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CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE
61 - 65 Institutional Area (Opposite 'D' Block), Janakpuri, New Delhi - 110 058, India
Tel.: +91-11-28521981, 28525982, 28525983, 28525831/52/62/83/97, 28520501, 28522524 • Fax: +91-11-28522965
Web site: www.ccrum.com • E-mail: unanimedicine@gmail.com & ccrum@rediffmail.com

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Contents

EDITORIAL

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

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

In view of an overwhelming response, the journal earlier published twice a year, its periodicity has now been changed to quarterly w.e.f. January 2008 to accommodate more articles for quick dissemination of research data among scientific community. The journal has sufficient room for invited articles from luminaries of modern medicine and sciences as well as scholars of Unani medicine. The broad areas being covered include clinical research on single and compound Unani drugs, validation of regimental therapy, clinico-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 14 original research and review papers in the areas of clinical research, drug standardization, vermitechnology, agronomy, pharmacology and ethnobotanical surveys contributed by eminent scholars in their respective fields. 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 standard and make HJUM the leading journal of Unani Medicine and related sciences. We sincerely hope and trust that the mission can be accomplished with active partnership of quality-conscious individuals and institutions. Through these lines we seek your cooperation and support in materializing our dreams about the HJUM. In this regard, we request you for your as well as your colleagues' contributions for publication in and subscription to the journal. Further, we will appreciate if the journal is introduced far and wide. We would also welcome esteemed suggestions for achieving the highest standards of quality for the journal.

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

Therapeutic Response of Coded Drugs UNIM-301 + UNIM-302 + UNIM-304 in Waja-ul-Mafasil (Rheumatoid Arthritis) – A Preliminary Clinical Study

Masroor Ali Qureshi and Mohammad Raza

Regional Research Institute of Unani Medicine (CCRUM), Sir J.J. Hospital Compound, Byculla, Mumbai-400008

Abstract

The efficacy of Unani Compound Coded formulations UNIM-301 + UNIM-302 + UNIM-304 was evaluated on one hundred and fifty five patients of Waja-ul-Mafasil (Rheumatoid Arthritis). The drug UNIM-301 was given in the form of tablets while UNIM-302 as hot fomentation and UNIM-307 for local application on the affected joints. The total duration of study was 90 days. The overall response of the trial drug showed complete remission in 40.65% and Partial remission in 32.26% of the patients. The study reveals that the Unani coded compound drug produces significant subsiding of sign and symptoms of Waja-ul-Mafasil (Rheumatoid Arthritis).

Key Words: Waja-ul- Mafasil, Unani Medicine, Rheumatoid Arthritis.

Introduction

Rheumatoid arthritis is a type of Wajaul Mafasil (Arthritis) and has become one of the most pressing public health problems globally. Acording to WHO statistic, it is estimated to be the 31st leading cause of non fatal burden in 0.8% in the world population (anonymous, Ynm). The prevalence in the general population is 0.5% to 1%, and women are at two to three times' greater risk for developing the disease. (Olsson *et. al,* 2007).

Rheumatoid arthritis is the commonest form of chronic inflammatory joint disease. In its typical form, it is a symmetrical, destructive and deforming poly arthritis affecting small and large synovial joints with associated systemic disturbance, a varity of extraarticular features and presence of cerculating antiglobulin antibodies (Rheumatoid Factors). Characteristically, the course of the disease is prolonged with exacerbations and remissions. (Kumar & Clark, 2002; Davidson, 1996; Lee and Weinblatt, 2001).

Early theories on the pathogenesis of rheumatoid arthritis focused on autoantibodies and immune complexes. T-cell-mediated antigen-specific responses, T-cell-independent cytokine networks, and aggressive tumour-like behaviour of rheumatoid synovium have also been implicated. More recently, the contribution of autoantibodies has returned to the forefront based on the pathogenic mechanisms, specific therapeutic interventions can be designed to suppress synovial inflammation and joint destruction in rheumatoid arthritis. (Firesteine, 2003). Although the pathogenesis of rheumatoid arthritis remains incompletely understood, much insight into the cellular and molecular mechanisms involved has been gained in the past decade. (Lee and Weinblatt, 2001; Judex *et. al,* 2005).

According to Unani physicians Wajaul Mafasil (rheumatoid arthritis) is caused by waste matter infiltrated in joints producing inflammations and pain. (Ibne Siena, 980-1037 AD; Ibn Hubal, 1122-1213 AD; Jurjani, 1042-1136 AD). The Unani

physicians classified Wajaul Mafasil according to different etiology/ pathology, causative factors and management. Eminent physician Razi explained the three causes of Wajaul Mafasil as Humoral causes, exciting factors and susceptibility. (Razi, 1860-925 AD). Physicians divided Wajaul Mafasil into two types (i) Sazij (Simple) without involvement of humor, which is found very rarely and (ii) Maddi (humoral). This kind of Wajaul Mafasil is very common in which a humorus always pathologically dominant. It may be single or compound form. (Ibn Siena 1887; Jurjani, 1887). The management of Wajaul Mafasil in Unani system is based on (a) diversion of infiltrated humor from affected joints (b) evacuation of dominant humor and strengthening of the joints against morbid humor. (Ibn Siena, 980-1037 AD; Jurjani, 1042-1136 AD).

In spite of advancement in the treatment of rheumatoid arthritis in modern system of medicine, there is no cure for rheumatoid arthritis in chronic stage. The available allopathic treatments produce severe side-affects on long term use. (Anonymous,ynm).

So there is need to search for some safe and effective remedies from natural sources either plants, minerals or animal source. The Unani drugs are proved to be effective in the treatment of Wajaul Mafasil (rheumatoid arthritis) for hundred of years. Hence a study was planed to evaluate the therapeutic value of certain Unani compound formulations in the treatment of Wajaul Mafasil (Rheumatoid arthritis).

Methodology

A Double blind Clinical trial was conducted to evaluate the efficacy of Unani Compound Coded formulations on one hundred and fifty five patients of Waja-ul-Mafasil (Rheumatoid Arthritis). The Patients were treated for a period of 90 days at Regional Research Institute of Unani Medicine Mumbai. The clinical assessment was done in term of relief in signs and symptoms. The criteria of selection of cases was done on the basis of RA test and Presence of the following sign and symptoms, Joint pain, tenderness, swelling, morning stiffness, swan neck deformity, loss of function, fever ,and palpitation. RA Negative cases were also included in the study on the basis of presence of above sign and symptoms. All the signs and symptoms were assessed on each follow-up. However, general response was assessed on the following parameters (a) Complete remission: 51% - 100% (b) Partial remission: 21%-50% and (c) No remission: Indicates less than 0-29% in the improvement of signs and symptom of disease.

Drug Dose and their Mode of Administration

Two tablets (500mg each) of the drug UNIM-301 were given orally twice a day, the drug UNIM-302 was used for hot fomentation on the affected joints. The patients were advised to boil 40 grams of the drugs in four liter of water for fifteen minutes

and filter it. This filtrate was used for hot fomentation on affected joints twice daily- in the morning and at bed time. The patients were advised to apply the oil UNIM-304 on the affected joints with gentle massage till it absorbed, twice daily in the morning and at bed times.

Follow up methods during and after treatment

During treatment observation were made on $15^{th,}$ 30^{th} , 45^{th}, 60^{th} 75^{th} and 90^{th} day's interval. After completion of treatment, follow up was done once in a month for two months.

Observations

One hundred and fifty five patients of either sex in the age group of 10 to 70 years were taken in the clinical trial, and the effect of Unani compound coded formulations, UNIM-301+ UNIM-302 + UNIM-304 was assessed depending on the above mentioned parameters. Out of one hundred and fifty five patients studies, 36 (23.22%) were males and 119 (76.77%) females. (Table 1)

Results and Discussion

One hundred and fifty five patients suffering from Waja-ul- Mafasil (RA) were treated with Unani compound coded drugs UNIM-301+ UNIM-302 + UNIM-304 for a period of 90 days, the response of the drug was assessed on the basis of clinical signs and symptoms the drugs was found very effective in the treatment of Waja-ul-Mafasil (Rheumatoid Arthritis).

It has been observed that maximum numbers of patients were in the age group of 41-50 years 57 i.e. 37%. (Table 1). The maximum numbers of patients were registered from middle class, 86 (55.48%) (Table 2). It has been observed that out of 155 patients, 81 (52.26%) were assessed Balghami, 34 (34.84%) Damvi, 13 (8.39%) Saudavi and 7 (4.51%) were Safravi temperament (Table 3). The maximum number of patients were females registered in the study, 48 (69.03%) but maximum relief was observed in males i.e. in 27 patients (56.25%) (Table 4). The maximum number of patients according to chronicity was registered whose chronicity was 1-2 years

Table-1. Showing Sex wise classification

S.No.	Sex	No. of cases	% age
1.	Male	36	23.22
2.	Female	119	76.77
	Total	155	100.00

Table-2. Showing classification according to Social status

S.No.	Social status	No. of cases	% age
1	Upper class	20	12.91
2	Middle class	86	55.48
3	Poor class	49	31.61
	Total	155	100.00

Table-3. Showing classification according to temperament

S.No.	Temperament	No. of cases	percentage
1	Balghami	81	52.26
2	Damavi	54	34.84
3	Safravi	7	4.51
4	Saudavi	13	8.39
	Total	155	100.00

Table-4. Showing response of drug according to age

S.No.	Age Group	Complete remission (71-100%)	Partially remission (30-70%)	No response (Less than 29%)	No. of Cases
1	10-20 years	1 (100)	–	–	1
2	21-30 years	6 (54.45)	3 (27.27)	2 (18.18)	11
3	31-40 years	16 (43.24)	12 (32.43)	9 (24.32)	37
4	41-50 years	21 (36.84)	20 (35.08)	16 (28.07)	57
5	51-60 years	14 (45.16)	9 (29.03)	8 (25.80)	31
6	61-70 years	5 (27.77)	6 (33.33)	7 (38.88)	18
	Total				155

i.e. 33 (21.29%) but maximum complete remission was showed in patients with disease duration of 1-6 month i.e. 7 (28.6%) (Table 5). It has been observed that out of 155 patient's 130 patients were relived of joint pain at the end of the treatment. Similarly out of 120 patients, 114 patients who were having tenderness at the base line phase, completely relived at 5th follow-up of the treatment phase. Out of 155

Table-5. Showing Response of drugs according to disease duration

Chronicity	Complete remission (71-100%)	Partially remission (30-70%)	No response Less than (29%)	Total No. of cases
0-1 month	–	1(42.1)	1(42.1)	2
1-6 month	07(28.6)	11(33.3)	12(38)	30
7-12 month	6(12.5)	4(55)	1(32.5)	11
1-2 years	16(9.5)	11(46.03)	06(45.4)	33
3-4 years	17(8.7)	07(56.5)	05(34.8)	29
5-6 years	0911.2	0958.3	0430.5	22
Above 6 years	0821.8	0737.5	1340.6	28
				155

patients 108 patients were found to have swelling at affected joints and, at the end of the treatment phase only 9 patients left whose swelling was not subsided. Similarly out of 155 patients 106 patients were complaining the loss of functions but at the end of the treatment phase only 7 patients with this complaint were left. The patients who were complaining the morning stiffness at base line phase were 130 patients in which 120 patients with relived of morning stiffness at the end of the treatment. It has been observed that only one patient Swan neck deformity at base line phase and he was relived at the end of the treatment phase (Table 6). Out of 155 cases 29 (18.70%) showed RA test positive in which 12 (7.741) cases showed complete remission, 9 (5.890%) Partial remission and 8 (5.16%) showed no response. Similarly 29 (18.70%) cases were found RA test Negative, 51(32.90%) showed

Table-6. Showing response of coded drugs on sign & symptoms

Follow-up	Joint pain	Tender-ness	Swelling	Loss of function	Swan neck deformity	Morning stiffness
Base line	155	120	108	106	1	130
Ist follow-up	144	105	105	90	–	121
2ndfollow-up	87	44	51	45	–	57
3rdfollow-up	58	26	31	33	–	34
4thfollow-up	35	10	8	15	–	17
5thfollow-up	25	6	9	7	–	10

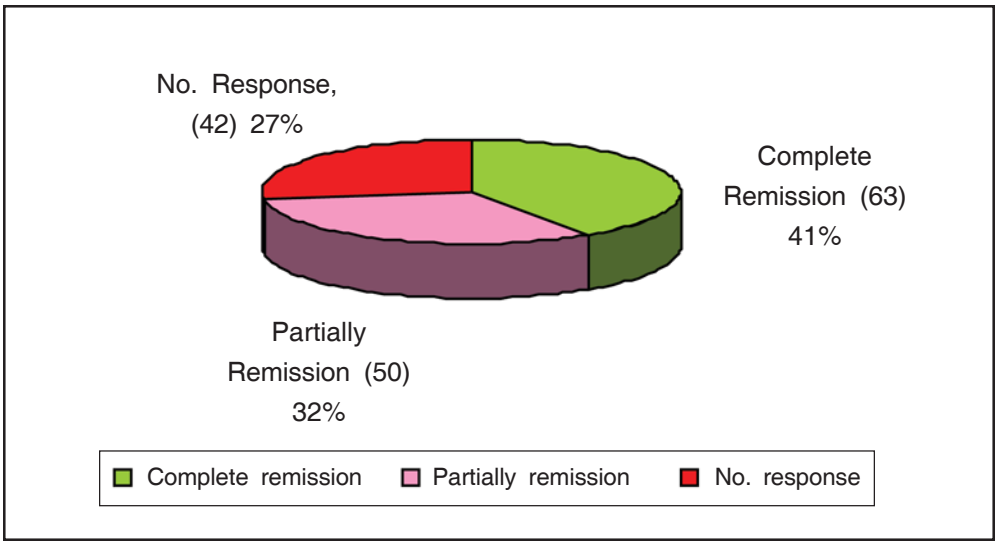
complete remission, 41 (26.45%) partial remission and 34 (21.93%) showed no response (Table 7). The result of the coded drugs UNIM-301 + UNIM-302 + UNIM-304 showed that out of 155 cases treated with coded drugs, 63 (40.65%) showed complete remission, 50 (32.26%) were showed partial remission and 42 (22.71%) patient showed no response (Table 8).

Table-7. Showing statistical data of RA test and response of drugs.

S.No.	RA test	Complete remission (71-100%)	Partially remission 30-70%	No Relief (Less than 29%)	Total No. of cases
1	+Ve	12(7.741%)	9(5.890%)	8(5.16%)	29(18.70%)
2	-Ve	51(32.90%)	41(26.45%)	34(21.93%)	126(81.29%)
					155

Table-8. Showing response of drug

S.No.	Coded Drug	Complete remission (71-100%)	Partially remission (30-70%)	No response Less than (29%)	No. of Cassese
1	UNIM-301 + UNIM-302 + UNIM-304	63 (40.65%)	50 (32.26%)	42 (27.10%)	155



Graph 1. Therapeutic response of coded drugs UNIM-301 + UNIM-302 + UNIM-304

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Pharmacopoeial Standardization and Phytochemical Appraisal of Darhald (*Berberis aristata* DC.)

Aisha Siddiqui, Tajuddin, Kunwar Mohammad Yusuf Amin and R.H. Zuberi

Pharmacology Section
Department of Ilmul Advia
A.K. Tibbiya College
Aligarh Muslim University,
Aligarh-202002

Abstract

The present communication deals with the Physicochemical standardization and Phytochemical study of Darhald (*Berberis aristata* DC.) along with the estimation of marker compound percentage and total alkaloid percentage to ensure the potency, purity and pharmacological comparibility of an important Unani drug, hitherto, not subjected to physicochemical characterization.

Keywords: *Berberis aristata*, Darhald and Berberine.

Vernacular Names

Arabic: Ambarbaris, Aagaris, Huziz-e-Hindi. **Bengali**: Darhaldi, Daruhaldi. **English:** Indian Barberry, Turmeric wood. **Gujarati**: Daruharidra. **Hindi**: Daruhaldi. **Persian**: Dar-chob, Zarishk. **Tamil**: Mullabula, Mullkakala **Urdu:** Darhald.

Description and Importance

For the purpose of quality assurance, the Physico-chemical characterization and Phytochemcial evaluation of Darhald has been undertaken to ensure the potency, purity and pharmacological comparability of the drug.

Darhald (*Berberis aristata* DC.) belongs to the family Berberidaceae. It is an erect, spinous, deciduous shrub usually 1.8 to 3.6 m high, found in Himalyas at an attitude of 1000-3000 m. Dark Yellow in colour. The texture is thick and strong. Its branches and roots yield a colour. Fruits are called Zarishk, smooth shiny, soft and green in colour when unripe and become blackish blue or blackish yellow on ripening and sweet in taste.

Methodology

The test drug the bark of *Berberis aristata* was procured from Delhi market and was identified by Raw Materials Herbarium and Museum, NISCAIR, New Delhi. The drug was coarsely ground in an electric grinder and was sieved using 60 mesh size to get a fine powder, and was then subjected to pharmacopoeial standardization for the evaluation of purity and strength.

Preliminary phytochemical screening of the drug was done according to the method mentioned by Afaq *et al*., 1994 and the results so obtained are given in Table 1.

Physico-chemical Studies

The Physicochemical studies were carried out based on Pharmacopoeial parameters (Anonymous,1968), which include the determination of successive extraction in

Table-1. Qualitative tests for various chemical constituents in *Berberis aristata* DC.

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

various solvents, alcohol and water soluble matter, ash values, loss of weight on drying, pH values, isolation of the marker compound, total alkaloids, colour reactions of powdered drug with different reagents and thin layer chromatography.

Determination of Extractive Values

The extractive values of the drug are reported in percentage after successive extraction of the drugs in soxhlet's apparatus using petroleum ether, di-ethyl ether, benzene, chloroform, alcohol and water as solvents (Anonymous, 1987) (Table 2).

Determination of Alcohol and Water Soluble Extractive

The soluble extractive of Alcohol and water of the drug were undertaken using an electrically operated mechanical shaker for 18-24 hours. The contents filtered and the percentage calculated after drying with respect to air dried drugs (Anonymous, 1968) (Table 2).

Ash Values and Moisture Content

The percentage of Total Ash, acid insoluble ash and water soluble ash were determined by conventional methods and statistically analyzed for standard error (Table 2). The moisture content was estimated as loss in weight on drying at 105°C (Anonymous, 1991).

Table-2. Physicochemical Data of Powdered Darhald

S.No.	Parameters	Results* (Mean +SE)
1.	**Organoleptic characters**	
	Appearance	Solid
	Colour	Yellow
	Smell	Odourless
	Taste	Slightly bitter
2.	**pH**	
	1% solution	4.24 ± 0.014
	10% solution	3.43 ± 0.011
3.	**Moisture content** (loss of weight on drying at 105°C	12.41% ± 0.01
4.	**Bulk density**	1.32 ± 0.01
5.	**Ash values**	
	Total ash	5.28% ± 0.01
	Acid insoluble ash	2.54% ± 0.01
	Water soluble ash	0.62% ± 0.01
6.	**Alcohol and water soluble extractive**	
	Alcohol soluble content	12.53% ± 0.005
	Water soluble content	15.71% ± 0.01
7.	**Successive extractive value**	
	Petroleum ether	0.48% ± 0.002
	Di-ethyl ether	0.36% ± 0.025
	Chloroform	1.55% ± 0.011
	Benzene	0.45% ± 0.020
	Alcohol	8.19% ± 0.012
	Water	9.62% ± 0.012
8.	**Total Alkaloid**	2.85%
9.	**Berberine**	1.2%

*The values are mean of three experiments

Fluorescence Analysis of Powdered Drug with Chemical Reagents

The yellow powder of the drug was treated with various chemicals and chemical reagents to see the specific change in colour, and its fluorescence study was also done under ultraviolet radiation. (Table 3).

Table-3. Colour reactions of the powder of *Berberis aristata* DC. with different reagents

S. No.	Powder + chemical reagents	Day light	U.V. Light (Short)	U.V. Light (Long)
1.	Powder + Conc HNO_3	Brown	Yellowish brown	Dark brown
2.	Powder + Conc H_2SO_4	Dark brown	Brown	Pale brown
3.	Powder + Conc HCl	Light brown	Brown	Dark brown
4.	Powder + Glacial acetic acid	Brown	Light brown	Dark brown
5.	Powder + Glacial acetic acid + HNO_3	Brown	Yellowish brown	Dark brown
6.	Powder + Iodine solution (5%)	Brown	Yellowish brown	Dark brown
7	Powder + Ferric Chloride solution (5%)	Brownish black	Yellowish brown	Black
8.	Powder + NaOH (10%)	Light brown	Yellowish brown	Dark brown
9.	Powder + NaOH (10%) + Conc HNO_3	Brown	Yellow brown	Black
10.	Powder + Dil HNO_3	Dull brown	Yellowish brown	Dark brown
11.	Powder + Dil H_2SO_4	Dull brown	Yellowish brown	Dark brown
12.	Powder + Dil HCl	Dull brown	Yellowish brown	Dark Brown
13.	Powder + Dragendroff's reagent	Blackish brown	Black	Black
14.	Powder + Mayer's reagent	Brown	Light brown	Yellowish brown
15.	Powder + Hager's reagent	Brown	Yellowish brown	Dark brown
16.	Powder + Wagner's reagent	Brownish black	Brown	Dark brown
17.	Powder + Benedict's reagent	Blackish brown	Brown	Dark brown
18.	Powder + Fehling's reagent	Greenish brown	Brown	Dark brown
19.	Powder + KOH (10%) Methanolic	Dark brown	Blackish yellow	Black
20.	Powder + $CuSO_4$ (5%)	Greenish brown	Brown	Yellowish brown
21.	Powder + Ninhydrin (2%)	Brown	Yellowish brown	Dark brown
22.	Powder + Picric acid	Yellow	Yellowish brown	Dark brown
23.	Powder + Lead acetate (5%)	Dull brown	Brown	Yellowish brown

Quantitative Estimation of Chemical Constituent

Estimation of Phytochemical constituent was done quantitatively to ascertain the nature of the drug and also as a tool to evaluate the quality and adulteration. The isolation of the marker compound 'Berberine" was done in addition to total alkaloids (Anonymous, 2005). (Table 2).

Thin Layer Chromatography

Thin layer chromatography of the extract for presence of chemical components was done on precoated Aluminum plates of silica gel GF_{254} (E. merck). Results thus obtained are presented in Table 4. R_f values were calculated precisely and these were matched with the reference sample (Anonymous, 2005).

Table-4. TLC Profile of *Berberis aristata* DC. extract

Extract	Solvent System	Detection/Observation		
		In day light R_f	R_f in UV 254 nm	R_f in UV 366 nm
Methanol	Chloroform:	0.38 (Y)	0.11 (G)	0.18 (LB)
	Methanol:	0.53 (Y)	0.24 (G)	0.37 (DY)
	Formic acid	0.75 (LB)	0.37 (B)	0.50 (FY)
	(8:1.5:0.5)		0.50 (DB)	0.72 (G)

G = grey, Y = yellow, B = brown, LB = light brown, DB = dull brown, DY = dull yellow, LB = light blue, FY = fluorescent yellow

Observations

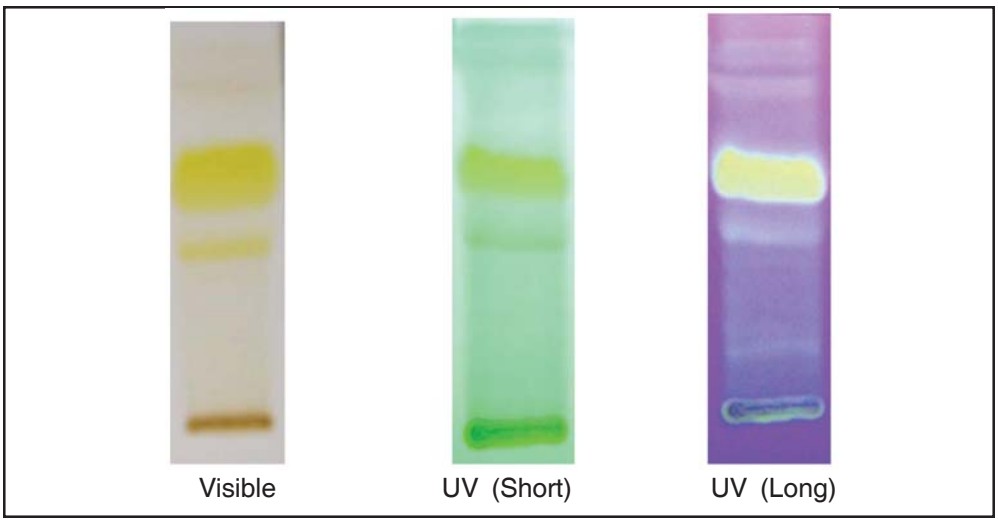

Fig. 1. TLC profile of *Berberis aristata* DC.

Results and Discussion

The results so obtained suggest that the test drug is of genuine quality and has not been exhausted for the water soluble matter. The Berberine content and the Total alkaloid fraction of the drug are found in accordance with the reported results. Moreover as detailed standardiazation of Physico-chemcial and Phytochemical studies of Pharmacopoeial parameters has not been reported, hence the TLC patterns and other parameters, fluorescence characteristic and ultraviolet chromatographic studies could be taken as reliable standard parameters for the establishment of the purity, identity and quality of the sample.

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Effect of Unani Compound Coded Drug (UNIM-352) on Zeequn Nafas (Bronchial Asthma) – A Preliminary Clinical Study

Munawwar H. Kazmi,
Masroor Ali Qureshi,
Nirmala Devi,
Parvez Khan
and
Humaira Bano

Regional Research Institute of Unani Medicine (CCRUM), Sir J.J. Hospital Compound, Byculla, Mumbai-400008

Abstract

The efficacy of Unani Compound Coded formulation (UNIM-352) was evaluated in one hundred seventy five patients of Zeequn Nafas (Bronchial Asthma). The Patients were treated for a period of 180 days in the OPD, at RRIUM, Mumbai. The clinical assessment and Peak Expiratory Flow Rate (PEFR) was done before, during and after the treatment. The clinical assessment was done in term of relief in sign and symptoms and reduction in number of asthmatic attack. The criteria of selection of cases were done on the basis of Presence of some or all the following sign and symptom. Cough, Chest pain, Dyspnoea, Sneezing, Wheezing sound, Vesicular breathing, Bronchial breathing, Changes in Vocal framitus, Rhonchi, Creptations. The clinical result suggested that the drug (UNIM-352) is effective in treating the mild moderate and sever type of Bronchial Asthma.

Key Words: Zeequn Nafas, PEFR

Introduction

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

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

Materials and Methods

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

The clinical assessment was done in terms of relief in sign and symptoms and reduction in number of asthmatic attack. The criteria of selection of cases were done on the basis of Presence of some or all the following sign and symptom. Cough, Chest pain, Dyspnoea, Sneezing, Wheezing sound, Vesicular breathing, Bronchial breathing, Changes in Vocal framitus, Rhonchi, Creptations. All the signs and symptoms were assessed on each follow-up and scored accordingly. However, general response was assessed on the following parameters (a) Complete Relief: 71% - 100% relief in subjective symptoms and objectives signs with reduction in physical signs as assessed on clinical examination with no history of relapse.(b)Partial Relief: 29%-70% relief in subjective symptoms reported by the patients and objective signs along with reduction in physical signs in lungs as assessed by clinical examination. (c) No Relief: Indicates less than 0-29% subjective or objective improvement. The following Investigations were carried out. Complete haemogram: Hb%, total red blood cell count, total and differential white cell count and ESR, Sputum Examination, Its amount, character, test for acid fast bacilli and other bacterial. Dynamic Pulmonary function test, Peak Expiratory Flow rate. Stool examination, Microscopic for any cyst and ova to rule out any parasitic infection

Drug Dose and Mode of Administration

Patients were given a coded Unani compound drug UNIM-352 in the form of *Majune* (paste) 10grams thrice daily orally after meal.

Follow up Methods During and After Treatment

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

Observations

One hundred seventy-five patients of either sex in the age group of 1 to 60years were taken in the clinical study and the effect of Unani coded formulation UNIM 352 and was assessed on the above mentioned parameters. Maximum number of patients registered in the age group of above sixty years (Table 1) Out of 175 patients 90% patients were non-vegetarian (Table 2). Out of 175 patients 48% patients were belongs to middle class group.

Table-1. Showing classification age and sex wise

S.No.	Age Group	Male No. % age	Female No. (% age)	No. of Cassese
1	1-10	7	3	10
2	11-20	47	5	52
3	21-30	24	4	28
4	31-40	50	7	57
5	41-50	68	9	77
6	51-60	75	20	95
	Total	175 (85%)	48 (15%)	175

Table-2. Showing dietary habit

S.No.	Dietary habit	No. of cases	% age
1	Vegetarian	18	10%
2	Non- Vegetarian	157	90%
	Total	175	

Table-3. Showing classification of Social status

S.No.	Social status	No. of cases	% age
1	Upper class	33	19%
2	Middle class	84	48%
3	Poor class	58	33%
	Total	175	

Table-4. Showing classification according to temperament

S.No.	Temperament	No. of cases	% age
1	Damvi	18	9%
2	Balghami	138	78%
3	Safravi	23	13%
4	Saudavi	04	2%
	Total	175	

Table-5. Showing Response of drug of Bronchial Asthma.

Coded Drug	Relieved 71-100%	Partially Relieved	No Relief Less than 29%	No. of Cassese
UNIM 352	20 (12%)	99 (57%)	56 (32%)	175

Table-6. Showing Chronicity wise Response of Bronchial Asthma

Chronicity	Relieved (71-100%)	Partially Relieved (30-70%)	No Relief Less than 29%	Total no. of cases
0-1	03 (15.8)	08 (42.1)	08 (42.1)	10
2-3 years	06 (28.6)	07 (33.3)	08 (38)	14
4-5 years	05 (12.5)	22 (55)	13 (32.5)	23
6-7 years	06 (9.5)	29 (46.03)	28 (45.4)	36
8-9 years	06 (8.7)	39 (56.5)	28 (34.8)	38
10-11 years	04 11.2	21 58.3	11 30.5	19
12-13 years	07 21.8	12 37.5	13 40.6	18
14-15 years	02 5.5	24 66.6	11 30.5	14
More than 15 years	–	2 50	02 50	03
Total				175

Table-7. Showing response in cough against the drug UNIM 352

Coded Drug	Before treatment	After treatment	No. of relieving cases
UNIM- 352	139 (100)	25 (18)	114 (82)

Table-8. Showing response in dyspnoea against the drug UNIM 352

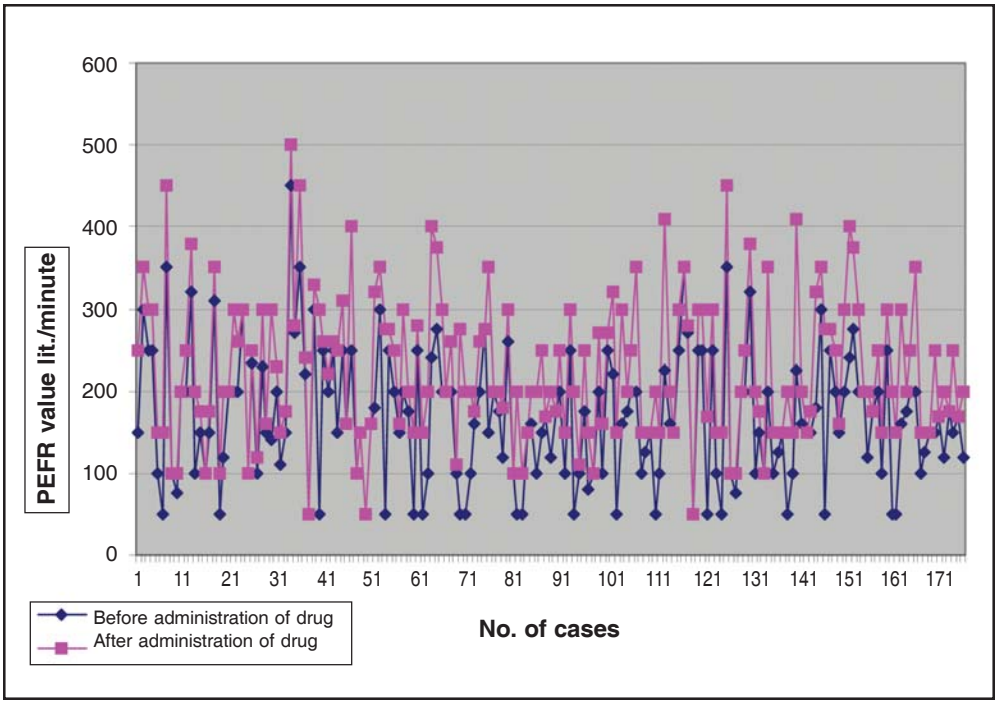
Coded Drug	Before treatment No. (% age)	After treatment No. (% age)	No. of relieving cases (% age)
UNIM-352	61 (100)	36 (59)	25 (41)

Table-9. Showing response in wheezing against the Drug UNIM 352

Coded Drug	Before treatment	After treatment	No. of relieving cases
UNIM- 352	76 (100)	24 (32)	52 (68)

Table-10. Showing response in Rhonchi against the Drug UNIM 352

Groups	Coded Drug	Before treatment No. (% age)	After treatment	No. of relieving
Group A	UNIM- 352	47 (100)	15 (32)	32 (68)



Showing response in PEFR in lit/min in Asthma patients treated with Unani coded drug UNIM-352

Results and Discussion

Three hundred nineteen patients suffering from Bronchial Asthma were treated with Unani compound coded drug UNIM -352 for a period of 180 days the response of the drug was assessed on the basis of clinical sing and symptoms and PEFR value and found very effective in the treatment of Zeequn Nafas (Bronchial Asthma). It has been observed that out of 175 patients 78% patients were assessed as *Balghami* temperament. The efficacy of UNIM -352 found very effective, out of 175 patients 12% patients relived 57% partially relieved and only 32% patients got no relief treated. It has been observed that 28% patients were relived in 2-3 years of chronicity group and 66% patients were partially relived in the group of 14-15 years chronicity and maximum no of patients who were found no relief in the chronicity group of more than 15 years. The response of drug was also observed in clinical sign and symptoms of bronchial asthma in the patients and observed that out of 139 patients, 82% patients with severe cough were relived treated by drug UNIM- 352. Similarly in Dysponea 41 % patients relived. (Table.8). Wheezing was completely disappeared in 68% of cases (Table.9). It has been observed that out of 47 patients, 68 % were found no Rhonchi treated by drug UNIM- 352. (Table.10).

It has been observed that there was 36% enhancement in the Peak Expiratory Flow Rate values after the treatment with coded drug (UNIM-352) in the patients of Bronchial Asthama.

During the drug study no adverse effects of the Unani drugs were noticed clinically. The clinical study concluded that the Unani compound coded drug (UNIM-352) is effective and safe in cases of Zeequn Nafas (Bronchial Asthma).

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Folk Medicinal Plants used by Tribals of Kanniyakumari District, Tamil Nadu

[1]K. Venkatesan,
[1]R. Murugeswaran,
[1]Gowher Sultana,
[2]Mohammed Khalid Siddiqui,
[2]V.K. Singh
and
[2]Aminuddin

[1]Regional Research Institute of Unani Medicine, No.1, West Mada Church Road, Royapuram, Chennai-13.

[2]Central Council for Research in Unani Medicine, 61-65 Institutional Area, Janakpuri, New Delhi-110058

Abstract

Medicinal plants are of great importance to the health of individuals and communities and the medicinal value of these herbs lie in some chemical substances that produce definite physiological action on human body. The tribals prefer the herbal medicine rather than modern medicine for curing their various ailments. Based on ethnopharmecological survey conducted in Kodaiyar, Zeropoint, Keeripari and Poothapandi tribal areas of Kanniyakumari district the paper presents 27 medicinal species used by the varions tribal groups of the area studied for treatment of many disease and conditions. Mode of administration, part used, precautions etc. have been listed for each recipe discussed.

Key Words: Kanniyakumari, Ethnopharmacological survey, Tribal groups, Folk medicine.

Introduction

The use of medicinal herbs in the treatment and prevention of diseases is attracting attention of scientists worldwide. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce definite physiological action on the human body. Exploration of traditional knowledge of medicinal plants is now a key issue the world over and it is our prime responsibility to safeguard traditional knowledge for misuse or overuse by modern societies (Rao, 1996).

Globally, about 85% of the Traditional Medicines used for primary healthcare are derived from plants. In India, approximately two million traditional health practitioners belonging to 4635 communities have been using some 7500 medicinal plant species for human and veterinary healthcare (Anonymous, 2002). Moreover, the country is tenth among the plant-rich countries of the world and fourth among the Asian countries (Hamilton, 1995). Medicinal plants, since time immemorial have been used virtually by all cultures as a source of medicine. The use of traditional medicines and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Anonymous, 1996).

The knowledge of traditional herbal medicine is very important for the people who generate it. While the rural household remedies can be obtained easily, the medicinal plants knowledge of tribals living in forest and hill area is not available so easily. For this reason, the ethnomedicinal documentation of tribal health-system will be of great advantage for further investigations to develop potential medicine for the treatment of many of the dreaded diseases, so the incurable in modern medicine. 80% of the rural population still depend on herbal/tribal medicine for their treatment and cure (Patel and Yadav, 2003).

Ethnomedicinal studies have become the subject of great medicinal importance. Therefore, frequent ethanobotanical survey were coundecting in forest area of Kanniyakumari district, Tamil Nadu. This indigenous knowledge is a potential tool in searching for new medicinal plants for some diseases. This may be obtained by personal interviews and field visit with inhabitant of particular locality. There are valuable regional records of indigenous plants to treat diseases, by tribal and villagers. But there is no specific study on medicinal plants used for various ailments in this district. So keeping all these things in mind, the present study was taken. The paper focus on the plants used by tribals and rural peoples of Kanniyakumari district of Tamil Nadu.

Kanniyakumari district is the southernmost part of Tamil Nadu situated between 77°10' and 77°35' East longitude and 8°5' and 8°35' North latitude. The Kanniyakumari forest division falls in the southernmost tip of the Western Ghats surrounded by Tirunelveli forest division in south by Kodayar left bank channel in South by Kerala state in West and by Tirunelveli district in East.

The present study undertaken presents first-hand field data on ethnopharmaedogical uses of 27 plant species collected and identified from the area investigated.

Material and Methods

Extensive ethnopharmacological surveys were conducted in rural and forest area of Kanniyakumari district during 2007. Based on field interviews and personal observations through repeated interactions with local and participatory rural appraisal (PRA), details on the ethnobotany of the plants pariticularly theose used by the folk population were gathered with tribal villagers using the methods described by Jain (1983). Botanical specimens of all folk drugs reported in the presenal study were collected, identified, herbarium specimens prepared and deposited in the herbarium of Regional Research Institute of Unani Medicine, Chennai, for future reference and study.

Data on folk medicinal plants are presented in tabular form (Table-1) provding information on the botanical name, family, voucher specimen number, Unani name, local name, mode of administration and other related information.

Results

In every ethnic group there exists a traditional health care system, which is culturally patterned. In rural communities health care seems to be the first and foremost line of defense. The WHO has already recognized the contribution of traditional health care in tribal communities. In the present work authors have collected 27 medicinal plant species from different study sites. These species contain valuable chemical substances useful to cure various diseases and conditions.

Table-1. Folk Medicinal Plants used by Tribals of Kanniyakumari District, Tamil Nadu

S. No.	Botanical Name/ Family/Voucher Specimen No.	Unani Name	Local Name/ Part used	Mode of Administration
1	*Ervatamia divaricata* (L) Burkill/ Apocyanaceae/ RRIUM-8873		Nanthiyavatai/ Latex	Latex is externally applied on wounds.
2	*Piper nigrum* L/ Piperaceae/ RRIUM-8876	Filfil siya	Kurumillagu/ Seed	10g crushed seed boiled in 100 ml water is take orally for body pain and fever.
3	*Cardiospermum helicacabum* L./ Sapindaceae/ RRIUM-8878	Qil qil	Mudakutan/ Leaves	50 to 100ml juice of leaves is orally given for joint pain and as laxative.
4	*Randia dumetorum* Lam./Rubiaceae/ RRIUM-8879	Mainphal	Karai/Fruit	5g to 10g fruit powder mixed with hot water is orally given twice daily for gastro intestinal disorders and worm infestation.
5	*Wrightia tinctoria* R.Br./Apocyanaceae/ RRIUM-8880	Inderjao shireen	Veppaly/ Latex and Fruit	1) Latex is externally on forehead applied for headache 2) 10g fruit powder mixed with 15g honey is orally given for general weakness.
6	*Calotropis gigantia* L.R. Br./ Asclepiadaceae/ RRIUM-8883	Madar	Erukku/ Latex	Latex is externally applied on affected part for rat bite and dog bite.
7	*Helicteres isora* L./ Sterculiaceae/ RRIUM-8884	Moror-phali	Edamburi valampuri/ Fruit	Seeds are soaked in coconut oil and applied on hair to reduce hair falling.
8	*Phyllanthus amarus* Sc & Th/ Euphorbiaceae/ RRIUM-8903	Bhui Amala	Kellanelli/ Whole plant	20g – 50g of juice of the whole plant orally given on empty stomach to reduce body heat and urinary infection.

S. No.	Botanical Name/ Family/Voucher Specimen No.	Unani Name	Local Name/ Part used	Mode of Administration
9	*Zizyphus rugosa* Lamk./Rhamnaceae/ RRIUM-8908		Kattu illanthai/ Fruit	10nos of fruits are eaten for indigestion and removal of sputum.
10	*Jasminum malabaricum* Wt./ Oleaceae/ RRIUM-8911	Gul	Kattumalli/ Flower	Required quantity of flower paste is applied on forehead to reduce headache.
11	*Hibiscus rosa sinensis* L./ Malvaceae/ RRIUM-8917	Gudhal	Sembarathi/ Leaf	1) Leaf paste is applied on boils. 2) 50ml flower extract with 20 ml honey orally given to prevent excess menstrual bleeding.
12	*Cassia fistula* L./ Caeselpinaceae/ RRIUM-8923	Amaltas	Sarakondrai/ Fruit	1) Fruit decoction 20-30 ml orally given for stomach disorder and stomach pain. 2) 10ml of fruit powder with warm water is given orally for asthma once daily.
13	*Tephrosia purpurea* (L.) Pers./ Caeselpinaceae/ RRIUM-8920	Sarphuka	Seed	Seed paste is applied externally on scabies and itching.
14	*Terminalia catappa* L./ Combretaceae/ RRIUM-8924	Badam	Badam/Latex	The latex is externally applied on affected part to reduce swelling.
15	*Abrus precatorius* L./Papilionaceae/ RRIUM-8929.	Ghungchi	Gundumani/ Leaf	Leaf paste is applied on forehead to reduce headache and on breast to treat breast pain.
16	*Cassia alata* L./ Caesalpiniaceae/ RRIUM-8930	Dadmar-dan	Seemai akathi/ Leaf and stem	1) Leaf paste applied externally for ringworm. 2) Stem piece is used as miswak to reduce toothache.

S. No.	Botanical Name/ Family/Voucher Specimen No.	Unani Name	Local Name/ Part used	Mode of Administration
17	*Andrographis paniculata* Ness/ Acanthaceae/ RRIUM-8931	Kalmegh	Nela vambu/ Leaves	1) 10g leaf powder is given orally with warm water for fever. 2) Leaf paste is applied externally for body itching.
18	*Cinnamomum zeylanicum* Blume/ Lauraceae/ RRIUM-8933.	Darchini	Lavangam pattai/Bark	50ml bark decoction is given orally to control vomiting.
19	*Ocimum basilicum* L. var. purpuresence/ Lamiaceae/ RRIUM-8903	Babari	Karunthulasi/ Whole plant	20–50g of juice of the whole plant is given orally on empty stomach for body heat and urinary infection.
20	*Wedelia sinensis* Willd./Asteraceae/ RRIUM-8937	Bhangra	Manjal karisalankani/ Leaves	10-20 ml leaf extract orally given to reduce cough and breathing problem.
21	*Amaranthus spinosus* L./ Amaranthaceae/ RRIUM-8941	Chaulai khardar	Mullu keerai/ Leaves	The leaf paste is applied externally on boils and inflammation.
22	*Euphoribia hirta* L/Euphorbiaceae/ RRIUM-8947	Dudhi	Amman pacharasi/ Whole plant	1) 5–10 g plant paste orally given to control worm infestation, body heat and urinary infection. 2) 25 ml leaf extract is given orally to control asthma. 3) Whole dried plant is inhaled as cigarette to control asthma.
23	*Solanum anguivi* Lam./Solanaceae/ RRIUM-8950	Oshtur-ghar	Mullu sundai/ Fruit	Fruit powder is applied on aching tooth for toothache.

S. No.	Botanical Name/ Family/Voucher Specimen No.	Unani Name	Local Name/ Part used	Mode of Administration
24	*Cassia tora* L./ Caesalpinaceae/ RRIUM-8951	Panwar	Thakarai/ Leaves	Leaf paste is applied externally to control ringworm and body itching.
25	*Rauwolfia serpentina* (L.) Benth. ex Kurz/ Apocyanaceae/ RRIUM-8959	Asrol	Serpangantha/ Root	1) Powdered root paste is applied externally on bite area and root decoction 50 ml orally given to snake bite. 2) 10g root powder with warm water is given orally to reduce chest pain.
26	*Aerva lanata* (L.) A. Juss. ex Schult/ Amaranthaceae/ RRIUM-8971	Biseri buti	Poolanbundu/ Root, Leaves	1) Root past is applied on forehead to reduce headache. 2) 10-20 ml leaf juice is given orally for kidney stone (renal calculi).
27	*Hygrophylla auriculata* (Sch.) Heine Acanthaceae/ RRIUM-8991	Talma-khana	Neermulli/ Leaves and Root	Equal part of leaf and root made into juice and 20-30 ml of this juice is given orally to treat jaundice.

As a result of survey, many interesting and useful information about the plants used in folk medicines by tribal communite of the study area were collected. More than 63 tribal pockets and forest villagers were surveyed for collecting the traditionally used medicinal species and gather data from forest villagers.

The species viz., *Ervatamia divaricata, Wrightia tinctoria, Calotropis gigantea* and *Terminalia catappa* are used in the form of latex. The plants such as *Piper nigrum, Randia dumetorum, Cassia fistula, Cinnamomum zeylanicum* are used in the form of decoction. Some plants like *Cardiospermum helicacabum, Phyllanthus amarus, Ocimum basilicum, Hygrophylla longifolia* etc. are used in the form of juice. Whereas, other plants are used as soaked extract, extract, paste, powder and edible fruits. Information on botanical name, family, voucher specimen number, Unani name,

local name, part(s) used, mode of preparation and dosage etc for each claim were collected through field interviews.

It is observed that these medicinal plants are currently used locally in Kanniyakumari forest villages for curing many diseases because these species are easily available and no side-effects reported.

Since modern medicine is not only expensive, and also not easily available in such areas, therefore, *in-situ* and *ex-situ* conservation efforts are needed immediately to maintain the plant-stock of such folk species widely used in traditional medicine in the region.

Discussion

In most of the forest villages of Kanniyakumari district, normally there is one elder who is familiar with the locally available medicines. The tribal folk medicines are practiced mainly by persons of over 50 years of age and with their long experience they are capable of treating common diseases. These people usually use dried plant parts as traditional medicine. The crude materials are used either singly or in combination with other materials. The final products used as medicine are *concentrated extract* and fresh *juice*. The medicines are either given orally or used externally as the case may be. Leaves are widely used as medicine followed by fruit, latex, flowers and seeds in folk treatment by tribals.

Protection of traditional knowledge of the local and indigenous communities seems to be one of the most contentious issues (Tripathi, 2003) and the collection, identification and documentation of ethnomedicinal data on biological resources are in envitable steps for bioprospecting (Rajendran *et al.,* 2002). To understand the therapeutic potential of the traditional medicine, there is a need for more studies of traditional healthcare practices through pharmacological and clinical research.

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Microscopical and Chemical Examination of A Polyherbal Formulation – Majoon-e-Yahya Bin Khalid

[1]S. Mageswari,
[1]Rampratap Meena,
[2]Shamsad Ahmed Khan,
[1]D. Ramasamy,
[1]Gowher Sultana
and
[2]Mohammed Khalid Siddiqi

[1]Regional Research Institute of Unani Medicine (CCRUM), 1 West Mada Church Street, Royapuram, Chennai-600013

[2]Central Council for Research in Unani Medicine, 61-65, Institutional Area, Janakpuri, New Delhi-110058

Abstract

Generally the herbal drugs are adulterated by various methods such as substitution with substandard commercial varieties, inferior drugs or artificially manufactured commodities. Many different procedures are available for the detection of adulteration for herbal products. But, the microscopical techniques play an important role to identify the genuine raw materials which are used in the preparation of Unani formulations. Microscopical examination for crude drug of plant origin is essential to identify certain characters from grounded or powdered materials. The powder crude drugs can be identified based on the presence or absence of different cell types like parenchyma, collenchyma, fibres, stone cells, vessels, trichomes, secretary cells, epidermal cells and also evaluating the cell inclusions such as starch grains, proteins, crystals and also the fixed oil, resins, gums. The data evolved from the present study can be considered for laying down pharmacopoeial standards for the drug Majoon-e-Yahya Bin Khalid.

Key Words: Majoon-e-Yahya Bin Khalid, Microscopy, Physico-chemical, TLC.

Introduction

Majoon-e-Yahya Bin Khalid is Unani polyherbal formulation listed under the category of Majooniath in the National Formulary of Unani Medicine, Part-II (Anonymous NFUM, 2007). The drug has many medicinal properties like Musakkin-e-Alam (Analgesic), Mohallil-e-Waram (Anti-inflammatory) and therapeutically useful in the ailment of Niqras (Gout), Waja-ul-Mafasil (Anthralgia). This herbal formulation consists of 6 plant ingredients. In order to lay down the pharmacopoeial standards, the drug was prepared in laboratory scale and subjected to microscopical, physico-chemical, TLC and microbial studies. Different batch samples were prepared at laboratory scale in Drug Standardization Research Unit, Regional research Institute of Unani Medicine, Chennai and analyzed to compare the data. The present paper describes the salient features of microscopical, physico-chemical, TLC and microbial studies.

Material and Methods

The preparation of the drug includes procurement of raw drugs, identification and authentication, removal of the adulterants if any, powdering to required sieve size, method of preparation, ash determination, extractable matter determination, identification, storage, maintenance, calibration, testing, preparation of reagents and solution, standardisation, raw data, heavy metal and microbial test. The raw ingredients were procured from different market places at Chennai and identified by the botanist using pharmacognostical method (Johansen, 1940).

Formulation/Composition

Majoon-e-Yahya Bin Khalid is a semi-solid preparation made with the following ingredients in the composition as given below:

S. No.	Unani Name	Botanical Name	Part used	Quantity used for preparation	Quantity as per NFUM
1	Asaroon API-VI	*Asarum europaeum* Linn.	Rhizome	75 g	75 g
2	Zanjabeel UPI-I	*Zingiber officinale* Rosc.	Rhizome	75 g	75 g
3	Zeera Siyah UPI-I	*Carum carvi* Linn.	Fruit	75 g	75 g
4	Filfil Daraz API–IV	*Piper longum* Linn.	Fruit	75 g	75 g
5	Suranjan Shireen (Wallis, 1997)	*Colchicum luteum* Baker.	Corm	75 g	75 g
6	Sana UPI-I	*Cassia angustifolia* Vahl.	Leaves	75 g	75 g
7	Qand Safaid	Sugar	–	1. 350 Kg	250 g

Procedure

Pharmacopoeial quality ingredients were taken to prepare the drug. Then all raw drugs were cleaned, dried, powdered separately and passed through sieve number 80. The specified quantity of sugar as per composition was dissolved in 1250ml of water on slow heat, at the boiling stage 0.1% of citric acid was added, mixed thoroughly and filtered through muslin cloth. Then prepared the quiwam of 79% consistency, while hot condition the mixed powders of all ingredients were added along with 0.1% sodium benzoate, mixed thoroughly to prepare the homogenous product.

Microscopy

10g of the sample was mixed with 50ml of water by gentle warming, till the sample gets completely dispersed in water. The mixture was centrifuged and supernatant decant. The sediment was washed with distilled water, centrifuged and a few mg of the sediment was mounted in 50% glycerine and observed under microscope. Camera lucida drawings were done for the salient features of the drug.

Physico-chemical analysis

The physico-chemical parameters such as total ash, acid insoluble ash, alcohol and water soluble extractives, moisture content, bulk density, pH values, reducing and non-reducing sugar (Ranganna, 1977) and heavy metals (Anonymous, 1955; Anonymous WHO, 1998) were carried out.

Thin Layer Chromatography

2g of sample was extracted with 20ml of chloroform and alcohol separately and refluxed on water bath for 30min. Filtered and concentrated to 5ml and used for thin layer chromatography. A known quantity of chloroform and alcohol extracts were spotted on TLC plates and developed in Toluene : Ethyl acetate (8 : 2 and 1 : 1.5) solvent system. After development the plates were allowed to dry in air and examined under UV 254nm and 366nm. Then the plates were dipped in 1% vanillin-sulphuric acid reagent followed by heating at 110° till the spot appears and observed under visible light (Wagner et al., 1984).

Microbial load determination

Total fungal count, Total bacterial count, *Salmonella*, *Enterobacteriaceae* and *Staphylocoocus aureus* were carried out as per WHO guidelines (Anonymous, 1998).

Results and Discussion

The drug is a semi solid, dark brown, agreeable odour and sweet in taste. The drug did not show any filth, fungus or objectionable extraneous matter when the sample was spread in a Petri dish.

Microscopic

The following microscopic characters were observed in different mounts (Fig. 1). Vessels with pitted thickening of length upto 200µ and breadth upto 50µ with oblique end walls and simple perforation plate (Asaroon – *Asarum europeaum* Linn.). Isolated starch grains, simple oval to round shaped measuring upto 70µ, hilum eccentric lamellae distinct, non-lignified septate fibres upto 30µ, reticulate vessels and fragments of reticulate vessels upto 100µ (Zanjabeel – *Zingiber officinale* Rosc.). Thin walled, transversely elongated parenchymatous cell layer with cells interlocked in a regular 'V' joint with neighbouring cells, fragments of vittae in surface view, groups of mesocarpic stone cell layer with polygonal cells not much longer than broad (Zeera Siyah – *Carum carvi* Linn.). Parenchyma cells in surface view with elongated or spindle shaped stone cells

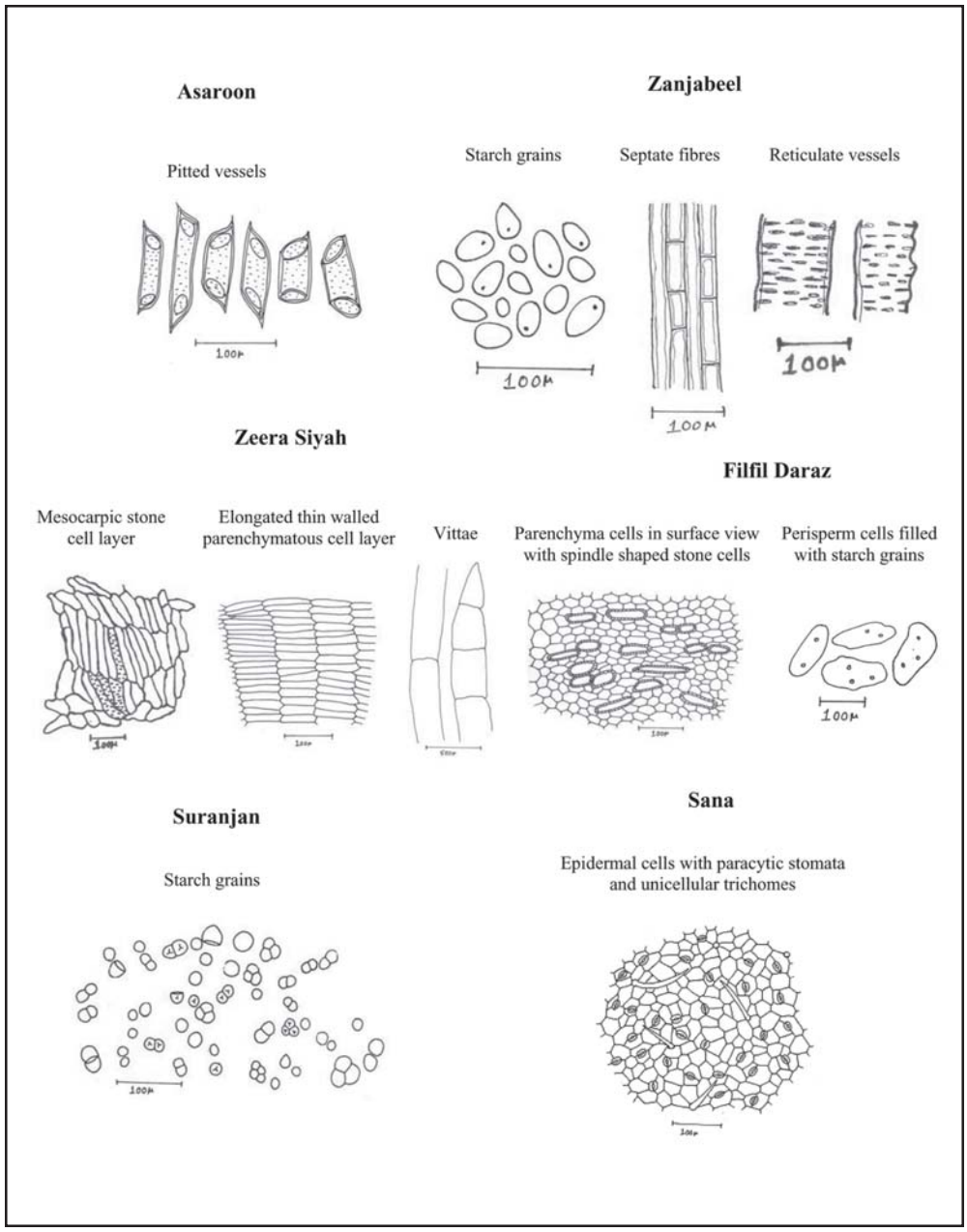


Fig. 1. Majoon-e-Yahya Bin Khalid

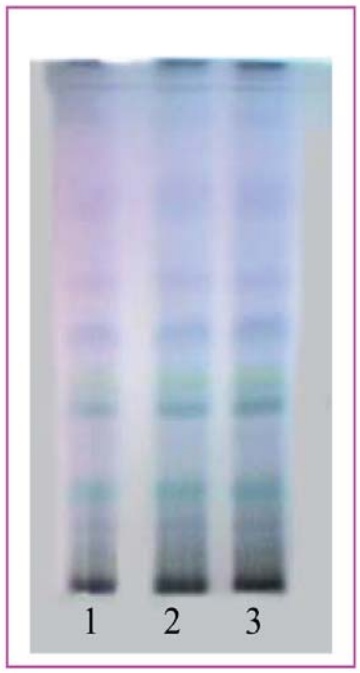
with broad lumen, stone cells isolated or in groups of 2 to 8, large polygonal perisperm cells isolated or in groups of 2 or 3 packed with simple and compound starch grains (Filfil Daraz – *Piper longum* Linn.). Starch grains simple or compound, simple starch grains round to oval with either a point or a two to three radiate split (stellate hilum), compound starch grains usually 2 to 3 sometimes 4 components, each components muller shaped with one or two flat facets (Suranjan – *Colchicum luteum* Baker.). Epidermal cells with straight walled in surface view with paracytic stomata and unicellular trichome (Sana – *Cassia angustifolia* Vahl.).

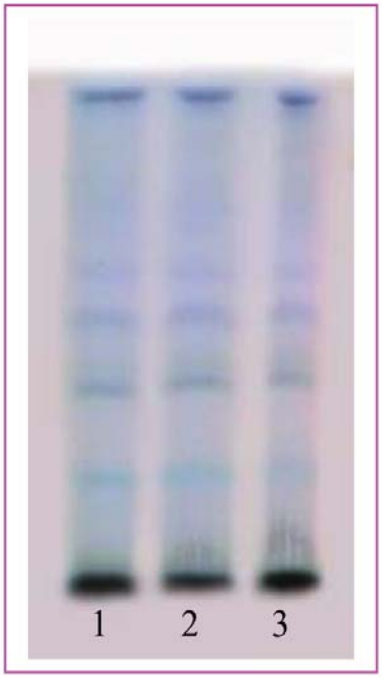
Thin Layer Chromatography

The thin layer chromatographic data of all the three batch samples (Fig. 2) of chloroform and alcohol are shown in Table-1.

Physico-chemical analysis

The Physico-chemical data, heavy metal and microbial test were carried out and the results are shown in Table-2, 3 & 4 respectively.





Chloroform Extract

Solvent system: Toluene : Ethyl acetate (8 : 2)

Detector: V.S. Reagent

Track 1. Batch - I

Track 2. Batch - II

Track 3. Batch - III

Alcohol Extract

Solvent system: Toluene : Ethyl acetate (1 : 1.5)

Detector: V.S. Reagent

Track 1. Batch - I

Track 2. Batch - II

Track 3. Batch - III

Fig. 2. Majoon-e-Yahya Bin Khalid (Thin Layer Chromatography)

Table-1. Thin Layer Chromatography

Extracts	Solvent systems	Rf Values		
		254nm	366nm	VS reagent
Chloroform	Toluene : ethyl acetate 8 : 2	0.96 Pink 0.57 Pink 0.50 Pink 0.39 Pink 0.35 Pink 0.25 Light blue 0.18 Pink	0.71 Red 0.47 Red 0.39 Light blue 0.34 Light blue 0.21 Light blue	0.86 Light blue 0.71 Light blue 0.57 Violet 0.48 Light blue 0.39 Yellowish green 0.34 Bluish green 0.17 Bluish green 0.11 Bluish green
Alcohol	Toluene : ethyl acetate 1 : 1.5	0.66 Pink 0.58 Pink 0.46 Pink 0.41 Pink 0.22 Pink	0.92 Red 0.84 Red 0.74 Light blue 0.53 Reddish blue 0.48 Light blue 0.41 Light blue 0.28 Light blue 0.11 Red	0.64 Light grey 0.45 Red 0.40 Red 0.22 Greenish yellow

Table-2. Physico-chemical parameters

Parameters Analysed	Batch Number		
	I	II	III
Organoleptic characters			
Appearance	Semisolid	Semisolid	Semisolid
Colour	Dark brown	Dark brown	Dark brown
Smell	Agreeable	Agreeable	Agreeable
Taste	Sweet	Sweet	Sweet
Extractives			
Alcohol soluble matter	52.36%; 52.60%; 52.88%	52.16%; 52.44%; 52.56%	52.71%; 52.85%; 52.96%
Water soluble matter	66.78%; 67.20%; 67.56%	66.92%; 67.32%; 67.84%	67.16%; 67.40%; 67.64%
Ash			
Total ash	0.31%; 0.33%; 0.39%	0.33%; 0.34%; 0.37%	0.32%; 0.36%; 0.41%
Acid insoluble ash	0.11%; 0.15%; 0.17%	0.09%; 0.12%; 0.13%	0.11%; 0.14%; 0.15%
pH values			
1% Aqueous solution	6.00; 6.10; 6.30	6.00; 6.20; 6.40	6.10; 6.30; 6.50
10% Aqueous solution	5.10; 5.20; 5.30	5.00; 5.10; 5.30	5.10; 5.20; 5.40
Sugar estimation			
Reducing sugar	38.23%; 38.27%; 38.61%	38.26%; 38.33%; 38.62%	38.42%; 38.57%; 38.66%
Non-reducing sugar	3.34%; 3.37%; 3.42%	3.13%; 3.33%; 3.64%	3.10%; 3.17%; 3.27%
Moisture	21.17%; 21.91%; 22.02%	20.86%; 21.52%; 21.83%	20.95%; 21.29%; 21.46%
Bulk Density	1.3452; 1.3593; 1.3649	1.3604; 1.3711; 1.4058	1.3552; 1.3685; 1.4107

Table-3. Heavy metals

S.No.	Parameter Analyzed	Results	Permissible Limits as per WHO
1	Arsenic	Not detected	10 ppm
2	Cadmium	Not detected	0.30 ppm
3	Lead	1 ppm	10 ppm
4	Mercury	Not detected	1.0 ppm

Table-4. Microbial load

S.No.	Parameter Analyzed	Results	Permissible Limits as per WHO
1	Total Bacterial Count	8,100 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	Nil /gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	Staphylococcus aureus	Absent / gm	Nil

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Clinical Trial of Unani Coded Drug ZS-9 on Type-II Diabetes Mellitus Cases – A Preliminary Study

[1]Mohammad Ataullah Shareef,
[2]S.T.H. Zaidi,
[3]Shariq A. Khan,
[2]S. Parveen,
[2]Zameer Ahmed,
[4]A. Latif
and
[2]M. Taiyab

[1]Central Research Institute of Unani Medicine, A.G. Colony Road, Erragadda, Hyderabad-500838

[2]Regional Research Institute of Unani Medicine (CCRUM), Aligarh-202001

[3]Clinical Research Unit (CCRUM), S.H. Unani Tibbiya College, Burhanpur-450331

[4]Department of Ilmul Advia, A.K. Tibbiya College, Aligarh Muslim University, Aligarh-202002

Abstract

Aim of the present study was to evaluate the therapeutic response of Unani coded drug ZS-9 in patients of Diabetes Mellitus. The major parameters adopted were the effect of the drug on clinical symptom alongwith quantitative estimation of glucose level in the blood (fasting and post parandial) and the qualitative analysis of urine for presence of sugar. Study consisted of forty-six clinically diagnosed cases of Non-Insulin dependent diabetes mellitus treated with ZS-9 in powder form given orally in the dosage of 3 grams twice a day after meals for a period of 180 days. Diabetic diet chart was provided to each patient with instruction to strictly follow the same. Blood and urine sugar tests alongwith LFT and RFT were carried out on every 30 days till the completion of trial. Results were encouraging as 80% of the patients responded to treatment, fresh cases (untreated patients) having responded better than others. No untoward affects were reported and liver and renal function tests remained unaltered. The detailed clinico statistical profile will be presented.

Key Word: Compound Formulation, Type II Diabetes Mellitus

Introduction

Diabetes Mellitus afflicts millions of Indians. While it was previously thought that diabetes is a disease mostly confined to western countries, recent studies have shown that Indians have infact a higher chance of developing diabetes due to their dietary habits, life style and physical and mental exertion. Infact, diabetes affects approximately 10% of adults.

Historical Background

Diabetes Mellitus has been known since ages and the sweatness of diabetic urine has been mentioned in Ebers papyrus. In pharaonic Egypt, diabetes mellitus was recorded to be of common occurrence, especially among wealthy people. Aereatus (150 CE) was the first Greek physician who gave the name to this disease as "DOLLAB" exactly corresponding to the Greek work as mentioned above.

Avicenna (980-1037 CE) a great Arab Physician held that Diabetes is caused by Su-e-Mizaj (dis-temperament) of kidney and also stated that it may be primary or secondary to any other disease. In Unani System of Medicine, Ziabetus (Diabetes) is classified into two types (1) Ziabetus-e-Sukari (2) Ziabetus-e-Sada. The symptoms of Ziabetus-e-Sukari as described by ancient Greek physicians as (1) Excessive thirst (polydipsia) (2) Excessive urination (polyurea), (3) Excessive hunger (Polyphagia), tiredness and loss of weight (Kabiruddin, 1924). According to Azam Khan (1885) in Ziabetus-e-har Sukari urine contains sugar and in Ziabetus-e-Sada, there is no sugar in the urine.

According to Charak and Shushruta ancient great Ayurvedic physician and surgeon had found out the passing of sugar in the urine. Acharya Shushruta described the colour of the urine in diabetes as honey like and Charaka examines the urine of diabetic with ants. He clearly says that the ant rush towards the urine in diabetes. Similarly in order to determine the excess of sugar in the blood he says that the flies are attracted by the sweatness of the smell of the body (Mishra, 1996).

In Unani Medicine the treatment of diabetes mellitus with herbal and mineral drugs is available and described in detail in unani classics. Hakeems have been using herbal drugs in the treatment of Diabetes Mellitus with success. Recently the hypoglyceamic effect of some chemical drugs has failed as reported in some countries and the hypoglyceamic activity of certain herbal drugs has been established and claimed to be very effective in Diabetes Mellitus in India (Pillai, *et al.,* 1981; Mukerji, 1957) and (Bhargava *et al.,* 1985).

There is no known cure of disease only some measures are to be taken to keep the blood sugar level under control. Many indigenous herbal drugs are reported to bring down the blood sugar level are as follows (Nadkarni, 1976; Ibne Baitar, Z.A.).

Material and Methods

46 patients suffering from Type II Diabetes Mellitus of either sex in the age group of 30-70 years were selected for trial with Unani compound formulation coded as ZS-9. Cases were selected from the out patient department of Clinical Screening Unit, Regional Research Institute of Unani Medicine, A.M.U. Aligarh during the years 1987 to 1991.

Criteria for Selection of Cases

Criteria for selection of cases was based upon the presence of clinical signs and symptoms of Diabetes Mellitus viz, polyurea, polydypsia, polyphagia, tiredness, giddiness, cramp in the muscles, numbness and tingling etc. with raised blood sugar level (post prandial not less than 180 mg%) and presence of sugar in urine. Patients fulfilling the above mentioned diagnostic criteria and willing to accept Unani medicine were selected for study. On admission thorough clinical examination was done and detailed clinical history was recorded in a special case sheet designed for the study. Laboratory investigations including complete urine examination, complete blood picture, biochemical investigations including blood sugar (fasting and post prandial), blood urea, serum creatinine, SGPT and SGOT, serum alkaline phosphatase and lipid profile were conducted as base line and after completion of trial for a period of 180 days. Cases of diabetes mellitus associated with other systemic diseases and complications were not included in the study.

S. No.	Botanical Name	Common Name	Parts used
1.	*Azaduracta indica* (Linn.) A. Juss.	Neem (Margosa)	Leaves
2.	*Gymnema sylvestre* (Retz) R.Br.	Gurmar	Leaves
3.	*Trigonella foenum graecum* Linn.	Methi (Fenugreek)	Seeds
4.	*Pterocarpus marsupium* Roxb.	Bijasar	Seeds & bark
5.	*Syzygium cumini* (Linn.) Skeets.	Jamun (Jambolava)	Fruits seeds
6.	*Allium cepa* Linn	Piyaz (Onion)	Bulb
7.	*Momordica charantia* Linn	Karela (Bitter gourd)	Fruit
8.	*Juniperus comminus* Linn	Abhal	Fruit
9.	*Eruca sativa* Mill.	Salad	Leaves
10.	*Allium sativum* Linn.	Lahsan (Garlic)	Bulb
11.	*Morus alba* Linn.	Tutt (White mulburry)	Leaves
12.	*Morus nigra* Linn.	Shahtut (Mulburry)	Leaves
13.	*Cichorium intybus* Linn.	Kasni (Chichorium)	Leaves
14.	*Phyllantus niruri* Linn.	Bhuiamla	Leaves
15.	*Arachis hypogea* Linn.	Mungpalli (Ground nut)	Seed
16.	*Citrullus vulgaris* Schrad.	Tinda	Fruit
17.	*Ficus glomerata* Roxb.	Gulnar	Root
18.	*Ficus religiosa* Linn.	Pipal	Root
19.	*Ceiba pentandra* Linn. Gaetn.	Qutun (Cotton tree)	Root
20.	*Vicia faba* Linn.	Baqla	Flower
21.	*Musa sapientum* Kuntze.	Kela (Banana)	Flower
22.	*Asteracantha longifolia* Nees.	Talmakhana	Root & Seeds
23.	*Anacardium occidentale* Linn.	Kaju (Cashew nut)	Seeds
24.	*Salvia officinalis* Linn.	Garden sage	Leaves
25.	*Brassica oleracea* Linn.	Bandgobi (Cabbage)	Flower
26.	*Grewia asiatica* Linn.	Falsa	Fruit
27.	*Psidium guyava* Linn.	Amrud (Guava)	Leaves
28.	*Cyamopsis tetragonoloba* Taub.	Gowar	Fruit & Seeds
29.	*Dregea volubilis* Benth.	Nakchikni	Fresh Leaves
30.	*Artemisia vulgaris* Linn.	Nagadouna	Leaves
31.	*Rosa canina* Linn.	Ward (Rose)	Leaves
32.	*Oleo europea* Linn.	Zaiytun (Olives)	Leaves
33.	*Ambrosia artimisiaefolia*	Ambrosiya	Herb

Drug, Dose and Mode of Administration

A compound Unani formulation coded as ZS-9 was administered orally in the dosage of 3 grams twice a day after meals.

Duration of Treatment and Follow-up

Duration of treatment was fixed as 180 days. The patient was registered and treated as out patient. Clinical follow up and biochemical investigations follow up was done on 30, 60, 90, 120, 150 and 180 days. Blood sugar fasting and post prandial and urine examination for sugar was done. After completion of trial for a period of 180 days post treatment follow up was done after every 3 months duration till one year. Patients were divided into two groups. Group-I Clinically diagnosed untreated cases. Group-II known case of diabetes taking oral hypoglyceamic drugs and insulin.

Assessment of Response

Assessment of response of the drug was done on the basis of improvement in clinical symptoms and reduction in blood sugar level and categorized as controlled, partially controlled, not controlled.

1. **Controlled –** Subsidence in all clinical symptoms with normalization of blood sugar level fasting and post prandial. Absence of sugar in urine.
2. **Partially controlled** – subsidence in the clinical symptoms, reduction in the blood sugar level (fasting and post prandial) and in the urine.
3. **Not controlled** – Clinical symptoms persists. No reduction in blood sugar level (fasting and post prandial) and urine remains positive for sugar.

Results and Discussion

46 patients suffering from Diabetes Mellitus (NID) of either sex in different age group from 30-70 year with relation to their occupation, socio-economical condition, dietary habits, family history and chronicity of disease were studied. The effect of Unani compound formulation coded as ZS-9 was assessed on Type II diabetic patients of different group like (1) Clinically diagnosed untreated cases (2) known case of diabetes taking oral hypoglyceamic drugs.

Diabetes occurs at all ages but most common in middle age and late life (Briedhl, et. al., 1985) and (Das, 1974). It was found during the trial in the series of 46 cases of diabetes mellitus the maximum number of cases registered were in the age group of 40-49 years i.e. 19 (41.3%) followed by 16 (34.8%) in the age group of 50-59 years. Males were more affected than the females i.e. 28 (60.87%) males and 18 (39.13%) females (Table-1).

Table-1. Showing age and sex wise classifications

Age in years	Sex		Total	Percentage
	Male	Female		
20 – 30	—	—	—	—
31 – 40	05	02	07	15.2
41 – 50	10	09	19	41.3
51 – 60	11	05	16	34.8
61 – 70	02	02	04	8.7
71 & above	—	—	—	—
Total Percentage	28 (60.87%)	18 (39.13%)	46	100.0

Occupation plays an important role in the occurrence of disease, physical and mental stress and strain may play a significant role in the causation of disease (Achal 1966)[12]. It is very clear with the present study that the occurrence of diseases is found more among employees of different categories like teacher, officials, engineers, lawyers etc. In this series of 46 cases of diabetes mellitus of which maximum 24 (52.2%) cases were occupationally employees. The next highest percentage of occurrence of disease was found among house wives i.e. 16(34.8%) and the remaining 5(10.8%) occupationally businessmen and 1(2.2%) were occupationally farmer (Table-2).

Diabetes occurs in preference to rich people over the poor, in comparison to manual labourers to sedentary and ideal life style, and intake of rich food. Out of 46 cases studied, 23 (50%) cases belonged to high income group followed by 16(34.7%) of middle income group and 7 (15.3%) to low income group. (Table-3).

Diet plays an important role in the occurrence of disease. It is observed with the present study that the occurrence of disease is more among non-vegetarians i.e.

Table-2. Showing classifications according to occupation

S.No.	Occupation	No. of cases	Percentage
1	Service	24	52.2
2	Business	05	10.8
3	House wife	16	34.8
4	Farmer	01	2.2
	Total	46	100.0

Table-3. Showing socio economical condition

S.No.	Income group	No. of cases	Percentage
1	High Income group (Rs.2001 & Above)	23	50.0
2	Middle Income group (Rs.1500 to 2000)	16	34.7
3	Low Income group (Rs.1500 & below)	07	15.3
	Total	46	100.0

38(82.2%) as compared to the patients having vegetarian dietary habit i.e. 7(15.6%) out of 46 cases (Table-4).

The type II diabetes (NID) which is by far the more common type arises in middle and older ages and affects people who are over weight and atleast initially do not need insulin for control. Factors, which predispose to this kind of diabetes mellitus include a positive family history of diabetes. Chronicity of disease was also studied in 46 selected cases of diabetes, of which 24(52.2%) cases had 1 to 5 years chronicity and 7(15.2%) cases had 5 years chronicity of disease and the remaining 15(32.6%) cases were having less than 1 year

Table-4. Showing Dietary habits

S.No.	Dietary habits	No. of cases	Percentage
1	Vegetarian	08	17.4
2	Non-vegetarian	38	82.6
	Total	46	100.0

Table-5. Showing Chronicity of disease

S.No.	Chronicity of Disease	No. of cases	Percentage
1	Less than 1 year	15	32.6
2	1 to 5 years	24	52.2
3	6 to 10 years	06	13.1
4	11 to 15 years	01	2.1
	Total	46	100.0

chronicity (Table-5). Family history of disease was studied in 46 cases registered for clinical trial of which 13 (28.3%) cases were having positive family history of diabetes mellitus. (Table-6).

In this series of 46 cases of diabetes studied of which the majority cases i.e. 32(64.56%) were having previous history of anti diabetic allopathic medical treatment and the remaining 14(30.44%) cases were fresh and untreated (Table-8).

Diabetes mellitus is a global problem of the day inspite of several oral hypoglycemic synthetic drugs available but there is no lasting cure. Hypoglycemic activity of certain herbal drugs is established and claimed to be very effective in diabetes. Clinical as well as pharmacological studies of some herbal drugs like *Allium sativum* (Lahsun), *Juniperus communis* (Abhal), *Trigonella foenum-graecum* (Methee), Azadiracta indica (Neem), *Syzygium cumini* (Jamun), *Momordica charantia* (Karela), *Cichorium intybus* (Kasni); (Pellai, 1981); (Bhargava, 1986) (Rehman, 1987) are available which favour their antidiabetic properties.

Pharmacological studies of ZS-9 coded Unani compound formulation was conducted at Pharmacology Research Unit, Regional Research Institute of Unani Medicine, A.M.U., Aligarh during the year 1987. Hypoglyceamic activity of coded drug ZS-9 as assessed on normal rabbits as well as alloxan induced diabetic

Table-6. Showing family history of Diabetes

S.No.	Family History	No. of cases	Percentage
1	Father	01	2.17
2	Mother	03	6.52
3	Father and Mother	01	2.17
4	Brother	03	6.52
5	Sister	01	2.17
6	Uncle (Maternal side)	02	4.34
7	Aunty (Maternal side	02	4.34
	Total	13	28.3%

Table-7. Showing treated and untreated cases

S.No.	Treated/Fresh (Untreated)	No. of cases	Percentage
1	Treated	32	64.56
2	Untreated (Fresh)	14	30.44
	Total	46	100.00

rabbits shows promising results i.e. 12% to 13% reduction in blood sugar level (Khan, 1987). The effect of Unani coded drug ZS-9 was assessed clinically as well as on biochemical parameters including Blood sugar fasting and post parandial in 46 cases of diabetes mellitus (NID). The response of coded drug ZS-9 was found very effective in clinically diagnosed untreated (Fresh) cases. Response of the drug was also found very encouraging in the cases who were taking oral hypoglyceamic drugs (Allopathic). Out of 46 cases treated with ZS-9 coded drugs 18(32.2%) cases were controlled and 19(41.3%) cases were partially controlled and the remaining 9(19.5%) cases did not respond with the treatment. (Table-8). The response of coded drug ZS-9 was found to be good on other biochemical parameters like Blood urea, serum creatinine, and liver function test. Post treatment follow up for a period of 1 year was done in maximum number of cases and showed satisfactory results in the cases who were following diet restrictions. Good improvement in clinical symptoms was found. The oral hypoglyceamic allopathic drug was tapered gradually. No adverse effects were observed during clinical trials and no severe hypoglyceamic symptom was seen in any case.

Table-8. Showing therapeutic response of unani coded drug ZS-9 in Diabetes Mellitus cases

S.No.	Duration of Treatment	Response	No. of cases	Percentage
1	180	Controlled	18	39.2
2	180	Partially controlled	19	42.3
3	180	Not controlled	09	19.5
	Total		46	100.0

Summary

46 selected cases of Type II Diabetes Mellitus of either sex between 30 – 70 years of age were treated with a compound Unani coded formulation ZS-9 for a period of 180 days as out patients. Clinical follow up during the course of treatment was done on every 30 days till the completion of treatment. Clinical symptoms were recorded and biochemical examination on blood sugar level fasting and post prandial were checked and recorded on each follow-up. After treatment clinical and biochemical follow up was done for a period of one year. Treatment outcome was evaluated on the basis of subjective and objective parameters like subsidence of clinical symptoms and reduction of blood sugar level post parandial. The treatment showed good result as out of 46 subjects 18(32.2%) cases were controlled and 19(41.3%) were partially controlled.

Acknowledgement

The authors are grateful to Dr. Mohammed Khalid Siddiqi, Director, Central Council for Research in Unani Medicine, New Delhi for providing facilities for the study.

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Botanical and Physico-chemical Studies of Unani Herbal Drug – "Qimbeel" (*Mallotus philippensis* (Lamk.) Muell. – Arg.)

[1]Sonali Sajwan,
[2]Kunal Sajwan,
[1]R.K. Negi,
[3]U.C. Aggrawal
and
[1]D.R. Lohar

[1]Drug Standardization Research Institute (CCRUM), PLIM Building, Sanjay Nagar, Ghaziabad, U.P.

[2]DSRU CCRUM, Jamia Hamdard, New Delhi.

[3]Department of Botany, D.S. College, Aligarh, U.P.

Abstract

The present study has been taken up to establish certain standards which may help in correct identification of drug while in crude form. The communication deals with macroscopy, microscopy; powder study, important physico-chemical studies and HPTLC. These parameters will be helpful for the standardization of drug.

Key Words: Standards, Standardization, Qimbeel, *Mallotus philippensis* (Lamk.) Muell.-Arg.

Introduction

The Unani single drug 'Qimbeel' is derived from glands and hairs of fruits of *Mallotus philippinensis* Muell (Family-Euphorbiaceae).These are obtained by rolling the fruits ina cloth and basket The drug plant species is a shrub or a small,much branched evergreen tree with a short and often buttressed bole found through out India occasionally ascending to 1,500m. in the outer Himalyas. It is used in India for the treatment of tapeworm infestation, abdominal disorders blood diseases, leprosy, skin diseases and also for treating poultry (Anonymous; 2005). In unani system of medicine Quimbeel is mixed with Roughan-e-Gul (an unani compound formaulation comprising *Rosa damascena* and *Sesamum indicum* as ingredients) for the treatment of boils, eruptions and in scabbies and pruritus (Anonymous; 2006).

Material and Method

The identified and authenticated plant material was resourced from natural habitat at Raiwala (Distt.Dehradun, Uttrakhand). Microscopic and physico-chemical studies were carried as per Standard methodologies prescribed in Unani Pharmacopoea (1998). For HPTLC fingerprinting, a Desaga HPTLC system equipped with a sample applicator AS 30 were used.

Observations

Macroscopic

Fine, granular, brick red, inodorous and tasteless powder, float on water surface.

Powdered Microscopy

Microscopic examination reveals the presence of numerous globular glands containing red resin and groups of unicellular trichomes which are generally curved or hooked at their ends. (Plate 1 and 2).

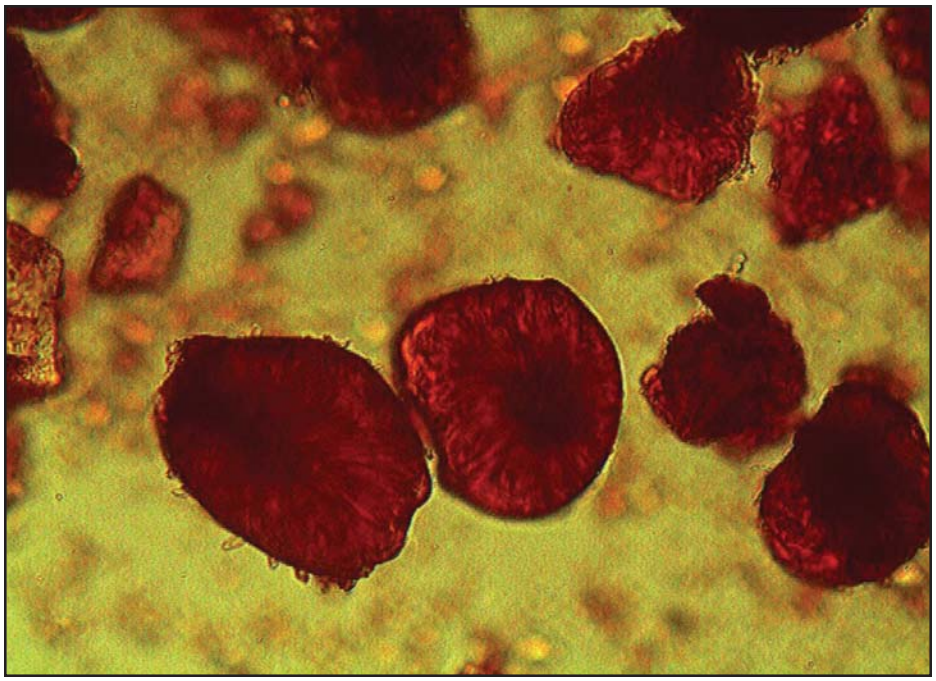


Plate 1. Qimbeel Powder showing glandular trichome, 40X.

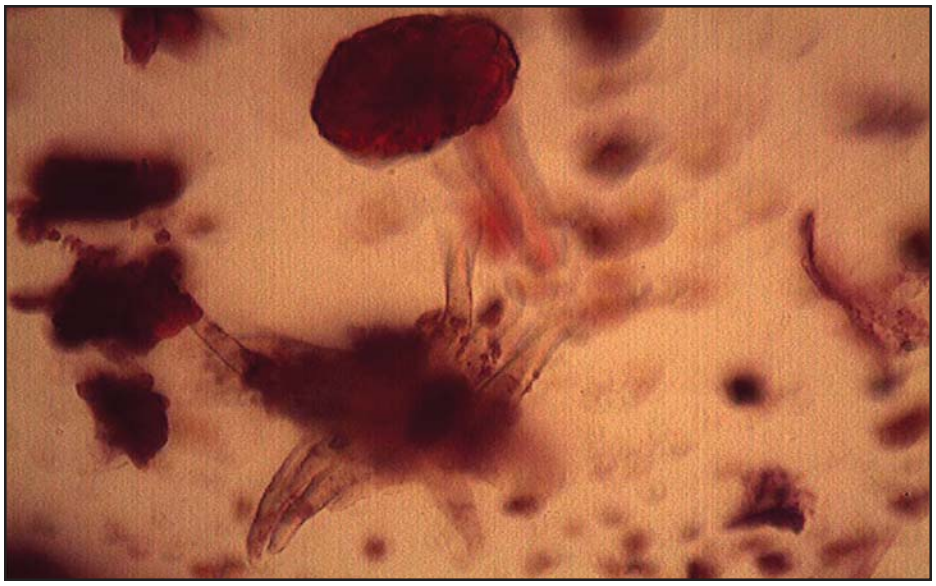
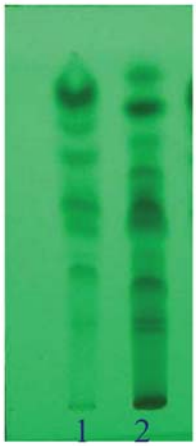


Plate 2. Qimbeel Powder showing glandular and stellate type of trichome, 40X.

MICROSCOPICAL FEATURES

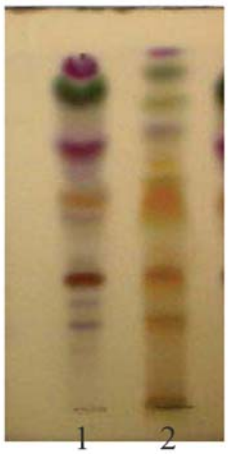
Physico-Chemical Studies

Total ash, (%) w/w	:	6.52
Acid Insoluble ash, (%) w/w	:	4.38
Ethanol Soluble extractive, (%) w/w	:	52.0
Water soluble extractive, (%) w/w	:	1.5



UV 254 nm

VISIBLE



AFTER DERIVATION

Sample : 1. Alcoholic Extract 2. Pet. Ether Extract
Solvent System : Toluene: Ethyl acetate (70: 30)
Spray Reagent : Vanillin Sulphuric Acid

Plate 3. TLC Profile of Quimbeel

HPTLC Profile

For TLC profile, the solution of the drug was prepared with dissolving 1 mg of the extract in 10 ml methanol. Extracts of the drug was applied on a pre-coated silica gel TLC plates (E. Merck) of uniform thickness with the help of Desaga HPTLC applicator AS 30 and the TLC was developed in solvent system Toluene: Ethyl Acetate (9:1) in the development chamber Photograph was taken with the help of Desaga Video documentation unit. Rf Values of Alcoholic Extract, 0.17, 0.25, 0.31, 0.37, 0.42, 0.54, 0.6, 0.73, 0.88, 0.94. Rf Values of Petroleum Ether Extract, 0.25, 0.37, 0.57, 0.63, 0.68, 0.77, 0.81, 0.94, 0.99. (Plate.3)

Results and Discussion

Qimbeel is one of the highly efficacious herbal drugs in Unani medicine. The drug is commercially exploited and often adulterated with brick powder and coloured wood dust. This adulteration can be detected by microscopy and physico-chemical tests suggested in present studies.

Acknowledgment

The authors are thankful to Dr. M.K. Siddiqui, Director, CCRUM, New Delhi for providing facilities and constant encouragement.

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Anti-inflammatory Activity of Earthworm (*Eutyphoeus incommodus* Beddard) Extract: A Preliminary Study

Shachi Bharati, Deepshikha Verma and Shweta

Vermiculture Research Station, Department of Zoology, D.S. College, Aligarh, U.P., India

Abstract

A drug preparation namely, Total Extracted Paste (TEP), prepared from earthworm *Eutyphoeus incommodus* Beddard has been investigated for its anti-inflammatory activity. The efficacy of drug has been observed to be at par with that of 'aspirin' in carrageenan induced oedema. Preliminary results are encouraging and further work is in progress.

Key Words: Antiinflammatory, *Eutyphoeus incommodus* Beddard, TEP.

Introduction

The Unani System of Medicine calls earthworm as *Kharateen*. According to Vohra and Khan (1978) Kharateen in the Unani medicine is used both internally and externally as a powerful aphrodisiac. Besides this, they have also reported the role of earthworms in the healing of wounds, chronic boils, piles and sore throat etc.

Hamdullah Mustanfi of Qazwin in the Nazhat-ul Qulub, a scientific encyclopedia written in 1340 AD mentions that earthworm baked and eaten with bread reduces the size of stones in the bladder and bring about its expulsion. When earthworms are dried and eaten they cure jaundice patients. In difficult labour they are reported to facilitate delivery.

Graff (1974) has reported significant anti-pyretic activity in the earthworms (*Lumbricus spencer* and *Perichaeta comunisima*) after testing them on rabbit made pyretic by injecting pyrogens from *Escherichia coli*. Because of its anti-pyretic properties earthworms are reportedly used in China and Japan in dry form for fever. Earthworms when taken orally for systemic effect, increase body heat and are valued in nerve disorders, bronchitis and tuberculosis (Tato *et al*., 2006). The anti-inflammatory property of anecic earthworm (*L. mauritii*) has been reported by Yegnanarayan *et al*. (1981, 1987). This demonstrates the great medicinal potential of earthworm in Unani Medicine. Based on this rationale, the present study has been undertaken with a view to scientifically investigate the anti-inflammatory effect of the drug TEP (prepared from earthworm, *Eutyphoeus incommodus*) by carageenan induced oedema test in albino mice and compare it with the standard anti-inflammatory agent 'Aspirin'.

Material and Methods

Earthworm (*Eutyphoeus incommodus*) were collected from garden soil of Malviya Library, Aligarh, transferred to laboratory, washed in running water, cleared of any debris, and allowed to feed on moist filter paper for 30 h to clear the gut.

Eutyphoeus incommodus Beddard

Length, 96 mm; 138 segments: clitellum annular and composed of five segments; proboscis tanylobic; First dorsal pore on 11/12 intersegmental; male pore on 17th segment and conspicuous; genital marking present; spermathecal pore on 8/9 intersegments and shortly stalked, ental diverticula in the form of a circle of 10 seminal chamber, Prostant gland tubular and nephridia holonephic (Fig. 1).

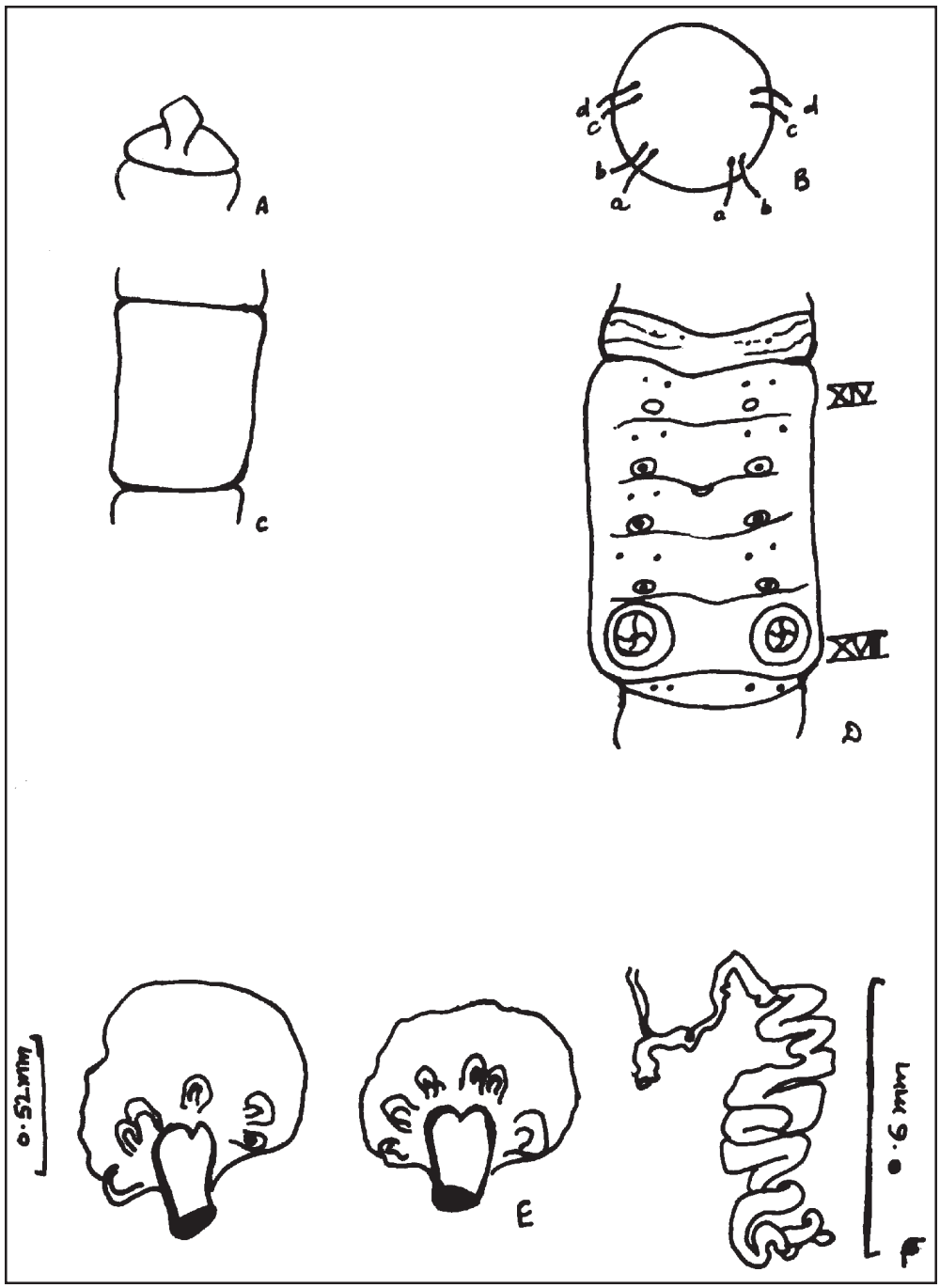

Fig. A-F. ***Eutyphoeus incommodus Beddard:*** **A. Prostomium, B. Setae, C. Clitellum, D. Genital region, E. Spermatheca, F. Prostate gland.**

The worms were then ready for the preparation of the drug referred to as TEP (Total Extracted Paste). Worms were again thoroughly washed in running tap water and if any worm was observed to be injured or unhealthy it was removed from the sample. Worms after being rewashed in distilled water were rolled on dry filter paper to remove as much moisture as possible without harming the worms. These worms were transferred into clean, dry, sterilized covered beakers.

Ethanol was applied for 10-15 minutes until the activity of the worms ceased (Edwards, 1988). Then, worms were taken out and placed in an oven at 50-52^{o}C. This preparation was dried with occasional stirring until a solid sweet smelling paste (TEP) was formed, which was cooled and stored in a desiccator. Petroleum ether fraction was used to assess the efficacy of the drug.

Carrageenin Oedema Test

Fifteen healthy albino mice (150-200 g) were selected and were divided into three groups of five mice each. Pedal oedema was produced by injecting 0.1 ml carrageenan in the left hind paw. Paw volumes were measured plethysmographically before and after 3 h of injection to record degree of inflammation. The first group offered (control) was administered with distilled water orally, the second group aspirin (100 mg/kg body weight) and the third group TEP (160 mg/kg body weight). The dose of 160 mg/kg body weight were standardized (Yegnanarayan *et al*., 1987). Paw volumes were measured after 1 h and 15 h of treatment. Means and standard deviation of triplicates were determined and all the figures presented include standard errors of the data. Analysis of variance (ANOVA) was carried out.

Table 1. The anti-inflammatory activity of the petroleum ether fraction of TEP in carrageenin Oedema Test

Experiment	Paw volume (ml)		
	Control	Aspirin	TEP
Normal	2.54 ± 0.17	2.47 ± 0.18	2.40 ± 0.09
3 h after carrageenan	3.06 ± 0.17	3.50 ±0.20	3.20 ± 0.20
1 h after treatment	3.60 ± 0.10	3.18 ± 0.20	2.90 ±0.09
15 h after treatment	2.62 ±0.17	2.50 ±0.19	2.39 ± 0.09

Results and Discussion

The petroleum ether fraction amounts to 5.4% of the TEP. Infra red spectroscopy analysis indicate the presence of steroids. Table 1 presents the anti-inflammatory activity of the petroleum ether fraction of the TEP.

Carrageenan-induced oedema is a model of acute inflammation and those agents which are responsible for reducing the oedema are useful as anti-inflammatory agents. The petroleum ether fraction of TEP shows a remarkable reduction in Paw volume after 1 hr treatment. Reduction in paw volume is by 6.67-13.88%, 7.04-13.33% and 2.53-5.2% after 1 h of treatment with the petroleum ether fraction of TEP, aspirin and controls respectively Table 1). The anti-inflammatory property of TEP is clear and the efficacy of the drug seems similar to that of aspirin. However, further investigations are needed to isolate and test the principles.

Acknowledgement

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The Study of Anti-Arthritic Activity of Suranjan-e-Talkh (*Colchicum luteum*) in an Animal Model of Rheumatoid Arthritis

Kunwar Mohammad Yusuf Amin and Minhajuddin

Pharmacology Section, Department of Ilmul Advia, A.K. Tibbiya College, Aligarh Muslim University, Aligarh-202001 (U.P.), India

Abstract

An ethnopharmacological survey showed that Suranjan-e-Talkh (*Colchicum luteum*) is used by physicians of Unani Medicine as the chief drug for Arthritis based on classical literature data but it has not been subjected to scientific study. So, 50% Ethanolic Extract of *C. luteum,* was studied at various doses for Anti-Arthritic activity in Freund's Adjuvant Arthritis (Established Type) Test in rats, which is considered to be a model of human Rheumatoid Arthritis whose results are likely to be reproduced clinically in man. Diclofenac sodium was used as a standard anti-arthritic agent for comparison. The study showed that *Colchicum luteum* possesses a striking, anti-arthritic effect, equal to that of Diclofenac sodium. The study, therefore, scientifically validates the Unani claim about the anti-arthritic activity of Suranjan.

Key Words: Rheumatoid Arthritis, Suranjan-e-Talkh, *Colchicum luteum*, Anti-arthritic.

Introduction

Arthritis is one of the top prevalent diseases and among the biggest health care problems, and is a common cause of physical impairment in the community. Tibb-e-Unani (Unani Medicine) claims to possess a number of effective and safe drugs useful in the treatment of arthritis. Despite the fact that arthritis require a long-term treatment, the drugs have not been found to cause any major side effect. The areas where western medicine is unsuccessful constitute priority areas of Research in Tibb-e-Unani. Thus, the development of effective and safe anti-arthritic drugs have been considered to be one such area (Amin, 1998). Many single and compound Unani drugs subjected to experimental and clinical studies have shown very promising results. For instance Sheer-e-Zaqqoom (*Euphorbia neriifolia*, Linn.) was demonstrated to possess anti-arthritic activity (Faridi *et al*., 1994). Buzidan (*Pyrethrum umbelliferum*, Boiss.) was shown to produce significant analgesic and anti-inflammatory effect (Tajuddin *et al*., 1982). Many compound Unani formulations such as Majoon Seer Alvi Khan (Usmani *et al*., 1997), Majoon Suranjan (Ahmad *et al*., 1997), Majoon Azaraqi (Khan *et al*., 1986) and Habb-e-Gul-e-Aak (Masarrat *et al*., 2004) etc. have been reported to possess striking anti-arthritic activity. Besides them many non-pharmacopoeal preparations that are used by Unani physicians have been shown to produce significant anti-inflammatory, analgesic and anti-arthritic properties (Ansari *et al*., 1990). However, a large number of drugs, which have been described in Unani materia medica and are widely used by the physicians of Unani medicine in the treatment of arthritis, have still not been studied scientifically for their reported effects.

Suranjan-e-Talkh (*Colchicum luteum*) is a reputed and well-known drug in Unani Medicine and is used as anti-inflammatory and anti-arthritic agent. However, it has not been subjected to a proper, multi-dose, mechanistic scientific study for this

action. Therefore, in the present study, it was tested for protective effect against articular inflammation by Freund's Adjuvant Arthritis Test (Established Type).

The 50% ethanolic extract of Suranjan-e-Talkh (*C. luteum*) was used because most of the potential constituents whether polar or non-polar are extracted out in water and alcohol. The doses for animals were determined by multiplying the Unani clinical dose by conversion factor of 7 for rats (Freireich, et al., 1966). The doses thus calculated were found to be 100 mg/kg and 180 mg/kg for Suranjan-e-Talkh (*C. luteum*). It is generally felt that the textual doses are usually lesser than the optimal ones (Amin, 1998) therefore one higher dose of each drug also used.

Diclofenac sodium, which is a well known and commonly used anti-inflammatory drug, was used as standard agent to make the study comparative.

Material and Methods

Preparation of test extract

The shade dried and powdered whole plant of Suranjan-e-Talkh (*C. luteum*) was extracted in 50% alcohol in Soxhlet's apparatus for 6 hours. The extract was filtered and dried by evaporation on water bath. The yield percentage was calculated with reference to crude drug and was found to be 10.90%. A fresh suspension of the extract was prepared in distilled water for oral administration.

Freund's Adjuvant Arthritis Test

The Test for established type of Adjuvant-induced arthritis was carried out by the method of Persico et al (1988). Albino rats weighing 150-200 gm were divided into 4 groups of 6 animals each. All the animals were injected in the right hind paw, with 0.075 ml of Freund's Adjuvant, (1.0 mg of heat-killed and dried *Mycobacterium tuberculosis:* F-5881, 37H8910 in 0.85 ml of paraffin oil and 0.15 ml of mannide mono-oleate). The thickness of the ankle joint was measured along the coronal plane by a micrometer on day 1 before injection of Freund's adjuvant and on day 11 and then on alternate days, from day 12 to day 17. Animals in Group I served as control and were administered with 20 ml/ kg distilled water, while the standard drug Diclofenac sodium (Dfc) was given to Group II in a dose of 5 mg/kg, orally. Animals in group III and IV were administered with 100 mg/kg and 180 mg/kg of *C. luteum* extract, respectively, daily for 5 days. On the concluding day i.e. day 17, final measurement was done, the findings analyzed statistically by ANOVA Test, followed by pair-wise comparison by LSD. The percentage inhibition was found out by the following formula:

$$i = 100 \left[1 - \frac{a - x}{b - y} \right]$$

where,

i = Percentage of inhibition

a = Mean right ankle thickness of Test / Standard animals on day 17

b = Mean right ankle thickness of control animals on day 17

x = Mean right ankle thickness of Test / Standard animals on day 11 before injection of Freund's Adjuvant

y = Mean right ankle thickness of control animals on day 11 before injection of Freund's Adjuvant

Observation and Results

Freund's Adjuvant Arthritis Test

The increase in thickness of the ankle joint was found to be 2.83 ± 0.04 mm in Control group while it was significantly reduced to 1.00 ± 0.13 mm ($p<0.0001$) with 5 mg/kg of Diclofenac sodium (64.6% inhibition), 1.25 ± 0.06mm ($p<0.0001$) with 100 mg/kg of *C. luteum* extract (55.83% inhibition) and 0.95 ± 0.09 mm ($p<0.0001$) with 180 mg/kg of *C. luteum* extract (66.43% inhibition) (Table-1).

Discussion

The The significant reduction in paw-volume in Freund's Adjuvant Arthritis test (Established Type) shows the drug to possess anti-arthritic activity. In Freund's Adjuvant test the lower dose is slightly less active (59.8% inhibition) than Diclofenac sodium (61.5% inhibition), but, the higher dose is slightly more active (63.2 % inhibition).

Table-1. Anti-arthritic effect of Test drugs in Freund's Adjuvant-induced Established Arthritis Test with Diclofenac sodium as standard drug (Ankle Joint Thickness)

Group	Increase in thickness in mm. (Mean ± SE)	% Inhibition of Inflammation
Control	2.83 ± 0.04	–
Standard (DFC 5 mg/kg)	1.00 ± 0.13*	64. 6
C. Luteum (100 mg/kg)	1.25 ± 0.06*	55.83
C. Luteum (180 mg/kg)	0.95 ± 0.09*	66.43

* = $p < 0.0001$

These findings corroborate the Unani reports regarding the anti-inflammatory (Ibne-Sina, 980-1030, Ibne-Baitar, 1197-1248) and anti-arthritic (Ibne-Baitar, 1197-1248) effect of Suranjan-e-Talkh (*C. luteum*).

The present study scientifically validates the Unani usage of Suranjan-e-Talkh (*C. luteum*) in Arthritis. It also adds to existing Unani knowledge by indicating the drug's effectiveness in Rheumatoid Arthritis by showing activity in Freund's Adjuvant test which is considered a good model of human Rheumatoid Arthritis (Turner, 1965). Further, by showing that Suranjan-e-Talkh (*C. luteum*) is equi-effective to Diclofenac, the study shows the test drug to be highly effective. By showing that the higher dose is more effective than the lower dose which corresponds to Unani clinical dose, the study suggests that higher doses of the drug are likely to be more effective during Unani therapy.

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A Review of the Unani Drug Cardamom – Phytochemical and Pharmacological Studies

[1]Anwar Jamal,
[2]Aisha Siddiqui,
[3]Tajuddin,
[4]M.A. Jafri,
[1]Mohd. Khalid Siddiqui

[1]Central Council for Research in Unani Medicine 61-65, Institutional Area, Janakpuri, New Delhi-110058

[2]Department of Ilmul Advia, Jamia Hamdard, New Delhi-110062

[3]Department of Ilmul-Advia, A.K. Tibbiya College, A.M.U., Aligarh-202002

[4]National Institute of Unani Medicine, Kottige Palaya, Magadi Main Road, Bangalore-560091

Abstract

The name **cardamom** is used for herbs within two genera of the ginger family Zingiberaceae, namely *Elettaria* and *Amomum*. Both varieties take the form of a small seed pod, triangular in cross-section and spindle-shaped, with a thin papery outer shell and small black seeds. Elettaria pods are light green in color, while Amomum pods are larger and dark brown. Both of the drugs are frequently used as a substitute for each other in various conditions.

Elettaria cardamomum Maton and *Amomum subulatum* Roxb. (Family: Zingiberaceae) are commonly known as Chhoti Elaichi and Bari Elaichi, respectively. These are important medicinal plants used in various formulations in Traditional Systems of Medicine. The fruit of plant of Elettaria and Amomum is called "Heel Khurd" and "Heel Kalan" in Persian language, respectively, which term is mostly used in Unani Medicine. Both of the plants have been extensively used for various disorders in the form of different formulations especially in Amraaz-e- Meda (Gastric Disorders) as Muqavvi-e-Meda (Stomachic), Muhallil (Resolvent), Hazim (Digestive), Mane Qai (Anti emetic) and Kasir-e-Riyah (Anti- Flatulent) property mentioned in Classical Unani Literature. These are also used as flavoring agents in both food and drinks. Keeping in view the medicinal importance of "Heel Khurd" (*Elettaria cardamomum* Maton) and "Heel Kalan" (*Amomum subulatum* Roxb.), the drugs were exhaustively reviewed including phytochemical and pharmacological works so far reported on the plants.

Key Words: Heel Khurd, *Elettaria cardamomum*, Heel Kalan, *Amomum subulatum,*

Introduction

Heel Khurd (*E. cardamom*) is tall, perineal herb, of about two meters height. It is generally found in Eastern and Northern areas of Kanara, Mysore, Coorg, Travancore and hills of Malabar. Nodes arise from the root and bear white and red flowers, which have aromatic odour like seeds of cardamom. Leaves are similar to Pomegranate and Basil. Surface of fruits are longitudinally striated. Seeds are enclosed in a pericarp similar to that of pista. Fruits are umbel in shape and divided into three compartments. They are smaller compared to *A. subulatum,* enclosed within a white delicate membrane and have aromatic odour. The seeds are obtuse in shape, one inch long with black outer surface and white inner surface. Taste is strongly pungent and odour is aromatic. They have low astringent power. The seeds, which are black in colour, are considered to be of best quality. The unripe fruits of *E. cardamomum* are green in colour, yellowish after ripening and become white on drying. The dried fruits are available commercially as cardamom and the seeds are used for their various properties (Kareem, 1879; Sheerazi, 1913).

The oil obtained from the seeds is pale yellow in colour having pungent taste and aroma, like cardamom. 12 gm of oil is obtained from 240 gm of seeds of *E.*

cardamomum. The seeds deteriorate in their quality in the presence of air. Therefore, they should be left in their pericarp, until they are used. The seeds are of best quality when they are fresh and bulky with strong fragrance. On drying, the fruits break open and seeds are visible. The life span of seeds is about three years (Kareem, 1879; Sheerazi, 1913, Anonymous, 2003).

Mizaj (Temperament)

The temperament of lesser cardamom mentioned by Unani physicians is hot and dry but there is a difference of opinion amongst them regarding the degree of heat and dryness.

1. Hot^2 Dry^2 (Khan, 1313A.H.; Kareem, 1879; Sheerazi, 1913; Ghani, 1917).
2. Hot^{1}Dry^2 (IbnSina, 1927; Ahmad, 1899).

Af'al (Action):

Both the varieties of cardamom viz.Heel Khurd and Heel Kalan mostly used as Muqavvie Meda (Stomachic), Mujaffif (Desicant), Muhallil (Resolvent), Habis (Retentive), Hazim (Digestive), ManeQai (Anti emetic) and Kasire Riyah (Carminative).

Istemalat (Therapeutic Uses)

It is used in the treatment of Hurqat Meda (Acid Peptic Disorders), Warme Meda (Gastritis) (Khan, 1940), Sue Hazm (Indigestion) (Kareem, 1879; Razi, 1980), Ghisyan (Nausea) (Khan, 1313A. H; Attar, 1888; Ghani, 1917) and Muteebe Dahan (Halitosis) (Kareem, 1879; Ghani, 1917).

Heel Kalan (*A. subulatum*) also is a tall, perennial herb of about two and a half meter height. Flowers are white or sometimes tinged with brown. Fruits are dark brown in colour. The pericarp is strong and rugged. Some times, when the fruits become ripe, the pericarp splits open. Seeds are numerous and strongly aromatic. When the seeds are fresh they are surrounded by a viscid fluid. When the outer covering is intact, the potency of the drug lasts till two years. Later on the taste and aroma decreases and the potency also goes on decreasing. When the seeds are stored after removing the seed coat, the potency lasts only for one year (Ghani, 1917; Kareem, 1879; Hassan, 1894).

Mizaj (Temperament):

The temperament of large cardamom is unimously mentioned as Hot and Dry but there is a difference of opinion amongst the Unani Physicians regarding the degree of heat and dryness.

1. Hot^{2} Dry^{2} (Rushd, 1980; Khan, 1314 A.H.).
2. Hot^{1}Dry^{2} (Sheerazi, 1913; Israili, 1907; Kareem, 1879).

Istemalat (Therapeutic Uses)

Large cardamom, being an aromatic substance is chewed to eliminate bad breath and body odour due to sweating and useful in nausea and vomiting because of anti emetic property (Ghani, 1917; Khan, 1314 A.H). It is also used in case of indigestion because of its stomachic property (Ali, 1301A.H; Razi, 1980) and in flatulence because of carminative (Khan 1314 A.H; Israili, 1907). It is used in gastric irritation. In this case the seed powder is prescribed in combination with misri (Crystallized Sugar) (Ghani, 1917).

Phytochemical Studies

A. Nutrients

Seed of small cardamom (Heel Khurd) is composed of volatile oil 2-8%, Protein 10.2%, moisture 20%, crude fiber 20%, minerals 5.4%, ether extracts 2.4%, carbohydrate 42.1%, calcium 0.13%, Phosphorous 0.16%, and iron 0.005% (Anonymous, 2003). It also contains starch, Potassium salts of oxalic acid, colouring matter fibres (Nadkarni, 1976; Ramashastri, 1983).

The constituents on analysis of the seeds of large cardamom (Heel Kalan) is composed of moisture 8.5%, protein 6.0%, volatile oil 2.8%, crude fiber 22.0%, starch 43.2%, ether extract 5.3%, alcohol extract 7.0%, ash 4%, phosphorous 61.0 mg/100 gm (Anonymous, 1985). It also contains fluoride 14.4 ppm, calcium 666.6 mg/100gm and phosphate 187.5 mg/100 gm (Nanda, 1972 and Anonymous, 1985).

B. Lipids

Total lipid contents of *E. cardamomum* seeds are 3.4%. The lipid content is mainly composed of Glycolipids 8.7% in seeds and 29.3% in pods and Phospholipids 1.9% in seeds and 4.4% in pods (Kataoka, 1987).

Phospholipids fraction of *E. cardamomum* seeds consists of Cadiolipin, Phosphatidylenthanolamine, Phosphalidyl-N, N-dimethylethanolamine (PE-dime), Phosphatidylserine (PS), Phosphatidylinositol (PI), Lysophosphatidylcholine, while sterol components of seeds are composed of 7 Ergesterol, Compesterol and Tocopherol. Two Phenolic acids (Caffeic acid and Vanillic acid) were also found in the seed (Variyar and Bandyopadhyay, 1995). Among nonsaponifiable lipid constituents there are mainly waxes of n–alkanes (C_{21}, C_{23}, C_{25}, C_{27}, C_{29}, C_{31}, C_{33}) and n–alkenes (C_{21}, C_{23}, C_{25}, C_{27}, C_{29}, C_{31}, C_{33}) and sterols (sitosterol, phytol & traces of eugenyl acetate) (Gopalakrishnan, 1990).

Glycosides of seed of *A. subulatum* consists of petunidin-3, 5-diglucoside [C_{28} H_{33} O_{17}, m.p.> 300°], leucocyanidine-3-O-β-D-glucopyranoside [C_{21} H_{24} O_{12}, m.p.>230° (dec.)] (Lakshmi and Chauhan, 1976) and Subulin (A new aurone glycosides), 6, 3', 4', 5'-tetrahydroxy-4-methoxy aurone-6-O-α- L-rhamnopyranosyl – (1 → 4) - β-D-glucopyranoside (Lakshmi and Chauhan, 1977).

C. Essential Oil

Cardamom oil of commerce is obtained by the distillation of the whole fruits of *Elettaria cardamomum*. It is a colorless or pale yellow liquid with a penetrating, somewhat camphoraceous odour and a strong persistent pungent taste. The oil has been reported to possess specific gravity 0.923-0,945, $[\alpha]^{20^{\circ}}_{D}$: (+) 20–48 0, 20-400, nD: 1.461 – 1.467, Acid value: 4.0, Ester value: 92 – 150, Saponification Value,: 96.5 – 156.4, Ester value after acetylation: 163 and solubility: 2 – 4 vols. of 70% alcohol (Anonymous, 2003; Nadkarni, 1976; Chopra, 1933). The main constituents of oil are a - pinene, 1, 8-cineole, a-terpinyle acetate (Anonymous, 2003). Terpineol, Terpinene (Baruah, 1973), Geraniol, a-terpineol, Sabinene, Linalyl acetate, Limonene, Myrcene, Terpineol, formic and acetic acid (Miyazawa and Kameokah, 1975), Nerylacetate, Neolidol, Heptacosane, β - terpineol, Cymene, Linalool acetate, 4-terpineol, β- pinene, Cineol, D-limonene, Meheptenone, Borneol, Geraniol, Nerol, Linalool, α-terpineol, Sabinene, α-pinene, Linalyl acetate, Limonene, 1, 8-cineole, α-terpinyle acetate, Myrcene, Terpineol, Terpinene (Rastogi and Mahrotra, 1993; 1995; Bin, 1999; Chopra, 1998; Chopra, 1986). Besides this it also contains Camphene, *P*-cymene, α-humulene (Rastogi and Mahrotra, 1993), Heptane, Menthone, β - phellandrene, (+) (S) nerolidol, β-phellandrene (0.2%), γ - terpinene (0.7%), Terpinolene (0.5%), Terpinen–4–ol (0.9%), Citronellol (0.3%), Methyl eugenol (0.2%) Trans-nerolidol (2.7%) (Korikanthimathm, 2000-2001).

Two new natural products, (E)-4,8-dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene was reported. These unusual C11 and C16 hydrocarbons were isolated from the essential oil of *Elettaria cardamomum* Maton var. miniscula and their structures confirmed by synthesis (Maurer, 1996).

Comparative analysis of the oil and supercritical CO_2 extract of cardamom was carried out which shows that main components were alpha-terpinyl acetate, 42.3%; 1,8-cineole, 21.4%; linalyl acetate, 8.2%; limonene, 5.6%; and linalool, 5.4% of oil while volatile fraction mainly made up of limonene, 36.4%; 1, 8-cineole, 23.5%; terpinolene, 8.6%; and myrcene, 6.6% (Marongiu, 2004). The identification of two new natural products, (E)-4,8-dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene is reported. These unusual C11 and C16 hydrocarbons were isolated from the essential oil of *Elettaria cardamomum* Maton var. miniscula Burkhill (Zingiberaceae) and their structures confirmed by synthesis (Maurer, 1986).

The volatile oil of *Elettaria cardamomum* (L.) Maton seeds was obtained by supercritical CO (2) extraction (SC-CO (2)). The effect of the extraction conditions

on the yield and composition of the resulting cardamom volatile oil was examined (Marongiu, 2004).

The essential oil of the seeds of Heel Kalan (*Amomum subulatum*, Roxb.) on steam distillation yield a dark brown oil (2.5%) having a characteristic odour of cineol. The essential oil has been reported to have specific gravity 29^0 (0.9142), n 29^0/D (1.4600), [á] D (18^0 3'), Acid value 2.9, Saponification value 14.53 and saponification Value after acetylation 40.2. The main constituents of oil are cineol 64.94%, bisabolene 3.6%, sabinene 6.6%, terpinene 10.7%, terpineol 14.53%, terpinyl acetate 40.2%, polymerized oil 1.91%, α-pinene 2.0%, β-pinene 2.4%, sabinene 0.2%, myrcene 0.3%, α-terpinene 0.2%, limonene 10.3%, 1,8-cineol 74.0%, γ-terpinene 0.2%, p-cymene 0.2%, terpinene-4-ol 2.0%, δ-terpineol 0.8%, α-terpineol 5.6%, nerotidol 1.0% (Anonymous, 1985; Lawrence, 1970).

The legal standards for whole cardamom are reported as ash value (5.49-6.05%.w/w), acid insoluble value (0.27-0.43%, w/w), volatile oil (0.50-0.82-1.50%, v/w) and for seed ash value reported as (3.45-3.95-4.57%,w/w), acid insoluble value (0.31-0.67-1.30%,w/w) and volatile oil (0.90-1.34-2.0%,v/w) (Chopra, 1986).

D. Chalcons and Flavanone

Chalcons and Flavanone of *A. subulatum* consist of cardamomin (2', 4'- dihydroxy-6'- methoxy chalcone) and Alpinetin (7-hydroxy-5-methoxy flavanone) (Rao, 1976).

Pharmacological Studies

Anti fungal activity

The ripe seeds of *E. cardamomum* have been reported to exhibit antifungal activity (Venkataraman, 1978).

Antifungal activity was tested against the food-borne fungi *Aspergillus terreus, Penicillium purpurogenum, Fusarium graminearum* and *Penicillium madriti*. The methanol and ethanol oleoresins gave the best results against *A. terreus* at 3000 ppm by the poison food method (Singh, 2008).

The essential oil from the seeds of *Amomum subulatum* was highly active against the growth of keratinophilic fungi (Jain and Aggarwal, 1978). The essential oil of fresh leaves of *Amomum subulatum* was collected in Varanasi and tested at 5000 ppm against the storage fungus Aspergillus flavus. The percent inhibition shown was 100%. The minimum concentration for complete inhibition was 3000 ppm. It is also reported that the oil possessed a broad range of fungi toxicity in tests with 20 plant pathogenic species and proved more efficacious than several standard fungicides. It had no significant adverse effect on seed germination in tests with oryza sativa (Mishra and Dubey, 1990).

Anti microbial activity

The essential oil from the seeds of *E. cardamomum* was found to have antibacterial properties (Pruthi, 1980) and cardamomum could prove to be good antibacterial treatment, particularly for *V. cholerae* and *Shigella* infections (Islam, 1990).

The essential oil exhibited strong antibacterial activity against the micro-organisms *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhi* at 3000 ppm by the agar well diffusion method (Singh, 2008).

The antimicrobial activity of *Elettaria cardamomum* against both Gram-positive and Gram-negative bacterial species was demonstrated. Likewise, its toxicity was investigated on Swiss albino's mice. Daily, mice were treated orally with 0.003 and 0.3 mg during 7 days. Plasmatic markers and antioxidant defense systems were assessed and histological alterations were evaluated. A significant increase in creatine phosphokinase level was observed. The microscopic evaluation shows that *E. cardamomum* induce morphological perturbation in mice's heart. The results show also an inhibitory effect of glyceraldehyde 3-phosphate dehydrogenase and an important increase in the level of thiobarbituric acid reactive substances, succinate dehydrogenase and catalase activities. Results show that E. cardamomum induces toxicity at 0.3 mg/g mouse and affect energy metabolism and oxidative stress (Malti, 2007).

Antioxidant activities

Anti oxidant activity of the essential oil and oleoresins of E. cardamom, studied in mustard oil by monitoring the peroxide value of the oil substrate, were comparable to those of the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at 0.02% concentration (Singh, 2008).

Anti-inflammatory, analgesic and antispasmodic activity

A comparative study of the anti-inflammatory activity of the oil extracted from commercial *Elettaria cardamomum* seeds, and indomethacin against acute carrageenan-induced planter oedema in male albino rats was performed, which proved to be marked. Moreover, investigation of the analgesic activity using p-benzoquinone as a chemical stimulus proved that the oil produced protection against the writhing induced by intraperitoneal administration of solution of p-benzoquinone in mice. In addition the antispasmodic activity was determined on a rabbit intestine preparation using acetylcholine as agonist, the results proving that cardamom oil exerts its antispasmodic action through muscarinic receptor blockage (Al-Zuhair, 1996).

Gastro protective activity

A crude methanolic extract and different fractions of *Amomum subulatum*, viz.

essential oil, petroleum ether (60-80^0), ethyl acetate and methanolic fractions, were studied in rats for their ability to inhibit the gastric lesions induced by aspirin, ethanol and pylorus ligature. In addition to their effects on wall mucus, output of gastric acid and pepsin concentration were recorded. The crude methanolic extract of *A. subulatum* and its fractions, viz. essential oil, petroleum ether and ethyl acetate, inhibited gastric lesions induced by ethanol significantly, but not those which were induced by pylorus ligation and aspirin. However, ethyl acetate fraction increased the wall mucus in pylorus ligated rats. The results suggest a direct protective effect of ethyl acetate fraction on gastric mucosal barrier. While the observation of decrease in gastric motility by essential oil and petroleum ether fractions suggest the gastroprotective action of the test drug (Jafri, 2001).

The essential oil and petroleum ether soluble fractions of small cardamom (fruits of *Elettaria cardamomum* Maton.) and large cardamom (fruits of *Amomum subulatum* Roxb.) were studied in rats for their ability to inhibit gastric lesions induced by aspirin and ethanol, and results were compared. Both the fractions of drugs inhibited gastric lesions significantly. Fractions of small cardamom were found to be better than large cardamom (Jamal, 2005).

The Methanolic extract (TM), essential oil (EO), petroleum ether soluble (PS) and insoluble (PI) fractions of methanolic extract, were studied in rats at doses of 100-500, 12.5-50, 12.5-150 and 450 mg/kg, respectively, for their ability to inhibit the gastric lesions induced by aspirin, ethanol and pylorus ligature. In addition their effects on wall mucus and gastric acid output were recorded. All fractions (TM, EO, PS, PI) significantly inhibited gastric lesions induced by ethanol and aspirin but not those induced by pylorus ligation (Jamal, 2006).

A study was carried out to rationalize cardamom use in constipation, colic, diarrhea, hypertension and as diuretic using in vitro and in vivo models. The results indicated that cardamom exhibits gut excitatory and inhibitory effects mediated through cholinergic and Ca+ + antagonist mechanisms respectively and lowers BP via combination of both pathways. The diuretic and sedative effects may offer added value in its use in hypertension and epilepsy (Gilani, 2008).

GIT regulators activity

The dose effects of peptic polysaccharide-rich extract from the food spice cardamom on intestinal environment were investigated. These findings suggested that the consumption of cardamom extract (at least 0.5 g/100 g diet or 40 mg/day) exert a favorable effect on improving the gastrointestinal milieu, and also provide a clue to substantiate its traditional therapeutic uses and dosage for intestinal health improvement (Huang, 2007).

In vitro susceptibility of *Helicobacter pylori* to *Elettaria cardamomum* (seed) extract used traditionally for the treatment of gastrointestinal disorders was found to possess MIC > 100 microgm/ml (Mahady, 2005).

Anti-cancer activity

The study was undertaken to identify the effects of a commonly consumed spice, viz., cardamom against azoxymethane (AOM) induced colonic aberrant crypt foci (ACF) in Swiss Albino mice. Whereas, secondary aim, was to explore the ability of cardamom to modulate the status of proliferation and apoptosis and to understand its role in altering cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS) expression. These results suggested that aqueous suspensions of cardamom have protective effects on experimentally induced colon carcinogenesis. Cardamom as a whole and its active components require further attention if the use of this spice is to be recommended for cancer prevention (Sengupta, 2005).

Cardio-vascular activity

The inhibitory activity of cardamom extract was studied on human platelets. Platelet aggregation and lipid per oxidation were evaluated with platelet rich plasma (PRP) and platelet membranes, respectively. It was found that aqueous extract of cardamom protect platelets from aggregation and lipid per oxidation (Suneetha and Krishnakantha, 2005).

Miscellaneous Pharmacological Activities of Essential Oil

The effect of the essential oil of *E. cardamomum* seeds on the cardiovascular system on rats, nictitating membrane of cats, isolated rabbit jejunum, isolated guinea pig ileum and the frog sciatic nerve preparation was investigated. The essential oil (5–20 μg/kg, i.v.) decreased rat arterial blood pressure and heart rate in a dose dependent manner which was antagonized by treatment of the animals with cyproheptadine (1mg/kg for 5 min). Electrically induced contractions of the cat nictitating membrane and spontaneously contracting rabbit jejunum were found relaxed in larger doses of essential oil. Large doses of the essential oil antagonized the stimulant effects of acetylcholine, nicotine and $BaCl_2$ on the rabbit jejunum. The essential oil (0.01–0.04 ml/ml) induced contractions of the isolated guinea pig ileum. This effect was antagonized by atropine or cyproheptadine. Exposure of the frog sciatic nerve to the essential oil (0.2–0.4 ml/ml) suppressed the frog limb withdrawal reflex suggesting a local anesthetic effect (Tahir, 1997).

Cardamomom oil as a skin permeation enhancer for indomethacin

Drug permeation through skin, extracts of crude drug was evaluated using in vitro and in vivo penetration techniques with rabbit skin as a model membrane. The acetone extract of *Elettaria cardamomum* had the best effect in enhancing the penetration of Indomethacin (Huang, 1993). The *in-vitro* and *in-vivo* effect of pretreatment by cardamom oil, a crude drug extract, in ethanol/water vehicles on the transdermal delivery of indomethacin was investigated. The permeation of

indomethacine was significantly enhanced after pretreatment with cardamom oil both in the *in-vitro* (rats, rabbit and human skin) and *in-vivo* (rabbit) studies. The results also showed that three minor components in cardamomum oil (α-pinene, 6.5%; β-pinene, 4.8%; α-terpineol, 0.4%) had a synergistic effect with 1, 8–cineole [eucalyptol] (59–3%) and d–limonene (29%) to enhance the permeation of indomethacin (Chopra, 1986).

Constituents of the fruits of greater cardamom (*Amomum subulatum*) were fractionated into three fractions, the dichloromethane extract, and the ethyl acetate-soluble and water-soluble fractions of the 70% aqueous acetone extract. The ethyl acetate-soluble fraction showed a high radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH). Four compounds were isolated from the ethyl acetate-soluble fraction, and their structures were ascribed to protocatechualdehyde (1), protocatechuic acid (2), 1,7-bis (3,4-dihydroxyphenyl) hepta-4E, 6E-dien-3-one (3) and 2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetrahydro-5H-benzocycloheptene (4) on the basis of spectroscopic evidence. This is the first isolation of these compounds from greater cardamom. In particular, 4 was a new type of cyclic diarylheptanoid. DPPH radical-scavenging activity of these compounds was measured by colorimetric analysis. Compounds 1 and 3 showed stronger activity than such natural antioxidants as alpha-tocopherol and L-ascorbic acid. Compounds 2 and 4 were comparable to alpha-tocopherol and L-ascorbic acid (Kikuzaki, 2001).

Dermal penetration enhancement of prednisolone by Cardamomom

The acetone extract of cardamom seed (*Elettaria cardamomum*) was found to possess penetration enhancer activity for the diffusion of prednisolone through mouse skin in vitro. It was observed that terpineol and acetyl terpineol are the active components in cardamom seed (Yamahara, 1989).

The effect of cardamomom extract on gastric acid secretion

The effects of volatile oil and water extract on the secretion of gastric juice, volume of gastric mucous blood flow and activity of SOD in gastric mucous membrane were studied and it was found obvious that volatile oil of *Amomum cardamomum* has better effects on the secretion of gastric juice and volume of gastric mucous blood flow than those of water extract (Qiu, 1999). Aqueous extract of *E. cardamomum* increased the gastric acid secretion in pentobarbitone anaesthetized rats (Vasudevan, 2000).

Conclusion

It may be concluded that both of the varieties have the property of Muqavvie Meda (Stomachic), Mujaffif (Desicant), Muhallil (Resolvent) and Habis (Retentive)

and proved as gastro protective remedies on scientific parameters justify the assert of Classical Unani literature. The essential oil of the cardamoms also used in bed breath due to phytochemical constituents present in essential oil and as GIT regulators having peptic polysaccharide-rich food spice on intestinal environment. So, on behalf of experimental data bases, so far carried out, further clinical research work may be taken in large number of patients in near future to investigate and validate the claim mentioned in Unani Classical literature.

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Response of Growth and Yield of Kalmegh (*Andrographis paniculata* (Burm.f.) Wall. ex Nees) to Different Organic Wastes Based Vermicompost

Ali Zafar and Shweta

Vermiculture Research Station,
Department of Zoology,
D.S. College,
Aligarh-202001, U.P., India

Abstract

Supplementing the nutrient requirement of crops through organic manures plays a key role in sustaining soil fertility and crop productivity and reducing use of fossil fuels. Field experiments were conducted for two years during 2007-08 at Vermiculture Research Station, D.S. College Aligarh, India, to assess the yield and vegetative growth of kalmegh (*Andrographis paniculata* (Burm.f.) Wall ex Nees) with the application of different organic wastes based vermicompost viz. *Parthenium*, water hyacinth and sugarcane trash with dung.

Key Words: *Andrographis paniculata* (Burm.f.) Wall ex Nees, Vermicompost, Organic Wastes.

Introduction

Andrographis paniculata (Burm.f.) Wall. ex Nees (Kalmegh) belongs to the family Acanthaceae, is one of the promising medicinal plants widely recommended for cultivation in India. There is also a great demand for the plant by pharmaceutical industries as raw dung mainly for export. It is a source of several diterpenoids of which andrographolide (alkaloid) is important. The drug is used for the treatment of general debility, dyspepsia, chronic malaria, jaundice and dysentery. Some scientists have observed that andrographolide has the potential to be included in the cocktail vaccine against AIDS by virtue of its antagonistic property with HIV II virus (Weibo, 1995). It is already being used in treating cancer as it promotes cell differentiation in tumour cells (Matsuda *et al.*, 1994). However, the leaves contain maximum andrographolide. The entire plant is used for extracting the active ingredient (Chiramek *et al.*, 2006).

Currently emphasis is on sustainable agriculture, which stresses for the use of less chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Vermicomposts are known to improve the nutritional status of soil growth and development of plants. About 5 million hectare of land has been invaded by *Parthenium* (Kohali and Rani, 1994) and 116.4 hectare by water hyacinth (Firehun Yirefu, 2007) in India. Both are obnoxious weed and have spread throughout India and have become menace in many forest nurseries and agricultural land. Hence, in the present investigation it was envisaged to screen and select an appropriate dose and manurial value of different types of vermicompost for optimum kalmegh cultivation.

Materials and Methods

Various types of experiments on agro-technological practices using different types of vermicompost for crude drug production on *Andrographis paniculata* were carried out during 2007-08. The experimental site lies between 27º54'50" N latitude and

78º4'26" E longitude, represents almost a dry climate, the cold weather lasts longer than in the eastern districts and it extends from middle of October to the end of March. The mean annual rainfall was 16.41 mm, the mean number of rainy days was 48 per annum. The mean maximum and minimum temperature were 30.75ºC and 18.6ºC respectively and relative humidity 66.93 per cent. The weather conditions prevailed during the experimental period are presented in Table 1.

Seed material was collected from Rajasthan Agro-Forestry Corporation Ltd., Sonamukhi Nagar, Jodhpur (Rajasthan) and nursery was developed under controlled conditions. Vermicompost was prepared from *Parthenium*, water hyacinth, sugarcane,

Table 1. Monthly mean weather data for the period from July 2007 to December 2008.

Month	Max. Temp. (°C)	Min. Temp. (°C)	Relative humidity (%)	Rainfall (mm)
		2007		
July	38.17	27.52	71.62	1.15
August	33.64	25.00	90.12	8.12
September	33.76	24.21	82.60	2.80
October	33.52	17.26	76.87	–
November	25.87	13.48	84.12	9.00
December	17.73	9.31	84.75	1.10
		2008		
January	21.71	8.96	88.87	–
February	22.22	7.80	79.62	–
March	29.71	14.77	68.12	–
April	38.22	20.07	45.87	–
May	38.08	23.68	52.62	–
June	41.75	26.61	55.62	2.80
July	32.52	25.06	78.97	87.90
August	31.98	24.47	87.12	82.50
September	33.51	22.81	79.00	17.20
October	26.22	18.36	73.85	0.40
November	23.70	12.03	80.20	6.20
December	24.44	9.27	24.44	–

cattle manure and organic wastes. The chemical composition of the experimental materials used in the study are given in Table 2.

The experimental design was RBD, the physical and chemical composition of soil were analysed during the study and are given in Table 3.

Table 2. Chemical composition of experimental material

Nutrient	Vermicompost		
	Parthenium based	Water hyacinth based	Sugarcane based
N (%)	1.87	1.79	1.91
P (%)	0.42	0.45	0.94
K (%)	0.72	0.76	0.78
C:N	17.4:1	15:1	16:1
pH	7.4	7.2	7.2

Table 3. Physical and chemical characteristics of the soil prior to the experiment

Parameters	Soil characteristics
Texture	Clay loam
Structure	Granular
pH	6.09
Available N (ppm)	144.48
Available P (ppm)	2.90
Available K (ppm)	68.00
Cu (ppm)	175.80
Mg (ppm)	16.07
S (ppm)	5.22
Fe (ppm)	20.36
Mn (ppm)	9.58
Zn (ppm)	1.75
Cu (ppm)	3.85

Table 4. Effect of different types of vermicompost on plant height (cm) of *Andrographis paniculata*

S.No.	Treatment	Plant height		
		2 MAP	4 MAP	6 MAP
1.	Sugarcane based vermicompost	34.86 ±4.61	44.46 ±4.12	45.36 ±2.54
2.	Water hyacinth based vermicompost	49.60 ±0.68	63.76 ±3.84	65.63 ±0.69
3.	*Parthenium* based vermicompost	42.43 ±1.94	51.13 ±1.41	53.66 ±0.21

MAP = Month after planting

Table 5. Effect of different types of vermicompost on number of branches of *Andrographis paniculata* per plant.

S.No.	Treatment	Number of branches		
		2 MAP	4 MAP	6 MAP
1.	Sugarcane based vermicompost	6.43± 0.92	10.50 ±3.61	14.2 ±1.21
2.	Water hyacinth based vermicompost	6.80 ±0.62	9.90 ±0.94	13.36 ±0.96
3.	*Parthenium* based vermicompost	5.72 ±0.63	8.86 ±0.58	12.3 ±1.07

Table 6. Effect of different types of vermicompost on number of leaves/ plant of *Andrographis paniculata*

S.No.	Treatment	Number of leaves		
		2 MAP	4 MAP	6 MAP
1.	Sugarcane based vermicompost	223.33 ±3.71	113.66 ±4.40	144.00 ±0.52
2.	Water hyacinth based vermicompost	258.33 ±3.71	126.33 ±3.98	131.33 ±2.12
3.	*Parthenium* based vermicompost	169.00 ±4.80	109.00 ±1.60	130.00 ±1.59

Table 7. Effect of different types of vermicompost on production of flowers of *Andrographis paniculata*

S.No.	Treatment	Flower (numbers)	
		5 MAP	6 MAP
1.	Sugarcane based vermicompost	5.66 ±1.45	46.00 ±1.59
2.	Water hyacinth based vermicompost	4.66 ±1.59	41.66 ±3.72
3.	*Parthenium* based vermicompost	6.00 ±1.59	25.00 ±1.59

Table 8. Effect of different types of vermicompost on number of capsules (fruits) of *Andrographis paniculata*

S.No.	Treatment	Capsule (number)		
		2 MAP	4 MAP	6 MAP
1.	Sugarcane based vermicompost	38.66 ±1.99	95.00 ±1.96	144.66 ±1.72
2.	Water hyacinth based vermicompost	55.66 ±1.29	97.00 ±2.65	138.00 ±2.26
3.	*Parthenium* based vermicompost	59.66 ±1.72	86.33 ±2.35	147.33 ±1.72

Table 9. Effect of different types of vermicompost on flower size (cm) of *Andrographis paniculata*

S.No.	Treatment	Flower (diameter)	
		5 MAP	6 MAP
1.	Sugarcane based vermicompost	1.03 ±0.12	0.90 ±0.11
2.	Water hyacinth based vermicompost	0.83 ±0.02	0.96 ±0.01
3.	*Parthenium* based vermicompost	1.00 ±0.11	1.00 ±0.11

Fig. *Andrographis paniculata* cultivated in different types of organic wastes.

Results and Discussion

Appropriate technological practices are recorded to ensure adequate availability of quality crude drugs and fetch high return to the farmers.

In the present investigation the plant height (cm) was found maximum (65.63± 0.69) in the 'water hyacinth' based vermicompost treatment at 6 MAP (month after planting) stage. While, the minimum were recorded (34.86±4.61) in the sugarcane based vermicompost at 2 MAP stage. The number of plant branches maximum (14.2 ± 1.21) recorded in sugarcane based vermicompost treatment at 6 MAP stage and minimum (5.72 ± 0.63) in *Parthenium* based vermicompost treatment at 2 MAP stage.

The number of leaves per plant was recorded maximum (258.33 ±3.71) in the water hyacinth based vermicompost at 2 MAP stage and minimum (109±1.60) in the *Parthenium* based vermicompost at 4 MAP stage. The number of capsules per plant was maximum (147.33 ±1.72), recorded in the treatment of *Parthenium* based vermicompost, while minimum (38.66 ±1.99) in sugarcane based vermicompost. The maximum number of flowers was recorded (46.00 ± 1.59) in the sugarcane based vermicompost at 6 MAP stage and minimum (4.66 ± 1.59) in the water hyacinth treatment at 6 MAP stage. The flower size (cm) was maximum recorded (1.03±0.01) in sugarcane based vermicompost at 5 MAP and minimum (0.83±0.03) in water hyacinth based treatment at 5 MAP stage.

A crop is cultivated not only for the quantity of yield but also for the quality of produce. Usually an inverse relation exists between quantity and quality, as the quality components are formed from quantity components and quality. Hence, the time of harvest needs to strike an ideal balance between the two on the one hand and be economically viable on the other hand. Singh *et al*. (2001) domesticated and cultivated three wild types of *Andrographis paniculata* and reported that the population exhibited wide variation among themselves with respect to growth, behaviour, maturity period dry biomass and leaf yield. Nemade *et al*. (2001) reported

that the growth and yield attributes of *Andrographis peniculata* were not influenced by the date of harvest but date of planting. Srivastava *et al*. (2000) noted that *Mentha arvensis* aged 3-4 months gave higher yields of high quality oil. Bahl *et al*. (2000) reported that the full flowering stage of *Ocimum basilicum* offered the most profitable time of harvest. Riba (2000) observed better growth of ginsing due to the addition of cow dung.

Singh *et al*. (2000) concluded that higher yield of *Plantago ovata* could be achieved by sowing in ridges with the application of organic fertilizers. Shiva and Mantolis (1998) further recorded that quality of medicinal plants was influenced by seasonal effects and hence they suggested that generally, drugs should be collected in autumn season. Pereira *et al*. (1998) noted that organic fertilization increased coumarin concentration in *Mikenia glomerata*.

On the basis of the present investigation the water hyacinth based vermicompost was found the best for the vegetative growth and to enhance the production of leaves in Kalmegh (*Andrographis paniculata*).

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Studies on the Effect of Organic and Inorganic Sources of Nutrients on the Productivity of Wheat (*Triticum aestivum* L.) – An Important Food & Medicinal Crop

Bharti Shachi, Ali Zafar and Shweta

Vermiculture Research Station,
Department of Zoology,
D.S. College,
Aligarh-202001 (U.P.)

Abstract

Field experiments were conducted on wheat (*Triticum aestivum* L.) during the two consecutive winter seasons of 2003 and 2005 in replicated split plot design. The treatments comprised 5 levels of organic manures *viz*., no organic manure (control), farmyard manure (FYM) at 10t/ha, vermicompost at 5, 7.5 and 10t/ha in main plots and 5 levels of N, *viz*., 0, 50, 100, 150 kg N/ha and recommended fertilizers (120N + $60P_2O_5$ + $25ZnSO_4$ kg/ha). The data on various yield attributes, and total biological yields along with harvest index were recorded in different treatments. The results revealed that application of vermicompost @ 7.5 or 10 t/h gave higher yield of crop. Integration of vermicompost at the rate of 10 t/ha or 7.5 t/ha or FYM 10t/ha with 100 kg N/ha produced both yield attributes and economic yields at par with recommended dose of inorganic fertilizers (120N + $60P_2O_5$ + $25ZnSO_4$ kg/ha) during both the years. Out of 5 combinations tried, maximum yield was recorded with vermicompost 10 t/ha mixed with 100 kg N/ha. The study indicted that Integrated Nutrient Management in wheat crop not only recoded significantly higher yield but also protected the soil from adverse effects of chemical fertilizers for a long term use.

Key Words: Wheat, FYM, Vermicompost, N-Fertilizers.

Introduction

Triticum aestivum L. (Syn. *T. sativum* L.), commonly known as Gehun, is widely cultivated in many parts of north India and the Deccan Peninsula, especially in the north-west, and upto 13,000ft in the Himalayas and Tibet. Besides food, the plant has medicinal value. The seeds are cooling, tonic, fattening; increase appetite and relish for food; useful medicine in general disorders of health (Chopra *et al*, 1956). In folk medicine the paste of the soaked grains is given with curd to treat spermatorrhoea (Anonymous, 2001). The young stems are used in the treatment of biliousness and intoxication. The ash as used to remove skin blemishes the fruit is antipyretic and sedative. The grains is used in the treatment of night sweats and spontaneous sweating. The seed is said to contain sex hormones and has been used in China to promote female fertility. The seed sprouts are ant bilious, antivirus and constructive. The are used in treatment of malaise, sore throat, thirst, abdominal coldness, constipation and cough. The plant has also anticancer properties (Yeung, 1985; Duke, 1985).

Integrating chemical fertilizers with organic manures has been found to be quite promising not only in maintaining higher productivity but also in providing greater stability in crop production (Montagu and Goh, 1989; Itnal *et al.,* 1996). Farmyard manure (FYM) is being used as a major source of organic manure in field crops. Limited availability of this manure is, however, an important constraint in its use as a source of nutrients. Vermicompost has been advocated as good organic manure

for use in integrated management practices in field crops (Robinson *et al.,* 1992; Jambhekar, 1992). It is a well known fact that wheat crop responds to applied nitrogen up to 120 to 150 kg N/ha (Doube *et al.,* 1997; Desai *et al*., 1999) and some substitution of plant nutrients particularly all nutrients except nitrogen may be achieved by using organic manures. Positive response of wheat to applied nitrogen along with FYM has been reported by several workers (Ranva and Singh, 1999; Khandal *et al.,* 2004). However, no work has been reported on the effect of vermicompost and nutrient substitution through this manure in wheat crop. Keeping this in view, field experiments were carried out to study the effect of integration of chemical nitrogen with organics on this important wheat crop exhibiting nutrient and medicinal valu.

Materials and Methods

The field experiments were conducted during winter seasons of 2003-05 at Jawalagarh Farm of D.S. College, Aligarh. The treatments comprised five levels of organic manures *viz*., no organic manure (control), farmyard manure (FYM) at 10t/ha, vermicompost at 5, 7.5 and 10t/ha in main plots and 5 level of N, *viz*. 0, 50, 100, 150 kg N/ha and recommended fertilizers (120N + 60P_2O_5 +25$ZnSO_4$ kg/ha) replicated thrice in split-plot design. The soil of the experimental field was sandy loam in texture, alkaline in reaction (pH 8.1), low in available nitrogen (160-160 kg/ha), medium in available phosphorus (9.5 to 10.8 kg/ha) and high in available potash (328 to 350 kg/ha). The FYM contained 0.68 and 0.70% N, 0.20 and 0.24% P_2O_5 and 0.75 and 0.82% K_2O during 2003-2004 and 2004-05, respectively. During both the years, FYM and vermicompost were applied about 3 weeks before sowing of the crop. Half of the nitrogen in the form of urea and full doses of phosphorus and zinc sulphate as per treatments, were applied at the time of sowing and remaining half nitrogen was top dressed after first irrigation. All other operations were performed as per the recommendations for the crop. The data on various yield attributes, grain straw and total biological yields were recorded in different treatments.

Results and Discussion

Effect of organic manures

The two years result revealed that all levels of organic manures improved the yield attributing characters (Table 1) and grain, straw and biological yields over no organic manure. Number of effective tillers, length of ears, number of grains/spike, grain weight/spike and 1,000 grain weight were maximum with vermicompost 10t/ha, but were statistically at par with vermicompost 7.5 t/ha. However, effect of organic manure was non-significant on ear length and number of grains/spike during first year as well as on 1000 grain weight during both the years.

Table-1. Growth and yield attributes of wheat as influenced by organic sources and fertilizer levels

Treatment	Effective tillers/m row length at harvest		Length of ear (cm)		No. of grains/ spike		Grain weight(g)/ spike		Test weight (g)	
	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05	2003-04	2004-05
Organic sources										
No organic manure	94.5	96.5	8.5	8.4	41.6	43.8	1.28	1.31	29.5	–
FYM at 10 t/ha	97.4	102.9	8.6	8.7	44.0	46.4	1.37	1.42	30.8	30.7
Vermicompost at 5t/ha	97.1	101.5	8.5	8.6	42.5	44.6	1.35	1.38	30.9	30.6
Vermicompost at 7.5 t/ha	99.6	105.1	8.6	8.8	43.9	47.8	1.42	1.48	30.9	30.9
Vermicompost at 10t/ha	101.4	107.3	8.9	9.1	46.6	49.7	1.47	1.52	31.1	31.0
CD (P = 0.05)	2.50	2.13	NS	0.37	NS	4.13	0.11	0.12	NS	NS
Fertilizer levels										
N0 (Control)	74.9	79.6	6.3	6.8	25.6	31.2	0.78	0.87	30.1	30.2
N50 kg/ha	91.4	96.9	8.5	8.5	43.0	44.2	1.41	1.45	30.4	30.3
N100 kg/ha	100.7	107.4	9.0	9.0	46.7	49.0	1.49	1.55	30.7	30.5
N150 kg/ha	110.6	113.7	9.6	9.6	51.0	53.3	1.57	1.58	30.8	30.7
N120 P60Zn25 kg/ha	112.4	115.8	9.8	9.8	52.2	54.6	1.64	1.66	31.0	31.0
CD (P = 0.05)	1.09	1.49	0.30	0.32	3.14	3.48	0.09	0.10	NS	NS

Application of FYM 10t/ha was also superior to vermicompost at 5 t/ha. These effects on yield attributes were reflected in grain yield and significantly higher grain yield was recorded with vermicompost 10t/ha over 5t/ha during 2003-04 and on pooled basis but it was statistically on par with rest of the organic manures during both the years as well as on pooled basis (Table 2). Vermicompost at 7.5 t/ha and FYM at 10t/ha were also better than vermicompost at 5 t/ha. On pooled basis highest grain yield of 3,835 kg/ha was recorded with vermicompost at 10 t/ha which was 16.5, 7.2, 3.4 and 1.6% higher than no organic manure, vermicompost at 5t, FYM 10t/ha and vermicompost at 7.5 t/ha, respectively. The beneficial effect of organic manures was also recorded in straw and total biological yields with vermicompost 10 t/ha which was closely followed by vermicompost at 7.5 t/ha and on pooled basis, percent increase in these two treatments in straw yields was 13.8 and 12.2 over control, respectively. The beneficial effect of organic sources of manures on yield attributes, grain, straw and biological yields could be attributed to the fact that after proper decomposition and mineralization of organic manure, the manures supplied made available plant nutrient directly to the plants and also had solubilizing effects on fixed form of nutrients. Better response of field crops to vermicompost was also reported by Ghosh *et al.,* 1999.

Effect of nitrogen and fertilizers levels

The applications of nitrogen and recommended fertilizers levels had significant effect on various yield attributes, grain, straw and total biological yields of the crop (Table 2). These parameters were increased significantly with each successive increase in fertilizer level in both the years and on pooled basis. Maximum yield of 4,385 kg/ha (pooled) was recorded with recommended fertilizers followed by 4,200 kg/ha with 150 kg/ N/ha. The per cent increase with recommended dose over 0, 50,100 and 150 kg N/ha was 83.4, 32.9, 12.1 and 4.4, respectively while increase due to application of 150 kg N/ha was 75.7, 27.3 and 7.4% over 0, 50 and 100 kg N/ha, respectively.

Effect of integrated nutrients supply

The integration of fertilizer nitrogen and recommended dose of fertilizers with FYM or various levels of vermicompost also exerted its effect on grain and straw yields of wheat (Table 3). The productivity of crops in terms of grain yield was minimum in no fertilizer treatment without organic manure. Response of crop to the increasing levels of fertilizers was more in no organic manure treatment and relative increase in grain yield with increasing levels of fertilizer nitrogen was lower in manurial treatments and least improvement was recorded under vermicompost 10 and 7.5 t/ha. Vermicompost at 10 t/ha and 7.5 t/ha in combination with 100 kg N/ha produced grain yield statistically at par with recommended fertilizer level of 120N + $60P_2O_5$ + $25ZnSO_4$ Kg/ha under no organic manures treatment. Similar effects of organic manures and fertilizer N were also visible on straw yields.

Table-2. Yield and harvest Index of wheat as influenced by organic sources and fertilizer levels

Treatment	Grain yield (kg/ha)			Straw yield (kg/ha)			Biological yield (kg/ha)			Harvest index	
	2003-04	2004-05	Pooled	2003-04	2004-05	Pooled	2003-04	2004-05	Pooled	2003-04	2004-05
Organic sources											
No organic manure	3185	3397	3292	4696	4741	4719	7881	8139	8010	40.2	41.7
FYM at 10 t/ha	3514	3905	3710	5146	5386	5266	8661	9290	8976	40.4	42.0
Vermicompost 5t/ha	3400	3756	3578	4963	5221	5092	8363	8977	8670	40.5	41.8
Vermicompost 7.5 t/ha	3573	3973	3773	5125	5462	5293	8698	9435	9067	41.0	42.2
Vermicompost 10 t/ha	3637	4032	3830	5178	5564	5371	8815	9596	9206	41.2	42.0
CD (P = 0.05)	296	206	161	171	289	148	355	450	258	NS	NS
Fertilizers levels											
N0 (Control)	2298	2483	2391	3516	3425	3471	5815	5908	5861	39.5	42.0
N50 Kg/ha	3153	3446	3300	4689	4769	4729	7842	8214	8029	40.2	42.0
N100 kg/ha	3730	4094	3912	5425	5689	5557	9155	9783	9469	40.7	41.8
N150 kg/ha	3932	4468	4200	5623	6133	5878	9555	10601	10078	41.2	42.1
N120 P60Zn25 kg/ha	4197	4573	4385	5855	6358	6107	10052	10931	10492	41.7	41.8
CD (P = 0.05)	77	90	64	126	154	82	108	67	70	1.03	NS

Table-3. Interaction effect of organic sources and fertilizer levels on grain yield (Kg/ha) of wheat

Organic sources	Fertilizers levels (Kg/ha)				
	No	N50	N100	N150	Recom-mended
No organic manure					
2003-04	2000	2822	3300	3700	4105
2004-05	1995	2851	3551	4153	4436
Pooled	1998	2837	3426	3927	4271
FYM at 10 t/ha					
2003-04	2254	3272	3865	3962	4218
2004-05	2487	3563	4279	4583	4612
Pooled	2317	3418	4072	4273	4415
Vermicompost at 5t/ha					
2003-04	2248	3056	3673	3914	4110
2004-05	2470	3445	4025	4380	4460
Pooled	2359	3251	3849	4149	4285
Vermicompost at 7.5 t/ha					
2003-04	2430	3296	3888	3996	4255
2004-05	2648	3663	4301	4598	4656
Pooled	2539	3480	4095	4297	4456
Vermicompost at 10 t/ha					
2003-04	2560	3318	3924	4088	6296
2004-05	2841	3706	4315	4624	4701
Pooled	2687	3512	4120	4356	4499
CD (P=0.05)		**2003-04**	**2004-05**		**Pooled**
Two fertilizers levels at same organic source		172	202		135
Two organic source at same or different fertilizer levels		412	273		229

Conclusion

From this study it may be concluded that for higher productivity of the wheat crop a combination of vermicompost at 10 t/ha and 100 kg N/ha was found optimum.

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A Study of *Nigella sativa* Linn. seeds for antimicrobial activity against multidrug resistant clinical strains of *Pseudomonas aeruginosa*

[1]Mohd. T. Salman,
[2]Rahat A. Khan
and
[3]Indu Shukla

[1]Department of Pharmacology, Era's Lucknow Medical College, Sarfarazganj, Hardoi Road, Lucknow-202003, India

[2]Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India

[3]Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India

Abstract

Nigella sativa (black cumin) seed oil and extracts were tested in varying dilutions against strains of *Pseudomonas aeruginosa* resistant to a number of clinically used antibiotics isolated from patients attending JN Medical College Hospital, Aligarh, using disc agar diffusion technique on inoculated Muellar Hinton agar plates under standard laboratory conditions. Both the oil and Methanolic extract showed remarkable dose dependant antibacterial activity against the tested strains upto a dilution of 1:50 as evident from the zones of inhibition. No cross resistance was noticed with any of the tested antibiotics.

Key Words: *Nigella sativa*, Black seed, *Pseudomonas aeruginosa*, Antimicrobial activity, Antibiotic resistance.

Introduction

Pseudomonas aeruginosa is an important cause of morbidity and mortality in hospitalized, critically ill patients and patients with underlying medical conditions such as neutropenia, and iatrogenic immunosuppression. Nosocomially acquired *P. aeruginosa* isolates tend to be more resistant to antimicrobials than community acquired strains, frequently displaying resistance to multiple classes of antimicrobials. It has become increasingly clear that resistance development in *P. aeruginosa* is multifactorial, with mutation in genes encoding porins, efflux pumps, penicillin binding proteins, and chromosomal â-lactamase (Livermore, 2002; Pai *et al*, 2001). As number of multidrug resistant *P. aeruginosa* strains including those resistant to all â-lactams, carbapenems, aminoglycosides and fluoroquinolones become increasingly larger (Gales *et al*, 2001; Livermore, 2002) the search for antimicrobial agents with alternative mechanisms of action has intensified. Recently, a rapid increase in frequency of multidrug-resistant clinical strains is being recorded, making the available therapeutic options very limited (McGowan, 2006).

Nigella sativa L. (Ranunculaceae) is an herbaceous plant whose seeds have been used for centuries for treatment of various ailments, including infectious diseases, and is an important drug of Unani Medicine. It has been recommended for use on a regular basis in Tibbe Nabwi (Prophetic Medicine) as is evident from the following tradition: Narrated Abu Hurairah, "I heard Allah's Apostle saying, 'There is healing in black cumin for all diseases except death'." (Al-Bukhari, 1976). The seeds have been thoroughly studied scientifically in the last 3-4 decades and have been reported to possess a number of medicinal properties (Ali and Blunden, 2003; Randhawa and Al- Ghamdi, 2002). Their crude extracts (Ali *et al*. 2001; Mouhajir and Pedersen, 1999) and essential oil (Halwani *et al*, 1999; Salman *et al*, 2008a.) have been shown to possess activity against several bacteria. However, little work had been done on their effect against multidrug resistant *P. aeruginosa* isolated from patients. Hence this study was undertaken.

Methodology

Acquisition of seeds and oil of *Nigella sativa*: Seeds of *N. sativa* (locally known as Kalonji) were procured from a local dealer at Aligarh and were authenticated by a botanist at Department of Botany, Aligarh Muslim University, Aligarh. *N. sativa* oil (Kalonji oil) was procured from Mohammedia products, Red Hills, Nampally, Hyderabad, Andhra Pradesh, India. As per manufacturer's information, it was prepared by steam distillation at Hyderabad, India.

Preparation of extracts

(a) *By Soxhlet extraction:* Three types of extracts were prepared: Methanolic extract- using methanol, Ether extract- using diethyl ether and Aqueous extract- using distilled water. Seeds were crushed and extracted with respective solvents in Soxhlet apparatus by heating at their boiling point till it clears.

(b) *By maceration:* Three types of extracts were prepared- Methanolic extract- using HPLC grade methanol, Ether extract- using diethyl ether and Aqueous extract- using sterile distilled water as described previously (Salman MT, 2008b). Briefly, 150 grams of ground seeds were soaked in 150 ml of respective solvents for 7 days at room temperature, followed by filtration and removal of solvent under aseptic conditions. The extracts thus prepared were transferred as aliquots of 1 ml each into sterile vials and stored at -20°C till further use.

Preparation of Drug impregnated filter paper discs

This was done by the method of Morley, 1945 with slight modification. Serial dilutions of aqueous or ether extract were prepared in distilled water or ether respectively and discs (6 mm diameter) having drug concentration of 25 to 400 ìg/disc were prepared.

Methanolic extracts and oil were diluted using methanol and Ethylene glycol respectively upto dilution of 1:100. During sensitivity testing, 4µl of methanolic extract or oil in pure or diluted form was kept on filter paper disc of 6 mm diameter, placed on Muellar Hinton Agar plate inoculated with bacteria.

Inoculation of plates: This was done using flood-inoculation technique (Acar and Goldstein, 1996). Bacterial suspension in Nutrient Broth having turbidity equivalent to 0.5 McFarland was freshly prepared and 2 ml of this was transferred onto the Muellar Hinton Agar plate and distributed gently over the surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate was kept in incubator at 37 ^{0}C for 30 mins for drying before application of discs.

Disc susceptibility testing: This was carried out by placing discs impregnated with test material on surface of inoculated agar plates (Bauer et al, 1966). For sensitivity

testing with standard antibiotics, commercial antimicrobial susceptibility testing discs obtained from HiMedia Laboratories Limited, Bombay were used. The plates were then kept in incubator at 37 ^{0}C for 18 hours and diameters of zones of inhibition were measured.

N. sativa oil and all the above extracts were tested in different dilutions against *P. aeruginosa* (ATCC 27853) and *P. aeruginosa* (NCTC 10662). All the experiments were repeated in triplicate. Ampicillin (10 ìg/disc) and Amoxicillin (10 µg/disc) obtained from HiMedia Laboratories Limited, Bhaveshwar Plaza, LBS Marg, Mumbai, India were kept as standard. Discs soaked in respective diluents were also kept as negative control.

Aqueous extracts and Ether extracts prepared by Soxhlation or maceration showed poor, variable and non-reproducible inhibition against the standard bacteria. Hence they were not tested further on clinical isolates. Since Methanolic extract prepared by maceration showed more pronounced activity as compared to Methanolic extract derived by Soxhlet, it was used for further studies on clinical isolates. *N. sativa* oil also showed pronounced antibacterial activity against standard strains and was studied further on clinical isolates.

Strains of *P. aeruginosa* were isolated from pus, blood, cervical swab and ear discharge of various patients attending Jawaharlal Nehru Medical College Hospital, Aligarh and tested for their sensitivity to a number of clinically used antibiotics. The concentrations of antimicrobial sensitivity testing discs used and interpretation of sizes of zones of inhibition were in accordance to Performance Standards for Antimicrobial Disk Susceptibility Tests, NCCLS, 2002 (Wayne, 2002) . The antibiotics tested and their concentrations used were: Ampicillin (10ìg/disc), Amikacin (30 ìg/disc), Cotrimoxazole (trimethoprim-1.25, sulphamethoxazole-23.75 ìg/disc), Cefaclor (30 ìg/disc), Ciprofloxacin (5 ìg/disc), Cephoperazone (75 ìg/disc), Ceftriaxone (30 ìg/disc), Cephotaxime (30 ìg/disc), Ceftazidime (30 ìg/disc), Gentamicin (10 ìg/disc), Gatifloxacin (5ìg/disc), Imipenem (10ìg/disc), Ofloxacin (5ìg/disc), Tetracycline (30ìg/disc) and Tobramycin (10 ìg/disc). Out of these, 21 strains which were resistant to 4 or more antibiotics belonging to at least 3 different classes were tested for their sensitivity to Methanolic extract and oil of *N. sativa* in various dilutions.

Statistical Analysis

Correlation between drug concentration and zone sizes was found using Pearson's test. Zone sizes obtained by Ampicillin and different doses of oil or Methanolic extracts against standard strains of *P. aeruginosa* were compared using One-way Anova followed by Dunett test. Clinical strains which were sensitive to oil or extract and those which were resistant were compared statistically for their sensitivity to various antibiotics using Fisher's Exact Test. Clinical strains sensitive or resistant to oil/extract were also compared for number

of antibiotics to which each of the strains was resistant and for number of groups of antibiotics to which each of the strains showed resistance using Mann Whitney U test. P value <0.05 was considered as significant. All analysis were done using SPSS 16.0 software.

Results

N. sativa seeds when subjected to Soxhlet extraction gave different yields with different solvents. It was highest with methanol (32.5%) followed by water (27%) and ether (16.7%). The yields were reduced to 28-31% of the above values when extraction was done by maceration with the respective solvents. The methanolic extract was a reddish brown liquid, oily in nature whereas ether extract was reddish brown semisolid, oily in nature. The aqueous extract was brownish black solid, greasy in nature.

Effect of Nigella sativa against standard strains

N. sativa oil as well as Methanolic extracts showed remarkable dose dependant antimicrobial activity against the standard strains (r 0.590 to 0.935, $P < 0.05$, Table 1). The methanolic extract obtained by maceration showed highest antibacterial activity. The antibacterial activity was observed upto 1:100 dilution, the least concentration tested. Zone sizes formed by 1:1 to 1:50 dilutions of *N. sativa* oil against *P. aeruginosa* (NCTC 10662) were significantly greater than that formed by Ampicillin (10 µg/disc) ($P < 0.05$, Table 1). Ether extract as well as Aqueous extract obtained by Soxhlet method or by maceration showed variable effects. The inhibition observed was not dose dependent. The diameters of zones of inhibition were of small size (7- 11 mm) and were not reproducible.

Effect of Nigella sativa against Clinical isolates

Out of 21 strains tested, 7 were resistant to 4-6 antibiotics, 8 to 7-9 antibiotics, 4 to 10-12 antibiotics and 2 to 13 antibiotics. Resistance was highest to Ampicillin followed by Gentamicin, Ciprofloxacin, Amikacin, Ceftazidime, Cefotaxime, Ofloxacin and Ceftriaxone (Figure 1). The oil was active against 11 strains, upto a dilution of 1:50 against 1, upto 1:10 against 4 and only in undiluted state against 6 strains. The Methanolic extract was active against 12 strains, upto a dilution of 1:50 against 4 strains, upto 1:10 against 5 and only in undiluted state against 3 strains. It showed zones of inhibition larger than oil at all concentrations (Table 2). No significant correlation was found between the resistance to *N. sativa* oil/extract and resistance to any other tested antibiotics (Fisher's test: $P > 0.1$, Table 3), number of antibiotics to which the isolate was resistant or the number of classes of antibiotics to which the isolate showed resistance.

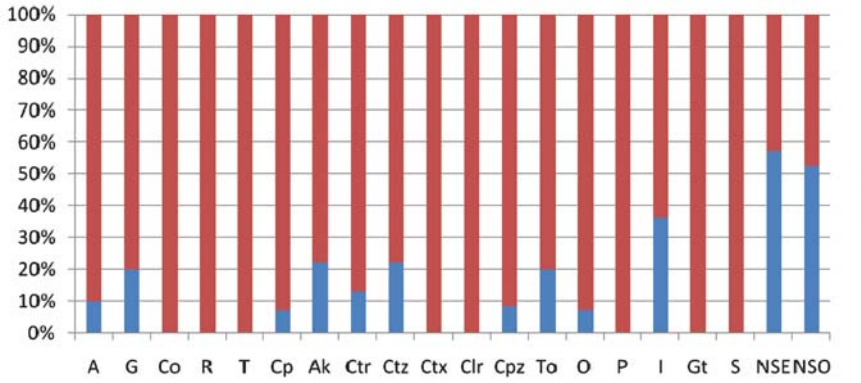
Table-1. Effect of *N. sativa* Methanolic extracts and oil against standard strains of *P. aeruginosa*

Conc./disc	Mean diameters of zones of inhibition in mm					
	Methanolic extract (Maceration)		Methanolic extract (Soxhlation)		Oil	
	P. aeruginosa (ATCC 27853)	*P. aeruginosa* (NCTC 10662)	*P. aeruginosa* (ATCC 27853)	*P. aeruginosa* (NCTC 10662)	*P. aeruginosa* (ATCC 27853)	*P. aeruginosa* (NCTC 10662)
1:1	19*,[a]	13[a]	18*,[a]	10[a]	24*,[a]	25*,[a]
1:10	17*,[a]	10[a]	14*,[a]	-	20*,[a]	20*,[a]
1:50	15*,[a]	10[a]	11[a]	-	20*,[a]	19*,[a]
1:100	12[a]	09[b]	08[c]	-	-	12[a]
r	0.918[d]	0.579[e]	0.897[d]	0.540[e]	0.902[d]	0.908[d]
Methanol	-	-	-	-		
Ethylene glycol					-	-
Ampicillin (10 µg/disc)	-	12	-	12	-	12
Anova FP	100.40< 0.001	23.30< 0.001	75.20< 0.001	32.13< 0.001	227.70< 0.001	154.50< 0.001

Comparison with blank (Methanol/ Ethylene glycon): [a]P < 0.001, [b]P < 0.005, [c]P < 0.05

r- correlation between drug concentration and zone size: [d]P < 0.01, [e]P < 0.05

* P < 0.05 when compared to Ampicillin



A- Ampicillin, Ak- Amikacin, Co- Cotrimoxazole, Clr- Cefaclor, Cp- Ciprofloxacin, Cpz- Cefoperazone, Ctr- Ceftriaxone, Ctx- Cefotaxime, Ctz- Ceftazidime, G- Gentamicin, Gt- Gatifloxacin, I- Imipenem, NSE- *N. sativa* extract, NSO- *N. sativa* oil, O- Ofloxacin, R- Roxithromicin, S- Sparfloxacin, T- Tetracycline, To- Tobramycin

Figure-1. Sensitivity pattern of Clinical isolates of *P. aeruginosa*

Table-2. Sensitivity of *P. aeruginosa* to *N. sativa* Methanolic extract and oil. (Figures show number of sensitive strains)

Test drug (4µl/disc)		Extract			Oil		
Dilution		1:1	1:10	1:50	1:1	1:10	1:50
Zone size (mm)	7-11	4	7	4	6	4	1
	12-16	6	2	0	4	1	0
	17-21	2	0	0	1	0	0
Total no. of sensitive strains		12	9	4	11	5	1

Discussion

Soxhlet extraction of *N. sativa* seeds gave different yields with different solvents. It was highest with methanol followed by water and ether. This is in confirmation with Boskabady and Shahabi (1997) who reported a yield of 24% of aqueous extract. The Methanolic extract was a reddish brown liquid, oily in nature. The ether extract made by Hanafy and Hatem (1991) by soaking the seeds for 6 hrs. was also dark brown and oily in nature but it was a clear liquid and the yield was 11% v/w. This difference could be due to difference in the seed sample, duration of soaking and/ or temperature at which extraction was done.

Among the different extracts, the Methanolic extract showed most pronounced antibacterial activity. The aqueous and ether extracts showed poor, variable and

Table-3. Sensitivity pattern of clinical isolates of *P. aeruginosa* sensitive to *N. sativa* Extract and/or Oil

Antibiotics	Exact significance (Fisher's test)		% strains showing resistance to antibiotics		
	Sensitivity to oil	Sensitivity to Extract	Strains sensitive to oil	Strains sensitive to Extract	Overall
A	0.77	0.71	88.9	90.0	89.9
G	0.71	0.63	80.0	81.8	80.0
Co	–	–	100	100	100
R	–	–	100	100	100
T	–	–	100	100	100
Cp	0.43	0.50	83.3	85.7	92.9
Ak	0.29	0.38	66.7	70.0	77.8
Ctr	0.34	0.43	77.8	80.0	86.7
Ctz	0.49	0.59	72.7	75.0	77.8
Ctx	–	–	100	100	100
Clr	–	–	100	100	100
Cpz	0.75	0.75	88.9	88.9	91.7
To	0.47	0.47	100	100	80.0
O	0.64	0.71	88.9	90.9	92.9
P	–	–	100	100	100
I	0.28	0.28	75.0	75.0	63.6
Gt	–	–	100	100	100
S	–	–	100	100	100

A- Ampicillin, Ak- Amikacin, Co- Cotrimoxazole, Clr- Cefaclor, Cp- Ciprofloxacin, Cpz- Cefoperazone, Ctr- Ceftriaxone, Ctx- Cefotaxime, Ctz- Ceftazidime, G- Gentamicin, Gt- Gatifloxacin, I- Imipenem, O- Ofloxacin, R- Roxithromicin, S- Sparfloxacin, T- Tetracycline, To- Tobramycin

non-reproducible inhibition against the standard bacteria. Since most of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through ethanol or methanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether.

However, tannins and terpenoids will occasionally be found in the aqueous phase, and may contribute to antibacterial activity in aqueous extract (Cowan, 1999).

N. sativa oil also showed good antibacterial activity. Since, as per manufacturer's information, it was prepared by steam distillation, it is the essential oil. Antimicrobial activity was highest in Methanolic extract and oil. This confirms the study of Toama *et al* (1974) who reported that the antimicrobial activity resided solely in the alcohol-soluble fraction of volatile oil.

N. sativa oil and Methanolic extracts derived by Soxhlet as well as by maceration, when tested in varying concentrations (1:1 to 1:100) showed dose dependent anti-bacterial activity ($p < 0.05$) against 2 standard strains of *P. aeruginosa* [*P. aeruginosa* (NCTC 10662) and *P. aeruginosa* (ATCC 27853)]. Out of 21 clinical strains of *Pseudomonas aeruginosa* tested, resistant to a number of antibiotics, the oil was active against 11 strains and Methanolic extract was active against 12 strains. In a study by Mouhajir *et al* (1999), 20 ìg of Methanolic extract was inactive against 2 strains of *P. aeruginosa* (one supersensitive and one wild). This may be due to difference of strains tested. Our observation is also in accordance with the study of Toama *et al* (1974) who found moderate activity of undiluted volatile oil (obtained by steam distillation of fixed oil) against one strain of *P. aeruginosa*.

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal (Zahner and Fiedler, 1995). This hypothesis is further supported by our study in which no significant correlation was found between the resistance to *N. sativa* oil/extract and resistance to any other tested antibiotics (Fisher's test: $P > 0.1$, Table 3) showing absence of cross-resistance with the tested antibiotics.

Thymol is a phenolic alcohol present in the essential oil (Randhawa and Al- Ghamdi, 2002) that has been reported to possess antibacterial activity (Karapinar and Aktug, 1987). Since Thymol is present in the methanol soluble portion of oil (Enomoto, 2001), it will also be extracted in the Methanolic extract. In a study by Kahsai (2002), thymoquinone present in volatile oil obtained from the crude extract exhibited remarkable inhibition of the growth of various strains of bacteria. Thymoquinone is present in the methanol soluble portion of N. sativa oil (Abou Basha *et al.* 1995) and thus will be extracted in Methanolic extract of seeds also. The seeds also contain tannins, which can be extracted by methanol (Eloff, 1998). A number of studies have reported antimicrobial properties of tannins (Scalbert, 1991).

It may be concluded that *Nigella sativa* oil as well as Methanolic extract are active against multidrug resistant strains of *Pseudomonas aeruginosa* and may be used, at least topically, in susceptible cases. Further research is needed to advocate its use in systemic infections.

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Studies on Biological Contamination of Commercial Samples of Herbal Drugs

Rajeev Kr. Sharma

Pharmacopoeial Laboratory for Indian Medicine, Opp. M-Block, Sector 27, Kamla Nehru Nagar, Ghaziabad-201002, India

Abstract

Biological contaminants (Bacterial, Fungal and insect) have been investigated in commercial samples of herbal drugs viz. *Acorus calamus* Linn., *Asparagus racemosus* Willd., *Calotropis procera* (Ait.) R. Br., *Cissampelos pareira* Linn. and *Cyperus rotundus* Linn. Bacteria, fungi and insect associated with these herbal drugs were isolated and reported. The presences of biological contaminants indicate poor post-harvesting and storage condition of herbal drugs which leads to deterioration of quality of the drugs.

Key Words: Biological contaminantion, Storage, Quality Control.

Introduction

During the past few decades, commercial exploitation of plants for medicaments has expanded rapidly. With this importance and recognition followed by the rapid expansion of the trade, the instances of lack of quality check have increased manifold. This has put a question mark on the quality of herbal drugs. The legislation enacted as 'Drugs & Cosmetic Act & Rules' has previewed this situation by promulgating schedule-T to ensure the quality of drugs. Raw materials used in the preparation of medicine have direct impact on the efficacy of the drug. To ensure the quality of herbal drugs, identity of raw material is prioritized where as freedom from biological contaminants (microbiological, fungal and insect) is restricted to finished products. Presence of biological contaminants in raw material is prime cause of deterioration which shortens the shelf life of a product. Post-harvest and storage practices play major role on this aspect. The existing work is carried on herbal drugs of root and rhizome origin resourced from *Acorus calamus* Linn, *Asparagus racemosus* Willd., *Calotropis procera* (Ait.) R. Br., *Cissampelos pareira* Linn. and *Cyperus rotundus* Linn. All these herbal drugs are attributed for different therapeutic activities and important medicinal plants used in a number of formulations of Ayurvedic and Unani System of Medicine (Anonymous,1940; 1948-1976; 1978, Chopra *et. al*, 1956).

Materials & Methods

The commercial samples of following herbal drugs were drawn from different drug dealers and pharmacies as per schedule recommended in USP XX (Anonymous, 1979) (Table 1).

Determination of Biological Contamination: The determination of biological contamination was established for fungi, bacteria and insects in the commercial drug samples. The methodology applied for the studies are as follows as per prescribed protocols (Anonymous, 1980a, 1980b).

Table-1

Sl.No.	Botanical Species	Herbal Drugs	Part Taken for Study
1.	*Acorus calamus* Linn	Vacha	Rhizome
2.	*Asparagus racemosus* Willd.	Shatavari	Root
3.	*Calotropis procera* R.Br.	Arka	Root
4.	*Cissampelos pareira* Linn.	Patha	Root
5.	*Cyperus rotundus* Linn.	Musta	Tuber

Sampling: The commercial samples of the drugs were kept in sterile container and brought to laboratory avoiding further contamination. The contamination studies were carried immediately after the sampling employing following steps for fungal, bacterial and insect contamination.

Bacterial Contamination

Sample Preparation: The drug samples were aseptically pulverised and 1 g of each sample was transferred to a dry sterile measuring cylinder (25 ml capacity). The volume was made up to 25 ml with sterile phosphate buffer (pH 7.2), after which the cylinder was covered with a sterilized glass stopper and manually shaken for about 20 min so as to liberate active, inactive mycelia, spores and propagules present in the samples.

Culture Media: Nutrient broth (for *Escherichia coli* and *Salmonella* enrichment culture), MacConkey's Agar (for *E. coli*) and Bismuth Sulphite Agar (for *Salmonella*).

Isolation of Pathogenic Bacteria – Preparation of Enrichment Culture: 1 g powdered drug samples were placed in 100 ml of nutrient broth and shaken well. The cultures were kept standing for one hr and shaken again. All cultures were incubated at. 37ºC for eighteen to twenty-four hours to promote the growth of expected bacterial contaminations.

Inoculation and Incubation: 1 ml quantity each of enrichment cultures of drugs samples were added to previously prepared MacConkey agar media and bismuth sulphite agar media aseptically. The plates were incubated at 36ºC to 38ºC for eighteen to twenty-four hours.

Identification of Pathogenic Bacteria: The colouration of colonies grown on cultural media plates were considered as confirmation of bacterial contamination of particular pathogenic bacteria. The development of pink to red coloured colonies on MacConkey's agar culture media and black to green coloured colonies on Bismuth

sulphite agar media were taken as indication of *E. coli* and *Salmonellae* respectively which was further confirmed by gram staining techniques (Wolf *et al.,* 1975).

Fungal Contamination

Sample Preparation: Same as in case of bacterial contamination.

Culture Media: Sabouraud Agar

Preparation of Culture Media Plates: The prepared sterile culture media was aseptically pipette (20 ml) into sterile Petri-dishes. The Petri dishes were covered and media was allowed to solidify at room temperature.

Inoculation: 1 ml of the prepared suspension was poured on the surface of the sterile media. The Petri-dishes were shaken gently to disperse the suspension throughout the culture media uniformly. Five culture media plates were prepared for each drug. Two culture media plates were kept blank without sample and rest of the three for the isolation of fungi in the drug.

Incubation: All the plates were kept in incubator at 25º-28ºC for 72 hours to 168 hours.

Identification of fungi: The fungal colonies arising on the plates were identified to the species level wherever possible. The identification of the fungal genera and species was carried out through the help of various specialized references (Gilman, 1957; Ellis, 1971; Barnett and Hunter, 1972).

Insect contamination

Isolation of Insect Debris: The drug samples were pulverized and 5 g of each sample was transferred to a beaker 100 ml of water added to the beaker and acidifieid with a few drops of hydrochloric acid. It was heated to boiling for 15 minutes with continuous stirring. The inner surface of beaker washed down with water and allowed to cool and quantity of 25 ml kerosene oil was mixed thoroughly with it on a magnetic stirrer for 2 min. The whole content of beaker was transferred to a 500 ml conical flask. The suspension kept standing for 5 minutes and a rod with a rubber stopper fixed to its one end was inserted into the conical flask. The rubber stopper was specified for close fitting in the neck of conical flask. The flask was filled with water to raise the kerosene oil layer well into the neck of the trap flask and allowed to stand for 30 minutes. After 30 minutes the kerosene oil layer was trapped by raising stopper as far as possible into the neck of the flask. The stopper held fastly and liquid above the stopper was poured off into a beaker. The rod and neck of the trap flask was rinsed out with water into beaker.

The neck of trap flask was washed down with water, and a further quantity of 15 ml kerosene oil was added and followed by sufficient to bring kerosene oil layer well

into the neck of flask and was kept standing for 15 minutes stirring every 5 minutes. After 15 minutes kerosene oil layer was trapped off for second time in the same manner into the same beaker and material washed on rod and neck of the flask with water. A final washing with absolute alcohol was also carried out. All the trappings were filtered by suction through a smooth, high wet strength, rapid acting filter paper. The beaker was washed with alcohol and the washings were filtered. The filter paper was removed to a petri dish and moistened with glycerol-alcohol (1:1) mixture for examination under suitable magnification. The filter paper was examined for insect fragments, hair, barbules, etc. with unaided eye under clear day light or 15x magnification under stereo zoom microscope (Anonymous, 1980b).

Identification of Insect: The insect infested drug sample was dissected to trap the insect and trapped insect was identified for the zoological identity with the help of specialized literature. The identity of the insect was authenticated from Entomology branch of Forest Research Institute, Dehradun (Basson, 1941).

Observations

Based on the studies conducted on the commercial samples of herbal drugs the following observations have been recorded (Table 2).

Discussion and Conclusion

The contamination of the drug material by biological contaminations viz. microbes, fungi and insects does not cause the destruction only but ultimately leads to health hazards to consumers. Sometimes, drug materials are so badly infested with biological contaminants that the possibility of the production of toxins can not be ignored. Among all the different toxins known so far, aflatoxins activity leading to strong hepatocarcinogenic activity, frequently occurs in high concentrations in stored drug materials. Considerable interest therefore lies in the investigations pertaining to the biological contaminants associated with the drug samples. The results of the present study are summarized in Table 2.

The result elucidates the various components of different contaminants associated with all the commercial samples of drugs studied. The identification of bacterial contaminants was only made regarding *Escherichia coli* and *Salmonella* species. Both these bacteria are pathogenic and are responsible for a number of human infections. *Escherichia coli* were found associated with some samples of Acorus *calamus* Linn and *Cyperus rotundus* Linn. *Escherichia coli* survive well in aquatic conditions. Water proves a good media for its transmission. Since both the drugs grow in marshy habitats, so the chances of presence of this pathogenic bacteria is more.

The presence of *Salmonella* species has been found in a few samples of drugs *Calotropis procera* R. Br. This pathogenic bacteria is quite common in soils where

Table-2. Components of Biological Contaminants and their distribution in commercial samples

Contaminants and their components	Incidence In Commercial Samples																			
	AC				AR				CLP				CSP				CR			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Bacteria (Pathogenic)																				
Escherichia coli	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+
Salmonella sp.	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Fungi																				
Aspergillus amstelodami	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
A. brunneo-uniseriatus	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
A. candidus	+	–	+	+	–	–	+	+	–	–	–	–	+	+	–	–	+	–	–	+
A. clavatus	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	+	–	–	–	–
A. flavos	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	+	+	+	–	–
A. fonsecaeus	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
A. fumigatus	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+
A. funiculosus	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. niger	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
A. sulphureus	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–
A. sydowi	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+
A. terricola	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–	–
A. ustus	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	+	–	–	–	–
A. versicolor	–	–	+	+	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–
Circinella simplex	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
Curvularia lunata	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Fusarium nivale	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	+	+	–
F. oxysporum	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+	+
Fusarium sp.	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–
Gilmaniella humicola	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
Monilia sp.	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+	+	+	+	–	–
Mucor fragilis	+	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–
M.mucedo	–	+	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–

Contaminants and their components	Incidence In Commercial Samples																			
	AC				AR				CLP				CSP				CR			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
M. pusillus	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+	+	–	+
Mucor sp.	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Penicillium chermesinum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
P. chrysogenum	+	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
P. decumbens	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
P. javanicum	–	–	–	–	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–
P. lanosum	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
P. pallidum	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P.purpurogenum	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–
P. spinulosum	–	+	+	+	–	–	–	–	+	+	–	–	–	+	–	+	–	–	–	+
Penicillium sp.	+	–	–	+	+	+	+	+	+	–	+	+	–	–	–	–	+	+	–	+
Rhizopus arrhizus	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
R. nigricans	–	–	–	–	–	–	–	–	+	+	+	–	+	–	+	–	–	+	+	+
R. oryzae	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	–	–	–	–
Rhizopus sp.	+	+	–	–	+	+	–	–	–	+	+	–	–	+	+	–	+	+	+	–
Sterile mycelium, black	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	+
Sterile mycelium white	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Insect																				
Lyctus africanus	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–

Abbreviations: AC- *Acorus calamus* Linn; AR- *Asparagus racemosus* Willd.; CLP- *Calotropis procera* R.Br.; CSP- *Cissampelos pareira* Linn. and CR- *Cyperus rotundus* Linn.;

'A' & 'B' – Samples drawn from drug houses of Dehradun and Hardwar
'C' & 'D' - Samples drawn from pharmacies of Hardwar
Indications: '-' Absence and '+' Presence

feacle matters are frequently available. In open and waste lands animal and human excreta is not uncommon. The contaminated samples of drugs are obtained from such plants which grow in open and waste land habitats. The occurrence of these pathogenic bacteria can be attributed to the above.

In the present study thirty-nine fungal species were isolated from the drug samples, and their respective distribution in each sample has been indicated in Table 1. The

dominant genera are *Aspergillus, Fusarium*, *Mucor, Penicillium* and *Rhizopus. Circinella simplex, Curvalaria lunata* and *Gilmaniella humicola* were found to be of restricted occurrence. A few species on the other hand viz. *Aspergillus clavatus, A. flavos, A. niger, Fusarium nivale, Mucor fragilis, Rhizopus nigricans R. oryzae,* black and white sterile colonies flourished well on the drug samples probably due to the availability of the suitable substratum in the environment. The preponderanance of deutromycetes especially Aspergilli might be due to their wide range of distribution and greater tolerance to various limiting factors.

The insect contamination in the drug was observed in three samples of *Calotropis procera* and identified as *Lyctus africanus* Lessne (Order- Colioptera and Family- Lyctidae). This insect is also known as powder post beetle as its infestation to the host result to powdering and badly damaging whole of the material. The contaminant insect and its larvae thrive well on woody material containing starch as it forms the chief source of food. The infested material is woody in nature and also consists of starch as one of the cell content. This type of insect contamination particularly from this insect is only possible through pre-infested material. It is evident that store premises may also lodged preinfested material which caused the dissemination of this insect in present samples of studied drug.

It can be concluded that out of the studied factors biological contamination in commercial drug samples is an increasing threat and this unawareness on either the part of trader or consumer can lead to health hazards. The bacterial contamination is found pathogenic in certain drug samples. Many of the fungi isolated are toxic but there are few fungal species which may be potentially toxigenic. The proper quarantine should be adopted at every level of drug collection to formulation of material for medicaments. The bacterial and fungal contamination can be reduced to appreciable extents through cleaning and drying of drug material. The store premises should be free from humid and damp conditions. The insect infestation can be avoided by using metallic containers having tight lids and periodical cleaning of the store. Besides these, in general a policy must be established for the mandatory checking of drug samples to ensure the absence of pathogenic contaminants and specific limits on the total counts of viable microorganisms and other contaminants should be set forth considering the potential hazards to the consumer.

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CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE
Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH)
Ministry of Health & Family Welfare, Government of India
61 - 65 Institutional Area, Janakpuri, New Delhi - 110 058, India
Tel.: +91-11-28521981, 28525982, 28525983, 28525831/52/62/83/97, 28520501, 28522524 • Fax: +91-11-28522965
Web site: www.ccrum.com • E-mail: unanimedicine@gmail.com & ccrum@rediffmail.com