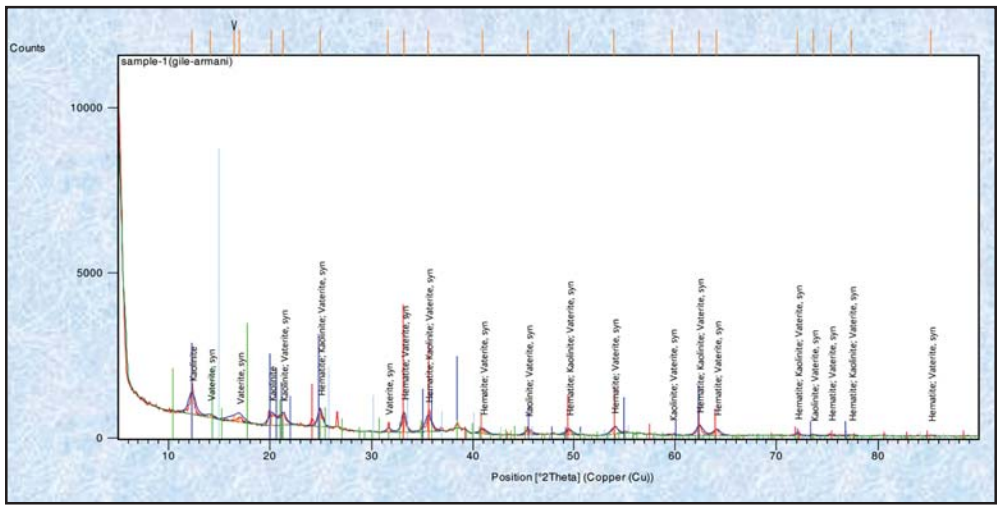

Fig. 4: Graph XRD for Sample No. 1

- Sample no. 1 and sample 3 contained Kaolinite, CaCo3 and hematite with no evidence of silica (quartz alpha). (Fig. 4, Fig. 6). On the other hand Sample 2 contained hematite, CaCo3 and quart alpha with no evidence of Kaolinite (Fig. 5).
- Sample no. 1 and 3 showed presence of Fe_2O_3-Hematite; $Al_2Si_2O_5$ $(OH)_4$ Kaolinite, Aluminium silicate; $CaCo_3$-Vaterite syn. The constitutents of sample 1 and 3 appeared similar to that of common chalk (Fig. 4, Fig. 6).
- Sample no. 2 (*Geru*) showed presence of Fe_2O_3-Hematite; Silica (SiO_2)-Quarts alpha; $CaCo_{3-}$ Calcite form and TiO_2- Titanium Oxide, Anatase, Sample no. 2 is different from 1 and 3 (Fig. 5, Fig.7).

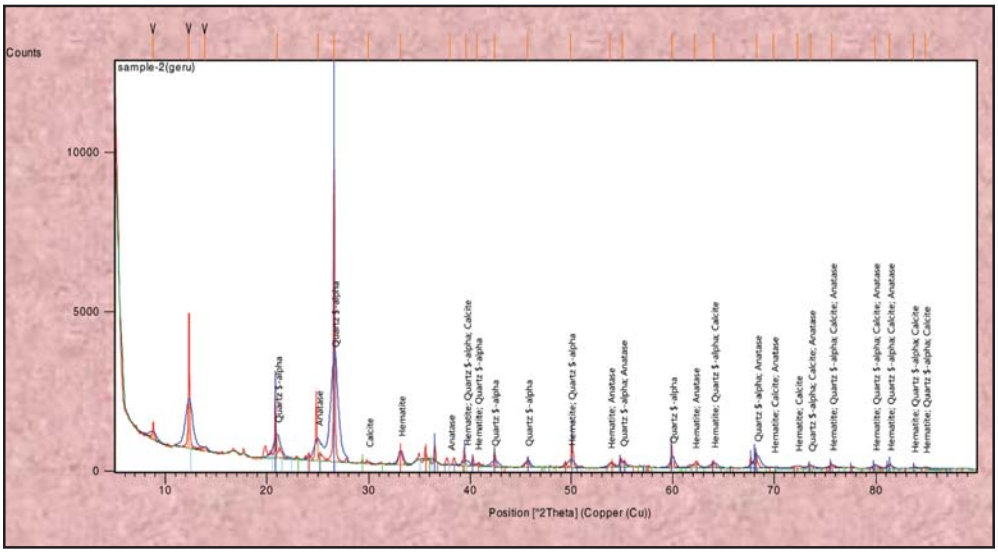

Fig. 5: Graph XRD for Sample No. 2

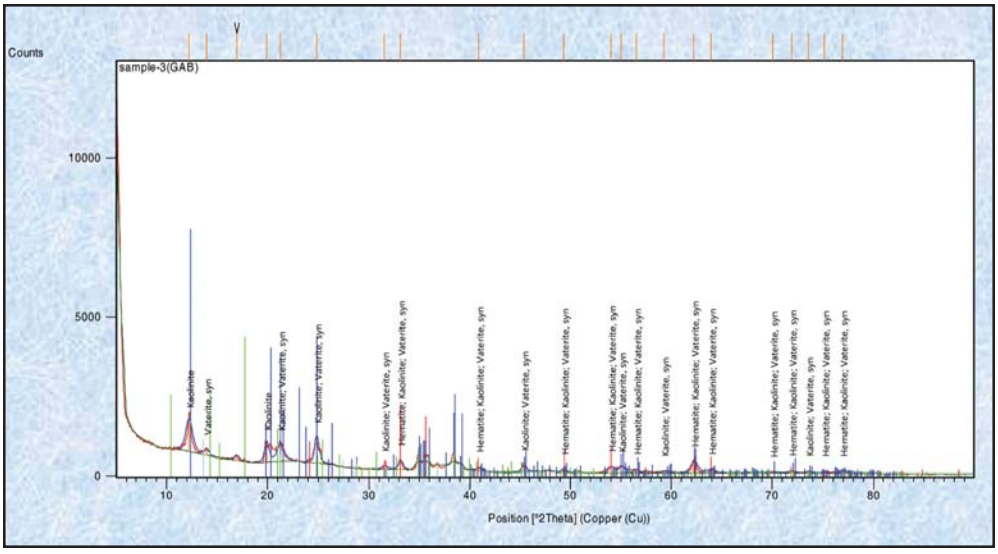

Fig. 6: Graph XRD for Sample No. 3

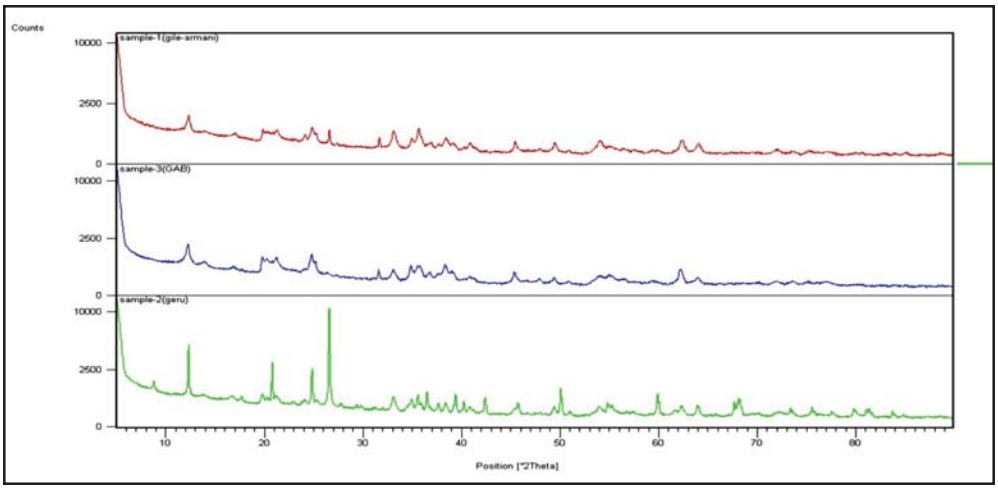

Fig. 7: Combined Graph of sample no. 1¨, 2¨ and 3¨

Discussion

Sample no. 2 was slightly yellowish red and soft, and sample no. 1 and 3 were red and soft. XRD findings of sample no. 1 and no 3 showed presence of Fe_2O_3-Hematite; $Al_2Si_2O_5(OH)_4$ Kaolinite, Aluminium silicate; $CaCo_3$-Vaterite syn. form, common chalk. Sample no. 1 appears to have the similar constitutents as that of the sample no. 3 and therefore both have great degree of resemblance in morphology. The description in the Literature that *Gile armani* is usually prepared by mixing pipe-clay or common chalk with oxide of Iron or red ochre (Nadkarni, 2009) was somehow confirmed by the findings of the present study as in the XRD analysis the presence of iron oxide (Fe_2O_3) pipe clay (Kaolinite) and common chalk ($CaCO_3$) has been shown in sample no. 1 and 3. Sample no. 2 shows presence of Fe_2O_3-Hematite; Silica (Sio_2)-Quarts alpha; $CaCo_3$- Calcite form and TiO_2- Titanium Oxide, Anatase. Its appearance looked like Yellow Ochre. Intensity of the peaks in XRD pattern shows that all three samples are crystalline but Sample no. 2 was having finer crystal structure as compared to sample 1 and sample 3. Sample no. 2 is different from 1 and 3, and its constituents resembled like Red Ochre as per its constituents mentioned in Ayurvedic Pharmacopeia (Anonymous, 2009).

By correlation of XRD findings with authentic literature it can be concluded that this clays sample appears to be of natural combination. Beside this, review of classical / relevant literature showed that Red Ochre was sold in the name of *Armenian Bole*, and also mixture of English Red Ochre or kind of pale red ocher and pipe-makers clay formed into cakes and dried are sold in the name of *Arminan bole.* Generally other clays were given the name of Bole Armenia but the fact is that true Bole Armenia is almost not available in the shops (Pomet *et al.,* 1570).

The characters mentioned in Unani literature of *Gile Armani* such as red coloured, multilayer, soft, slippery, sticks to the tongue etc. (Kabeeruddin, 2007; Ghani, YNM; Ibn Sina [980-1037A.D.]; Mustehasan and Ali, 2004; Anonymous, 2003) matches with several clays including a type of Red ochre (*Geru*) used internally. Review of *Geru* (Red Ochre) enable us better understanding of relation of *Gile Armani* with *Geru*. *Geru* contains oxide of iron, it is a natural mineral pigment found with other iron-titanium oxide minerals in igneous and metamorphic rocks as accessory hematite mineral, associated with magnetite, which is generally found mixed with clay and some other impurities (Anonymous, 2009). Two types of ochre are found in the country, one is red ochre (*Gairika, geru*) contain anhydrous iron oxide Fe_2O_3. 15-65% and other one is yellow ochre containing hydrated iron oxide 15-30% (Nadkarni, 2009; Anonymous, 2003, 2009). According to other classification *geru* is of two types, one is *Pasana* (hard) and other is *Swarna* (soft) and latter is preferred for medicinal use (Vohora and Athar, 2008). One which is red and pure, is called *Soun geru*, second one is light red and impure, commonly known as simply *geru* (Ghani, YNM). *Geru* has been mentioned as a substitute of GA. while GA has also been mentioned as the substitute of *Geru* (Kabiruddin, 2007; Ghani, YNM; Hakeem, 2002). Red ochre is widely distributed in India whereas GA is not found. *Geru* contains silicate of aluminium and iron oxide whereas GA mainly contains silicate of aluminium, magnesium and iron oxide (Vohora and Athar, 2008), Geru may also contain 1% Magnesium and 1% Titanium (Anonymous, 2009). In some recent literature *Geru* is even mentioned as an Indian type of *Gile Armani* (Mustehasan and Ali, 2004). Majority of pharmacological actions of *Gile Armani* and *Geru* have also been described to be similar (Kabiruddin, 2007; Rafeequddin, 1985; Ghani, YNM; Hakeem, 2002).

Findings of the present work and review indicates that *Armenian Bole* sold in the market is either (*Geru)* Red Ochre or dried cakes formed by mixture of Red Ochre and pipe maker's clay. To some extent drug name *Armenian bole* is a case of shift from a locality-related name to a general type mark '*Armenian bole*', which was later used for any clayey red material (Hradila *et al.,* 2003). Probably no such clay is available in market that is brought from *Armenia*. Sample no. 2 of GA can be taken as a genuine substitute of *Gile armani.*

Conclusion

It can be established by review of *Gile Armani* and XRD findings of the samples studied that clay available in market in the name of *Gile Armani* is doubtfully associated to be procured from Armenia. Clay sample no 2 looks closer to the natural combination and its constituents resemble component of Red Ochre. Therefore, it can be concluded that among the various sample of GA available

in the market only sample no. 2 can be said to be the genuine one. None of the available samples have been updated for Armani.

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Pharmaco Botanical Studies on Some Powdered Herbal Drugs for Their Diagnostic Characterization-I

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Abstract

Identity of herbal drug is a paramount aspect of quality and established on the basis of pharmaco- botanical studies which comprise organoleptic, macro and microscopic characterization studies. In the present studies, powdered herbal drugs viz *Cissampelos pareira* Linn., *Cyperus rotundus* Linn. and *Desmodium gangeticum* DC. are subjected to pharmcao-botanical studies leading to their diagnostic characterization. These findings are a tool to establish the identity of powdered ingredients in a formulation or dosages form.

Keywords: *Cissampelos pareira* Linn., *Cyperus rotundus* Linn., *Desmodium gangeticum* DC., Powdered herbal drug.

Introduction

Herbal drugs are powdered for the manufacturing of different dosages forms in Ayurveda, Siddha and Unani medicines.Most common powdered dosages forms are Churna, Kvatha Churna (in Ayurveda), Sufoof (in Unani system of medicine), Churnam and Kudineer Churnam (in Siddha system of medicine). These medicinal powders are single ingredient or multi ingredients (combination of plant, mineral/metal or animal origin drugs). Besides medicinal powders, powdered ingredients are further processed to formulate other dosages forms of respective systems and modern dosages forms.The quality of powdered ingredients is foremost to ensure the quality of medicine. In the exercise of quality assessment, identity of the ingredients is an essential requirement. In this communication diagnostic characteristics of powdered root or rhizome of *Cissampelospareira* Linn., *Cyperus rotundus* Linn. and *Desmodium gangeticum* DC. are studied. These herbal drugs are specifically used in a number of formulations of Ayurveda, Siddha and Unani systems of medicine (Table-1).

Cissampelos pareira Linn. (Family-Menispermaceae) is known as 'Patha'. The drug consists of dried roots of this twining perennial shrub. The roots are also commercially exploited as 'false pareirabrava'. However, the true 'pareirabrava' is reported to be derived from *Chondodendron tomentosum* Ruiz et Par. (Family-Menispermaceae), which is a tropical African species. *Cissampelos* Linn. genus (*Kissos*-ivy and *ampelos*-a vine) have the characters of ivy in its resembling branches that of the vine from the fruits being in recemes. *C. pareira* Linn. is native of south America. *C. pareira* Linn. is anthelmintic, antidote to poison, antilithic, astringent, cardiac, carminative, diuretic, expectorant, febrifuge, sedative, supportive and toxic in action. It is medicinally used for asthma, cold and cough, colic, diarrhoea and dysentery, fever, indigestion, inflammatory

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Table 1: Status of Herbal drugs in different official compendium and systems of medicine

Sl. No.	Botanical Name	Official Name	Pharmacopoeia	Formulary
1.	*Cissampelos pareira* Linn	Patha	Ayurvedic Pharmacopoeia of India, Part-I, Vol.-I	Ayurvedic Formulary of India, Part-I
2.	*Cyperus rotundus* Linn.	Ponmusuttai	-	Siddha Formulary of India, Part-I
		Musta	Ayurvedic Pharmacopoeia of India, Part-I, Vol.-III	Ayurvedic Formulary of India, Part-I
		Sad kufi	Unani Pharmacopoeia of India, Part-I, Vol.-I	National Formulary of Unani Medicine, Part-I
		Korai	-	Siddha Formulary of India, Part-I
3.	*Desmodium gangeticum* DC.	Salparni	Ayurvedic Pharmacopoeia of India, Part-I, Vol.-III &IV	Ayurvedic Formulary of India, Part-I
		Peraamalli	-	Siddha Formulary of India, Part-I
		Desmodium-gangeticum	Homoeopathic Pharmacopoeia of India, Vol.-VI	-

affections of the bladder and kidney (chronic cystitis), nephritic disorders, piles and ulcers. Some authors equate 'Laghupatha' with *C. pareira* Linn. and 'Patha' with *Stephania hernandifolia*Walp. In South India *C. pareira* Linn. is accepted as medicinal plant, but not as 'Patha'. *Cyclea peltala* Diels and other allied species belonging to family Manispermaceae are used as patha (Herman, 1868, Kirtikar & Basu, 1933; Anonymous, 1950, Aiyer and Kolamal 1953-66 Chopra *et al*., 1956; Day, 1980).

Cyperus rotundus Linn. (Family- Cyperaceae) is an annual weed of the pasture lands, road sides and other moist places and grows throughout Indian sub-continent. The dried tubers of plants are officially regarded as 'Musta' in Ayurveda and 'sad kufi' in Unani system of Medicine. Besides its medicinal potentialities, it is also used in certain dye preparations to impart perfume to the fabrics. In

Bengal, dried and pounded tubers are largely used as perfume in the weddings of natives. The generic title of the plant '*Cyperus'* is supposed to be derived from '*Cypris'* – a name of lord venus, as the underground parts of some of the species of *Cyperus* being aphrodisiacal. It is reported that Romans used it as emmenagogue in uterine complaints. The drug is mentioned in AshtangHridya, Bhav Prakash Nighantu, Charak Samhita, DhanvantariNighantu, Sushruta Samhita etc. and also mentioned as 'Nagarmotha' in Unani system of medicine, The drug is often adultrated with allied species and other generic members of family Cyperaceae. (Herman, 1868, Watt, 1889-93; Anonymous, 1969; Chunekar, 1972).

Desmodium gangeticum DC. (Family **-** Leguminosae) is most valued in Ayurveda, being an ingredient of the famous Ayurvedic combination of 'Dasmool' (combination of roots of ten different medicinal plant species). It is officially titled as 'Shalparni'. This drug is elaborated with its medicinal potentialities in Ashtanghridaya, Bhav Prakash Nighantu, Nighantu Ratnakar, Sushruta Samhita, etc. of Ayurvedic classical literature. The identity of the drug has been a subject of controversy. In Travancore –Cochin, *D. gangeticum* is considered as 'Prisniparni and *Pseudarthia viscida* W. & A. as 'Shalparni. Other species such as *D. latifolium* DC. and *Uraria lagopoides* DC. are also used as 'Shalparni'. However, majority of workers have mentioned *D. gangenticum* as Shalparni (Anonymous, 1952, 1978; Aiyer and Kolamal, 1953-66; Chunekar 1972).

Material and Methods

The herbal drugs selected for present study were collected from the natural habitats and authenticated by complying the macroscopoicalcharteristics of these drugs with that of standard reference drug samples available in the museum-cum-herbarium of the Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad, India. To study the powder microscopy, the drugs were first washed under running tap water to remove any dust or soil particles and then air dried for few days at room temperature or in shade,. The dried drugs were then powdered and pass through 120 µm sieve. The fine powder obtained through sieve 120 µm was then subjected to various histo-chemical tests and the temporary mounts of powder prepared to observe under light microscope (Jackson and Snowdon, 1968; Johansen, 1940; Youngken, 1951).

Results and Conclusion

The herbal drugs (entire and powdered) selected for present study was subjected for organoleptic characteristics (Table-2). The powder microscopy was also carried out and characteristics cellular elements and ergastic contents observed in these drugs are given in Table-3.The characters observed may serve as

Table 2: Organoleptic characteristics of herbal drugs

Sl. No.	Botanical Name	Organoleptic Characteristics	
		Entire drug	**Powdered drug**
1.	*Cissampelos pareira* Linn	The drug occurs in the from of dried, cylindrical cylindrical pieces of perennial and seldom branched matured tap roots. The drug varies in size and measures 15.0-24.0 cm in length and 1.0-2.5 cm in diameter. The pieces of roots obtained from the closer portion of shoot system are woody in comparison to other portions obtained from deeper parts of the root. The other portions are generally more fleshy and tuberous. The dried roots are brownish to grey in colour, corky in texture, compressed, entire or splitted longitudinally. The external minute pits and wavy. It also shows vertically branched cracks or fissures. The older pieces of drug exhibits longitudinally ridged surface with transverse cracks. The fracture of the root is short and splintery. There is faint aromatic odour. The taste is at first sweetish and then bitter.	The powdered drug is brown in colour with faint aromatic odour. It has bitter taste which is at first sweetish on chewing.
2.	*Cyperus rotundus* Linn.	The drug comprises of dried tubers of varying sizes. The tubers are oval to spindle shape, somewhat compressed and tapered at both the ends spreading the root system. The tubers generally range from 1.5-3.5 cm in length and 0.5-2.5 cm in diameter. The tubers are unbranched and sometimes flattened or uniformly cylindrical with comparatively longer center portion. These are slightly semi-succulent when fresh, but turn hard in nature after drying. These are dark brown to black in colour and are covered with numerous rootlets. Some of the tubers have scares or remains of rootlets.Tubers are not easily breakable due to smaller size and hardened nature. The fracture is short exposing white interior with light brown dots. The tubers have an aromatic fragrance and a slightly agreeable taste.	The powdered drug is brown in colour with aromatic odour and agreeable aromatic taste.
3.	*Desmodium gangeticum* DC.	The dried matured tap roots are utilized as drug. The roots are simple, branched, long, irregularly curved, light yellow in colour and are of varying length usually 10.0 – 30.0 cm long. The roots are cylindrical and have cord-like appearance. The diameter of roots ranges from 0.5 – 2.5 cm. The whole root system is usually cut into smaller and convenient sizes or occasionally formed as compact bundle consisting of whole root system. The surface of the roots are smooth bearing irregularly distributed small brown lenticels. It breaks with short and fibrous fracture. It has no characteristic odour, but the taste is slightly sweetish and mucilaginous.	The powdered drug is dull yellowish brown in colour with slightly sweetish and mucilaginous taste. It is devoid of any characteristic odour.

Table 3: Diagnostic microscopic characteristics of powdered herbal drugs

Sl. No.	Botanical Name	Diagnostic Microscopic Characters		
		Cellular elements	Ergastic contents	
			Starch Grains	Calcium Oxalate Crystals
1.	*Cissampelos pareira* Linn	Fairly common fragments of phellem which occur in both surface and transactional view, thin walled phelloderm cells containing starch grains and occasional crystals of calcium oxalate, small groups of selerenchymatous cells which are not abundant and fairly common rectangular, thin walled medullary ray cells, containing starch grains. The vessels and trachieds are singly or in groups but usually fragmented. Vessels have articulations with simple pits on wall	Starch grains are mostly simple and some of them are compound with three to five components. Individual starch grains are round to oval some of them are cup shaped.	The occasional calcium oxalate crystals are found scattered or enclosed in cells and are usually in the form of single prisms.
2.	*Cyperus rotundus* Linn.	Occasional fragments of epidermis, a few of them adhering to the cells of hypodermis, abundant thin walled compact, parenchymatous cells of cortical and steler region filled with starch grains; rarely, cells of endodermis associated with parenchymatous or sclerenchymatous cells and occasional moderately thick walled fibres with tapering or blunt ends. The vessels often fragmented occur singly or usually in groups and have reticulate thickening. Parenchymatous cells containing brown tannin content are also fairly common in powdered drug.	Starch grains are simple and abundant in occurrence.	Absent
3.	*Desmodium gangeticum* DC.	Fragments of thick brown phellem cells with or without prismatic crystals of calcium oxalate, a few of fragments are associated with parenchymatous cells of phelloderm, abundant thin walled parenchymatous cells of phelloderm, some of the cells contain starch grains or calcium oxalate crystals or resinous mass, thick walled lignified cells of xylem parenchyma, particularly adhering with vessels, medullary ray cells and abundent fibres which are generally in groups. Fibres are usually fragmented, lignified thickened with narrow lumen and also found associated with thin walled or thick walled parenchymatous cells.	Fairly distributed starch grains are simple, elliptical or spherical having central hilum.	The prismatic calcium oxalate crystals are rarely found scattered independently, mostly enclosed in cells and twin crystals are also present.

diagnostic for identification of these drugs in a formulation.TLC/HPTLC are frequently used for detecting and identifying herbal ingredients in formulations, but the pharmaco-botanical evaluation to confirm the presence or absence of the herbalingredients in the formulations has advantage over chemical methods as later is simple and inexpensive. In addition, the pharmaco-botanical evaluation of herbal preparations is also helpful to detect any deviation from the official formulation not declared on the label.

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