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Contents

Editorial

During last three decades, a large number of traditional drugs, mainly herbal, have been subjected to clinical, pharmacological, phytochemical and pharmaceutical studies in an effort to validate them and prove their medical efficacy and safety. Methodological advances coupled with availability of modern scientific tools, in recent years, have further brought much awaited spur in such investigations in India and abroad. All these studies have yielded extensive and valuable findings and insights, and there is a need for wide exchange of this information among scientists engaged in the development of new drugs of natural origin.

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

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

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

The current issue of this journal provides 12 original research and review papers in the areas of clinical research, drug standardization, pharmacology, ethnobotanical surveys and allied disciplines contributed by eminent scholars in their respective fields. It is hoped that data presented will contribute significantly in R&D sector of traditional drugs and prove to be an excellent exposition of current research efforts of scientists in this direction. Council acknowledges the authors for their contributions included in this issue and hope for their continued support in this endeavor. We wish to ensure the readers to bring out the future issues of the journal on time.

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

(Prof. S. Shakir Jamil)
Editor-in-Chief

Evaluation of antiinflammatory activity of Habbe-Gule-Aakh in albino rat

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Abstract

In the present study, Habbe-Gule-Aakh (HGA)-a Unani pharmacopoeial preparation, was studied for anti-inflammatory effect. Carrageenan-induced rat paw edema test and Cotton pellet implant test were used to determine the effect against acute and sub-acute inflammation, respectively in different groups of albino rats treated with HGA in the dose of 400, 750 and 1000 mg/kg. Diclofenac sodium was used as standard drug. In Carrageenin-induced rat paw edema, the maximum effect was observed at three hours when HGA produced 26.32%, 47.3% and 50% inhibition of inflammation, respectively. In Cotton pellet implant test, it was found to induce inhibition of 30.94%, 47.78% and 49.80% of granuloma formation. HGA demonstrated significant anti-inflammatory activity in both acute and sub-acute inflammatory condition. The effect in sub-acute inflammation was found more pronounced

Keywords: Habbe-Gule-Aakh, Anti-inflammatory activity, Carrageenin, Granuloma

Introduction

The arthritis is among the most prevalent conditions and one of the largest health care problems, as about one third of all adults over the world suffer from one or other form of arthritis (Denqueker, 1999). Since it is a disease of varied etiology and diverse pathological and symptomatic manifestation that transform soon into chronic stage therefore its management is complex and frequently ends up in a poor prognosis. Despite tremendous development in understanding the complexities of the disease and the rationale of its treatment, in allopathic medicine, successful treatment and cure are still elusive (Gaitonde *et al*., 2000). The partial success to the extent of symptomatic relief claimed, appears to be achieved at very high cost, considering the adverse effect of the medications used in the treatment of various forms of arthritis (Chopra *et al*., 1994). That is why even the practitioners of modern medicine at times prescribe drugs of alternative medicine without necessary understanding of their role (Gaitonde *et al.*, 2000) and about 68% of the patients of chronic arthritis have been estimated to approach non-allopathic physicians for the treatment (Shekhran *et al.*, 2000). Inflammation is the hallmark symptom of arthritis and many other joint diseases and most of the therapeutic regimens evolve antiinflammatory and analgesic attributes of a drug intended to be used in joint disorders. Unani Medicine claims to possess a number of effective

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and safe drugs useful in the treatment of arthritis. These drugs are in use since centuries in various forms of arthritis with a good recuperation rate and without any major side effect. One such pharmacopoeal preparation is Habbe Gule Aakh (HGA) which has been described to possess analgesic and antiinflammatory effect; useful in arthritis and other inflammatory conditions of joints (Lubhaya, 1979; Khan, ynm). It contains four ingredients viz. Gule Aakh (flowers of *Calotropis procera* R. Br.) Zanjabil (*Zingiber officinale* Rosc.), Filfil Siyah (*Piper nigrum* Linn) and Barge Bans (leaves of *Bambusa arundinaceae*, Retz) in equal proportion. Anti-inflammatory and analgesic activity of all the ingredients have been reported in certain recent studies (Jana *et al*., 1993; Mascolo *et al*., 1988; Muniappan *et al*., 2003; Lee *et al.*, 1984). Further, *P. nigrum* is a known bioavailability enhancer (Shoba *et al.*, 1997), while *Z. officinale* has anti-ulcerogenic (Kawai *et al.*, 1992) and anti emetic activity (Kawai *et al*., 1993), thus the combination is having added value of improving some of the side effects frequently associated with anti-inflammatory agents. In view of the above, HGA was studied for its anti-inflammatory effect in acute and sub acute and experimental models.

Materials and Methods

Drug material

'Zanjabil', 'Filfil siyah' and 'Gule aakh' were procured from Dawakhana Tibbiya College, AMU, Aligarh, while 'Barge-Bans' was procured from Forest Research Institute (F.R.I.), Dehradun (Uttrakhand) (now Indian Council of Forestry Research ICFRI). All four plant materials were identified by Dr. Athar, Associate Professor, Department of Botany, Aligarh Muslim University, Aligarh.

Diclofenac sodium (Dfc) was used as standard drug while distilled water was used as vehicle.

Preparation of test drug

All the ingredients were dried under shade and ground in an electrical grinder to get the powder. Powder of each drug was taken in equal proportion and was put together and mixed. The mixture was then extracted in hydroalcoholic solution (50% each) with the help of Soxhlet apparatus. The extract was filtered and evaporated on a hot plate till it dried. The yield percentage of the extract was calculated with reference to crude drug and it was found to be 40.23%. At the time of administration, fresh suspension of extract was prepared in distilled water and administered orally to the animals with the help of gastric cannula.

Dose

The dose for albino rats was calculated by extrapolating the human dose of HGA by conversion factor of 7 (Freidrich et al., 1966). The dose of extract thus calculated, was found to be 400 mg/kg. Two higher doses i.e., 750 mg/kg and 1000 mg/kg were also used in rats to study the dose dependant response.

Animals

The experiment was carried out on healthy albino rats of Wistar strain weighing 150-200 g of either sex divided in to 5 groups of 6 animals each. Prior to the experiment, the animals were allowed to get acclimatized for one week. They were maintained under standard laboratory conditions throughout the experiment period and were provided with standard diet and water *ad libitum*. They were housed in clean propylene cages and maintained at a temperature of 25 $\pm$ 2^0C and humidity of 45-55% with 12 h light and 12 h dark cycle.

Carrageenin-induced rat paw edema test **(***Winter et al., 1962)*

The thickness of right hind paw of albino rats was measured in ml with the help of a plethysmometer (UGO Basile 7041), by dipping the paw up to the indelible mark just below the knee joint. Liquid displaced by the paw gives the paw volume. Animal in group I, II and III were treated with test drug in the dose of 400 mg/kg, 750 mg/kg and 1000 mg/kg, respectively. The animals in group IV served as control and received vehicle, while the standard drug Diclofenac sodium was given to the animals in group V in the dose of 5 mg/kg, orally. One hour after the drug/vehicle treatment, animals were injected 0.1 ml suspension of lambda type carrageenin in normal saline under the planter aponeurosis of right hind paw. The thickness of paw was again measured at 1,2,3,4 and 5 hours after the injection of carrageenin. The percent inhibition in paw volume in test and standard control was calculated in comparison with plain control group by the formula described by Newbould (1963).

Cotton pellet implant test **(***Winter and Porter, 1957)*

Sterile cotton pellet (50 mg ± 1 mg) soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) was implanted subcutaneously in axilla, bilaterally, under ether anesthesia in 5 groups of 6 animals each. Animal in group I, II and III were treated with test drug in the dose of 400 mg/kg, 750 mg/kg and 1000 mg/kg, respectively. Animals in group IV served as control and received distilled water, while the standard drug diclofenac sodium (5 mg/kg) was given to the group V, daily for 10 days. On the 11^{th} day the pellet were dissected out. After removal of fat and extraneous tissues, the pellets were

dried overnight at 60^0C and the weight of dried pellets was determined. Mean weight of pellets of different groups was calculated.

Data analysis

Findings were tabulated and analyzed statistically using Student's't' test. P-value less than 0.05 was considered as significant.

Observations and Results

HGA produced a dose dependant inhibition of carrageenin induced paw edema. Oral administration of 400 mg/kg, 750 mg/kg and 1000 mg/kg of HGA produced significant effect at 2 hours that continued till 4 hours but the maximum effect was observed at 3 hours when it produced 26.32% (NS), 47.37% ($p<0.01$) and 50.00% ($p<0.01$) inhibition, respectively as compared to plain control group. Diclofenac sodium (5 mg/kg) showed 82.89% (<0.001) inhibition after the same period (Table 1, Fig. 1). No significant effect was observed after 5 hours of the administration of test drug. Intergroup comparison showed no difference between the two higher doses but the two doses demonstrated significant effect as compared to the lower dose. The effect of the standard drug was also found significantly increased as compared to the all three dose of test drug.

The percentage of inhibition observed in granuloma formation in cotton pellet implant test was 30.94% ($p<0.02$), 47.78% ($p<0.001$) and 49.80% ($p<0.001$) at the dose of 400 mg/kg, 750 mg/kg and 1000 mg/kg, respectively. Diclofenac sodium (5 mg/kg) showed 59.78% ($p<0.001$) inhibition (Table 2, Fig. 2). Intergroup comparison showed no difference between the two higher doses of the test drug. There was significant difference between Diclofenac sodium and the two lower doses of test drug but no difference was found when it was compared with higher dose. Difference was also not found in between the two higher doses of test drug. The lower dose however induced significantly lesser response as compared to the higher dose.

Discussion

Results of the present study demonstrated dose dependant anti-inflammatory activity possessed by HGA. In Carrageenin-induced rat paw edema test the lower dose of the drug did not induced significant response but the two higher doses were found effective. The effect started from the first hour but was found statistically significant at 2, 3 and 4 hours of the study and the peak effect was observed at 3 hours. The effect of two doses was not found significantly different from each other suggesting that the dose of 750 mg is sufficient to

induce the antiinflammatory effect. Thus the finding demonstrated that the two higher doses HGA possess significant antiinflammatory activity in acute inflammatory condition. The effect of diclofenac was found significantly better than the test drug in acute phase of inflammation.

The test drug also produced significant effect against sub-acute inflammation in Cotton pellet implant test. All the three doses of test drug demonstrated a dose dependent decrease in the weight of cotton pellets ($p<0.02$, $p<0.01$ and $p<0.001$). The two higher doses i.e. 750 mg/kg and 1000 mg/kg produced greater response than the lower dose. Further, the effect produced by the two higher doses was comparable with diclofenac sodium suggesting that the test drug is equally effective as that of the standard drug in reducing the sub-acute inflammation. The study also suggests that overall effect of test drug is more pronounced in sub acute condition as compared to the acute phase of the inflammation and even the lower dose of the test drug is effective against sub acute inflammation.

All the 4 ingredients of the HGA are described in Unani literature to possess anti-inflammatory and analgesic (Attar, 1988; Ibne Baitar, 1999). The antiinflammatory activity of Gule-Aakh (Mascolo, 1988), Zanjabil (Vendruscolo, 2006; Penna, 2003) Filfil siyah (Bang, 2009; Kumar, 2007) and Barge-Bans (Muniappan, 2003) has also been reported in several studies. However, the test combination has not been formulated simply by mixing the four antiinflammatory agents but all the ingredients are meant to serve specific purpose in this combination. The dominance of phlegmatic humor or derangement of its quality is an important cause of arthritis (Kabeeruddin, 2009); this combination was formulated to provide symptomatic relief on one hand and remove the causative factors on the other. By virtue of their anti-phlegmatic effect, all the ingredients help to resolve and remove the phlegm from the body (Ibn Sina, 1887) and on account having antiinflammatory and analgesic effect they provide symptomatic relief to the inflamed joint. In addition, *P. nigrum* has been reported to increase the bioavailability of other drugs and *Z. officinale* possesses antiulcerogenic and anti-emetic effect, thus the former help increase the concentration of the drugs at the site of action while the later protect the gastric mucosa from the likely ulcerogenic effect of an anti-inflammatory agent.

Conclusion

In view of the above, it can be concluded that HGA possesses significant anti inflammatory effect in both acute and sub acute phase of inflammation. The effect is dose dependant and is more pronounced in sub acute inflammatory condition. This combination can be used in arthritis and other inflammatory diseases of joints as described in Unani Medicine.

Table 1: Carrageenean-induced rat paw edema test

Groups	1 h			2 h			3 h			4 h			5 h		
	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value	Increase in paw volume (mean±SE)	% Inhibition	'P' value
Control	0.62±0.04	-	-	0.70±0.04	-	-	0.76±0.02	-	-	0.70±0.20	-	-	0.65±0.06	-	-
Dfc 5mg/kg	0.23±0.05	62.90	< 0.01	0.15±0.05	78.50	< 0.001	0.13±0.02	82.89	<0.001[a]	0.15±0.04	78.57	< 0.001[a]	0.18±0.04	73.31	<0.001
HGA 400 mg/ kg	0.61±0.05	11.60	N.S	0.59±0.05	15.70	NS	0.55±0.11	26.32	N.S	0.62±0.04	11.43	NS	0.64±0.06	11.54	N.S
HGA 750 mg/ kg	0.50±0.07	17.74	N.S	0.49±0.05	30	<0.01[a,b]	0.40±0.08	47.37	<0.01[a] <0.05[b]	0.42±0.06	40.00	< 0.05[a,b]	0.55±0.06	15.38	N.S
HGA 1000 mg/ kg	0.50±0.05	19.35	N.S	0.42±0.09	40	< 0.01[a,b] < 0.05[c]	0.38±0.06	50.00	<0.01 <0.05[b]	0.40±0.03	42.86	< 0.05[a,b]	0.51±0.11	21.54	N.S

n=6

a= compared with plain control; b= compared with standard control; c= inter comparison between two higher doses

Table 2: Cotton pellet implant test

Groups	Weight of cotton pellet (Mean ± SE)	% inhibition	'P' value
Control	94.00± 5.6	-	-
Standard Dfc 5mg/kg	37.81± 3.4	59.78	<0.001[a]
HGA 400 mg/kg	64.91± 3.16	30.94	<0.02[a] <0.01[b]
HGA 750 mg/kg	49.09 ± 3.89	47.78	< 0.01[a] <0.05[b,c]
HGA 1000 mg/kg	47.19± 3.58	49.80	<0.01[a] <0.05[c]

n = 6
a= compared with plain control; b= compared with standard control; c= compared with the lowest dose

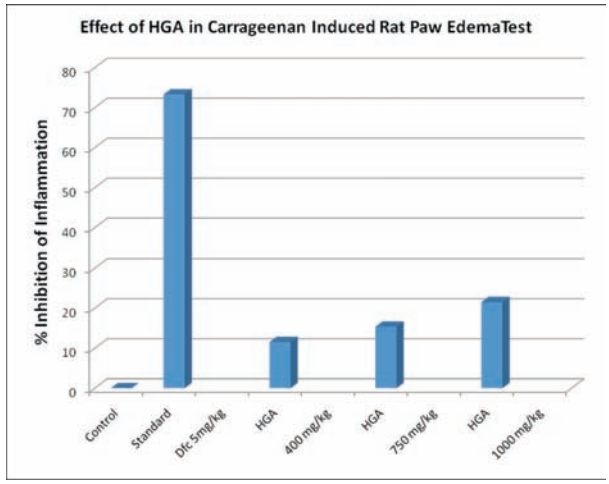

Fig. 1

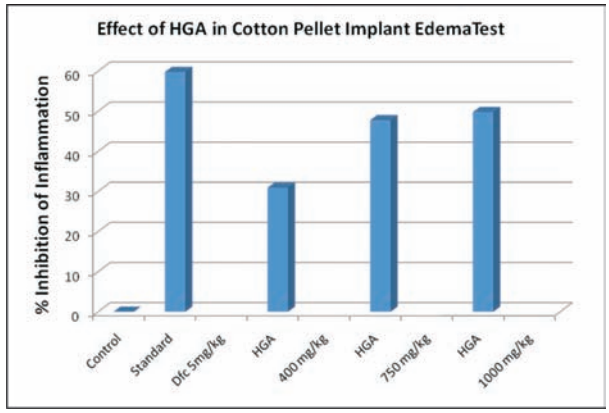

Fig. 2

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The Efficacy and Safety of Unani Coded Drug UNIM-046 in Cases of Bars (Vitiligo) - A Preliminary Clinical Trial

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Abstract

Vitiligo is an acquired idiopathic depigmentary condition characterized by the appearance of depigmented patches on the skin and/or mucous membrane. These patches may appear at various sites of the body, may be localized, segmental or generalized, of various colours e.g. hypopigmented, milky white or pink. This may affect both sex and all age groups. Though it does not affect the general health but considered a social stigma which leads to sociopsychological problems. This study was designed and carried out to evaluate the efficacy and safety of Unani drug UNIM-046 in vitiligo cases in RRIUM, Aligarh.

Out of 25 patients studied, 7 patients showed 40% repigmentation, 12 patients 41 to 70% repigmentation, 3 patients 71 to 90 % repigmentation and 3 patients 91 to 100% repigmentation on different affected parts of the body. In biochemical studies a significant reduction on the levels of Serum Total Protein ($P<0.01$) and Serum Albumin ($P<0.01$) was found; however UNIM-046 significantly increased the levels of Serum Globulin ($P<0.05$); while decreasing the A/G ratio ($P<0.001$) during follow-up in Bars patients. UNIM-046 significantly increases the levels of Serum Glutamate Pyruvate Transaminase (SGPT) ($P<0.01$), While a reduction in the level of Serum Glutamate Oxaloacetate Transaminase (SGOT) $P<0.01$) was observed whereas a significant increases in the levels of Serum Alkaline Phosphatase enzyme, (within normal limits) ($P<0.001$) was observed when compared with pre-treatment values. In haematological studies, a significant decrease in the levels of Erythrocyte Sedimentation Rate (ESR) ($P<0.01$), Red Blood Corpuscles (RBC) count ($P<0.01$) and lymphocyte Count ($P<0.01$) was observed. However, significant increase was observed in Eosinophil Count ($P<0.05$). Thus, test Unani formulation is suggested to be effective as well as safe for Bars patients.

Keywords: Bars, Vitiligo

Introduction

Vitiligo is an acquired idiopathic depigmentary condition characterized by the appearance of white patches on the skin or mucous membrane (Agrawal *et. al.*, 2001). These patches might be localized, segmental or generalized of various colours from milky white to pink. This disease does not affect the general physiology of the body but seriously threatens the social well being. It is a social stigma which leads to social embracement and psychological

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turmoil. It has been estimated that 1-2% of the world population is affected with this agony (Moser *et. al.,* 1999). In India the incidence among dermatology outdoor patients is estimated to be between 3 and 4 percent (Satish & Wahia*.,* 2000). The onset of the disease is reported before 20 years of age in 50% of the cases (Majumdar *et. al.*, 1993) and one third of the total cases of vitiligo were found having positive family history (Das *et. al.*, 1985).

In Unani system of medicine, the disease has been referred in the classics since antiquity and considered a skin disorder produced due to weakness of Quwwat-e-Mughayyara and Mushabbiha at tissue level due to coldness and / or preponderance of phlegm. Galen 130-200 AD) has the same view (Tabri Ahmad BinMohammed, ynm), Rabban Tabri (810-895 AD) (Tabri Abdul Hasan Ali Bin Sahl, 1981), underlined the Fasad-at-Dam (Impurity of blood) and Buroodat-at-Dam (Coldness of blood) to be responsible to this pathology. Avicenna (980-1037 AD) (Ibn Sina, 1906) also emphasizes the importance of Quwwat-e-Ghazia (Nutritive metabolism) which includes the Q. mughayyera and Q. Mushabbiha in pathogensis of the disease. The weaknesses of these faculties ultimately affect the natural process of the synthesis of melanin, a pigment in the melanocytes and responsible for normal skin colour. When the synthesis of melanin or distribution of melanocytes got affected, depigmentation or hypopigmentation occurs leading to vitiligo. Though the various theories regarding the pathogenesis of the disease have been put forward including immunological (Ongenae, *et.al.*; 2003), genetic (Xue *et.al.*, 2005), neural (Orecchia, G.; 2000) and biochemical but complete explanation is still obscured.

Keeping in view the sociopsychologiocal importance of the disease, the therapeutic potential of Unani drugs in this condition and the classical approaches of streamlining of the melanin synthesis. This study was designed and carried out as a clinical open trial with Unani Coded drug UNIM-046 in the cases of vitiligo. The objective of the study was to evaluate the therapeutic efficacy as well as the safety of the coded Unani drug UNIM-046 in the cases of vitiligo.

Materials and Methods

Subjects Selection

Forty eight patients attending in the out patients department (OPD), Regional Research Institute of Unani Medicine (RRIUM), Aligarh of either sex, age (10-60 yrs) were screened to assess the different biochemical and haematological

parameters. Out of forty eight patients, twenty five patients were selected for clinical trial. They were informed about the nature and objectives of trial and a written consent was obtained before enrolling them into the trial. UNIM-046 capsule and UNIM-046 cream were obtained from Central Council for Research in Unani Medicine, New Delhi.

Inclusion Criteria

Patients suffering from Bars (vitiligo) belonging to both sex and different age group (10-60 years) were selected for study. White patches on surfaces of skin neither elevated nor depressed having no exudation or scaling and no itching with hyperpigmented/ hypopigmented margin was taken as vitiligo patches without loss of sensitivity. Bars (Vitiligo) cases free from other systemic diseases, skin diseases and intestinal infestation were included in the study

Exclusion criteria

Pregnant women and patients with hepato-renal, cardiac and pulmonary malfunction, patients on active vitiligo treatment with other drugs, subjects with other skin diseases such as Leprosy, Pityriasis and albinism, subjects with known allergies, subjects who were unwilling to come for regular follow-up for the entire duration of the study and non-cooperative patients were excluded.

Diet restriction and recommendation

Diet plays an important role according to the Unani System of Medicine. As Unani classics relate Bars as a phlegmatic disorder which is attributed to cold and wet, hence any food articles which produces coolness and moistness in the body qualities were strictly prohibited.

Restricted Food Articles

Articles which produce Balgham (Phlegm) are milk and milk products, lemon and lime, tamarind, orange/ citrus fruits, parsley, custard apple, guava, prunes, cashew nuts, melon, water melon, Chinese dates, sour tomatoes and amla e.t.c. and articles which are supposed to bring changes in blood and make blood impure (Fasad-ud-dam) i.e. egg, fish, beef, brinjal and heavy and light mixed food was restricted.

Recommended Diet

Recommended food articles included Wheat, Indian Millet, Pulses, pure ghee obtained from butter, broad beans, French beans, Spinach, Bitter gourd,

Onion, Beet root, Carrot, Chillies, Black pepper, Maize, Figs (fresh and dry), Almond, Walnut, Dates, Mango, Apricots, Grapes, Potatoes, Rice, Papaya, Turnip, Mutton, Bird's flesh. Finally the diet was prescribed according to the patients need.

Collection of blood serum

Blood samples were collected by puncturing the vein at each investigation. 1.0 ml of blood with ethylene diamine tetra acetic acid (EDTA) was used for various haematological parameters and other 2.0-2.5 ml of blood samples were allowed to clot and serum was separated by centrifugation, which was used for various biochemical parameters. Biochemical and haematological investigations were carried out as follows.

Biochemical analysis

Biochemical parameters carried out are as follows. Serum Total Protein, Serum Albumin and Serum Globulin, Serum Glutamate Pyruvate Transaminase (SGPT, E.C. 2.6.1.2) and Serum Glutamate Oxaloacetate Transaminase (SGOT, E.C. 2.6.1.1.), Serum Alkaline Phosphatase enzyme (ALP).

Haematological analysis

Haematological parameters include: Haemoglobin (Hb %), Erythrocyte Sedimentation Rate (ESR), Total Leucocytes Counts (TLC), Red Blood Corpuscles (RBC) and Differential Leucocytes Counts (DLC): Polymorphs, Lymphocyte and Eosinophil Counts.

Drug, Dose and mode of administration

Compound Unani formulation coded drug UNIM-046 capsule, two capsules (500mg each) twice daily was given orally with water after meal to the patient. UNIM-046 cream was locally applied on affected area with exposure of early morning sun rays for 2-7 minutes daily.

Duration of treatment and follow-up

Duration of treatment of patients was 12- months. After registration of patients, a pre-treatment (0 days) and follow-up (3-months, 6-months, 9-months and 12-months) observations were made as per clinical diagnosis and by investigating Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphatase

enzyme (ALP), Serum Total Protein and Serum Albumin, Serum Globulin and A/G ratio were done in biochemical investigations and Haemoglobin (Hb %), Erythrocyte Sedimentation Rate (ESR), Total Leucocytes Counts (TLC), Red Blood Corpuscles (RBC) and Differential Leucocytes Counts (DLC): Polymorphs Lymphocyte and Eosinophil Counts were done in haematological investigations.

Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dennett's' test. The values were considered significant when the P- value was less than 0.01.

Results and Discussions

Demographic Findings

Out of 37 patients of vitiligo, 29.73% patients were male and 70.27% female, which shows that female have higher incidence as compared to male. Shajil *et.al.*, 2006 had reported similar type of observations. 11-20 years of female and 21-30 years age group of male are more susceptible to vitiligo. Non-vegetarian had more incidences (56.75%) than vegetarian (43.24%). Middle income group had more incidences (59.45%) than low income group (40.54%). 26 cases (70.27%) have 0-5 years and 9 cases (24.32%) duration of this disease. The incidence was found more in Balghami patients 26 (54.05%) followed by Damvi 12 (32.43%).

Table 1: Showing distribution of patients according to Age and Sex.

Age group (in years)	Number & % of males	Number & % of Females	Total number & %
0-10	—	02 (5.41%)	02 (5.41%)
11-20	03 (8.11%)	15 (40.54%)	18 (48.64%)
21-30	05 (13.51%)	03 (8.11%)	08 (21.62%)
31-40	02 (5.41%)	02 (5.41%)	04 (10.81%)
41-50	01 (2.70%)	03 (8.11%)	04 (10.81%)
51-60	—	01 (2.70%)	01 (2.70%)
Total	11 (29.73%)	26 (70.27%)	37 (100.00%)

Table 2: Showing distribution of patients according to Dietary Habits.

S. No.	Dietary habits	Number of cases	%
1	Vegetarian	16	43.24%
2	Non-Vegetarian	21	56.75%
	Total	37	100.00%

Table 3: Showing distribution of patients according to Social status.

S. No.	Social status	Number of cases	%
1	HI group	—	—
2	MI group	22	59.45%
3	LI group	15	40.54%
	Total	37	100.00%

Table 4: Showing distribution of patients according to Chronicity of disease.

S. No.	Chronicity of disease (In years)	Number of cases	%
1	0-5	26	70.27%
2	6-10	9	24.32%
3	11-15	—	—
4	16-20	—	—
5	21-25	01	2.70%
6	26-30	—	—
7	31-35	01	2.70%
Total		37	100.00%

Table 5: Showing distribution of patients according to Temperament.

S. No.	Type of Temperament	Number of cases	%
1	Damvi	12	32.43%
2	Balghami	20	54.05%
3	Safravi	05	13.51%
4	Saudavi	—	—
	Total	37	100.00%

Pigmentation response

After completion of the treatment with Unani coded drug UNIM-046 (12th month), 7 (28 %) out of 25 patients showed 40% pigmentation, 12 (48%) patients showed 41-70% pigmentation, 3 (12 %) patients showed 71 to 90% pigmentation, 3 (12 %) patient showed 91 to 100% pigmentation affected on the different parts of the body in vitiligo patients.

Biochemical Studied

Serum Proteins

UNIM-046 significantly reduced the levels of Serum Total Protein 7.98% (P<0.01), 8.64% (P<0.001) and 6.12% (P<0.001) (3rd, 6th & 9th months), Serum Albumin 9.03% (P<0.05) and 14.0% (P<0.001) (3rd & 12th months), and A/G ratio 28.49% (P<0.05) (12th months) (Table-6), when compared with pre-treatment to the different (3rd to 12th months). A significant increase in the levels of Serum Globulin 13.7% (P<0.001) (12th month) (Table-1) were observed in Bars (Vitiligo) patients. *Verma et. al*., (2012) had reported similar type of observations in vitiligo patients treated with Unani coded drug UNIM-045.

Liver Function Tests

A significant increase in the level of the Serum Glutamate Pyruvate Transaminase (SGPT) 26.61% (P<0.05), 18.15% (P<0.01) and 12.21% (P<0.05) (3rd, 6th & 9th months), Serum Glutamate Oxaloacetate Transaminase level (SGOT) 14.67% (P<0.05), however significant decrease 11.94% (12th month) but within normal level was observed, when compared with pre-treatment to the different follow-up (Table-7). UNIM-046 significantly increased but within normal level of Serum Alkaline Phosphatase enzyme, 21.11% (P<0.05), 24.40% (P<0.001) and 28.33% (P<0.001) (6th, 9th & 12th months) were observed when compared with pre-treatment to the different follow-up (Table-7) in bars (vitiligo) patients.

Haematological Studies

In haematological studies UNIM-046 significantly decrease the levels of haemoglobin 3.72% ((P<0.01)) and 5.31 % (P<0.01) (9th & 12th months), Red blood corpuscles 7.27% (P<0.01) and 5.20% (P<0.05) (3rd & 6th months) and lymphocyte counts 11.85% (P<0.05), 16.71(P<0.001) (6th & 9th months) however a significant increase in the level of eosinophil counts 51.69% (P<0.01), 31.46% (P<0.05) and 30.30% (P<0.05) (3rd, 9th & 12th months) (Table-8) were observed, when compared with pre-treatment to different follow-ups in bars (vitiligo) patients. No concrete inference might be taken from these findings since sample size is small. However, the drug was found safe in general.

Conclusion

The drug UNIM-046 showed considerable repigmentation effect in vitiligo cases and it was found safe on biochemical as well as haematological

parameters. No untoward effect of the drug was noticed on clinical, biochemical and haematological parameters during the course of treatment

Table 6: Effect of Unani coded drug UNIM- 046 (Oral and local) on the level of Serum Total Protein, Serum Albumin, Serum Globulin and A / G ratio.

Group → / Parameter ↓	(Pre-treatment)	3 -Month	6-Month	9-Month	12-Month
Serum Total Protein (gm/dl)	7.52 ± 0.16	6.92 ± 0.90**	6.87 ± 0.14***	7.06 ± 0.16*	7.19 ± 0.17▪
Serum Albumin (gm/dl)	4.43 ± 0.14	4.03 ± 0.14*	4.19 ± 0.10▪	4.14 ± 0.11▪	3.81 ± 0.10***
Serum Globulin (gm/dl)	2.92 ± 0.18	2.93 ± 0.16▪	2.76 ± 0.16▪	2.67 ± 0.17▪	3.32 ± 0.15*
A/G Ratio	1.72 ± 0.16	1.49 ± 0.11▪	1.71 ± 0.15▪	1.76 ± 0.14▪	1.23 ± 0.08***

*P<0.05 significant **P<0.01 significant, ***P<0.001 highly significant

Table 7: Effect of Unani coded drug UNIM- 046 (Oral and local) on the levels of SGPT, SGOT and Serum Alkaline Phosphatase.

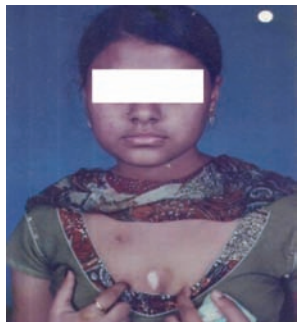
Group → / Parameter ↓	(Pre-treatment)	3 -Month	6-Month	9-Month	12-Month
SGPT (IU/L)	26.95 ± 2.06	34.12 ± .22**	31.84 ± 2.05**	30.24 ± 1.80*	26.32 ± 1.24▪
SGOT (IU/L)	29.99 ± 1.22	34.39 ± 1.63*	31.23 ± 2.64▪	28.16 ± 1.33▪	26.41 ± 0.81**
Serum Alkaline Phosphatase (IU/L)	69.60 ± 4.92	81.52 ± 5.87▪	84.29 ± 5.57*	86.58 ± .84***	89.32 ± .82***

*P<0.05 significant, **P<0.01 significant,***P<0.001 highly significant

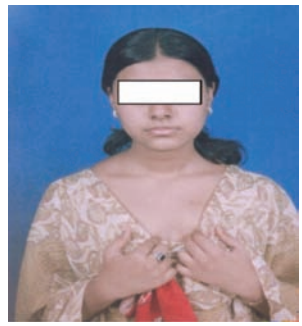
Table 8: Effect of Unani coded drug UNIM- 046 (Oral and local) on Haemogram.

Group → / Parameter ↓	(Pre-treatment)	3 -Month	6-Month	9-Month	12-Month
Haemoglobin (gm %)	12.63 ± 0.17	12.29 ± 0.24▪	12.19 ± 0.26▪	12.16 ± 0.20*	11.96 ± 0.21**
ESR (mm /hr)	22.16 ± 1.77	13.28 ± 1.70**	24.20 ± 3.11*	20.88 ± 3.14▪	18.36 ± 2.56▪
R.B.C. (10 6 /mm3)	3.85 ± 0.08	3.57 ± 0.09**	3.65 ± 0.09*	3.79 ± 0.08▪	3.74 ± 0.08▪
T.L.C. (103/mm3)	6.75 ± 0.32	6.46 ± 0.47▪	6.48 ± 0.45▪	6.39 ± 0.41▪	6.30 ± 0.35▪
Polymorphs (%)	62.64 ± 1.88	58.24 ± 1.98▪	66.40 ± 1.56▪	66.00 ± 1.73▪	62.56 ± 1.71▪
Lymphocyte (%)	33.76 ± 1.85	36.72 ± 1.96▪	29.76 ± 1.41*	28.12 ± 1.34**	33.12 ± 1.59▪
Eosinophil (%)	3.56 ± 0.32	5.40 ± 0.58**	4.08 ± 0.33▪	4.68 ± 0.5*4	4.64 ± 0.44*

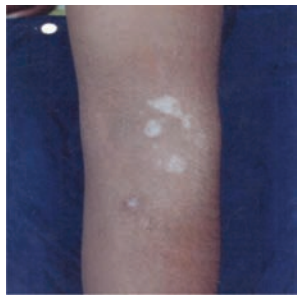
*P<0.05 Significant, **P<0.01 significant



Pre-treatment



After-treatment
(12-Month)



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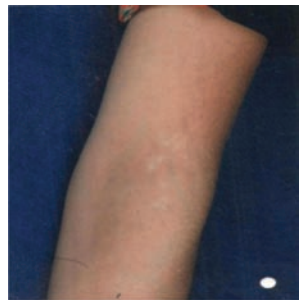
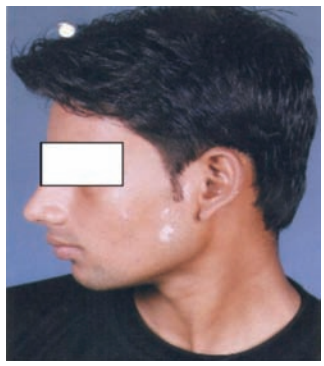
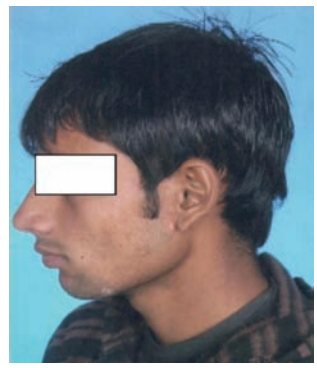


After- treatment
(12-Month)



Pre-treatment



After- treatment
(12-Month)

Fig. 1 Photographs showing response to the Unani coded Drug UNIM-046 in Bars (Vitiligo) lesions

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Standardiztion of Unani Ointments: 'Marham Kafoor'

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Abstract

Marahim (Ointments) are the important formulations of Unani System of Treatments, used as topical applicant for cuts, pains and abrasions etc. Most of the ointments contain mineral and/or plant products that vary from formulation to formulation. No work on standardization of 'Marahim' (Ointments) has been done till date, therefore, a series of the work on different ointments of Unani System of Medicine have been started and in the present paper the works on standardization and quality assurance of an ointment (Marhaam kafoor) are reported. The parameters that are selected are those that are recommended by National Unani Pharmacopoea Committee. 'Marham Kafoor' is a white, semisolid compound with camphorous smell. Its action in mentioned as 'Mubarrid' and 'Daf-e-Taffun', in Unani literature and the mode of administration is topical (Anonymous, 1971; Anonymous, 2008). The parameters that are studied are Total ash (8.33%), Acid insoluble ash (0.82%), Water soluble ash (0.15%), Alcohol soluble matter (5.84%), Water soluble matter (1.00%), Pet. ether soluble matter (36.23 %), Water content (4.22%), Loss on drying (5.5%), Total Zinc as Zinc oxide (0.8-0.9%) and Congealing point (62 - 65^{0}C). Thin Layer Chromatography (TLC) profile are also used for finalizing the marker compounds. The heavy metals, aflotoxins and pesticidal residue are not detected. No microbs were noted in the final product. In addition HPLC profile of 'Marham Kafoor' are also recorded for future reference.

Keywords: Marham Kafoor; Standardization; Quality control; Ointment

Introduction

With the increase of global interest in traditional system of medicine, issue of quality, efficacy and safety of Ayurvedic, Siddha and Unani drugs has attained the renewed attention of scientists, and there is need of sufficient scintific data in order to enforce acceptance of these traditional medicines in masses of India and other countries. Marahim (Ointments) are the important preparation of Unani Medicine, used as topical applicant for cuts, pains and abrasions *etc*. Most of the ointments contain mineral and/or plant products that vary from formulation to formulation. No work on standardization of such type of drugs has been done till date, therefore, a series of work has been initiated to standardize the ointments for maintaining the quality and efficacy. For the present study 'Marham Kafoor' is selected and standardization is made. The work on others ointments will be reported else where. The parameters that are selected are those which are recommended by National Unani Pharmacopoea Committee.

Materials and Methods

Raw Materails:

The formulation contains the ingridients (Table 1) that are mentioned in part Vth of National Formulary of Unani Medicine (Anonymous, 2008). The raw materials were purchased from the market and their identity, purity and strength were checked as per reference, given in table 1.

Preparation of Ointment

In an open mouth bottle the egg albumin and methyl alcohol were added and the content stirred till egg albumin dissolved in alcohol. After filtration with muslin cloth the content was preserve for further processing. In another bottle small pieces of recommended quantity of camphor were dissolved in methyl alcohol and preserved for further processing. In a stain less steel pan, oil of 'Kunjud' (*Sesamum indicum* Linn.) was boiled and in hot oil, the desired quantity of bees wax was added and kept on gas burner till wax was completely dissolved. The pan was removed from the burner and Zinc oxide (Safeda Kashgari) was added and stirred till it takes the consistency of an ointment. The egg albumin and camphor which was dissolved in methyl alcohol was mixed and stirred to get the homogenous mixture.

Physicochemical Parameters

Physicochemical studies like total ash; acid insoluble ash; water soluble ash; alcohol, petrolium Ether and water soluble matter; water content; loss on drying; total zinc as oxide and congealing point were determined quantitatively according to methods recoeded in Indian Pharmacopoeia, WHO guidelines and methods mentioned by Afaq *et al* (Anonymous, 1978(a); Anonymous, 1978(b); Anonymous, 2005 and Afaq *et al*, 1994). Thin Layer Chromatography was conducted (Harborne, 1973). The HPLC for determination of pesticidal residue and atomic absorption for heavy metals determination was used. The presence of aflotoxins and microbial load were studied as per revised recomendation of WHO mentioned in its bulletin (Anonymous, 2005).

Estimation of Zinc oxide

The ointment was ignited in a porcelain crucible till free of carbon. 1.5 g material was dissolve and 2.5 g of ammonium chloride in 50 ml of 1N sulphuric acid. Excess of acidic solution was titrated with I N sodium hydroxide using solution of methyl orange as indicator. Each ml of I N Sulphuric acid is considered as equivalent to 0.04069 mg ZnO (Anonymous, 1978(a))

HPLC analysis

Common pesticide (Chloropyriphos, DDT, Parathion, Malathion and Endosulphan) were obtained from Sigma-Aldrich and dissolved in acitonitrile. This standard was injected in the C18 column (30 cm) fitted in the HPLC instrument (Cyber lab, USA) and software driven peaks obtained. The pressure was 6.5 Pa and temperature was 250C.The Flow rate was 1.0 ml/min. The detector was UV and the wavelength was 254 nm. The mobile phased was acitonitrile: water (75:25). The drug samples were also injected and the peaks appears were compared with the peaks of pesticides (Fig. 1), considering the retention time in the same conditions. The general HPLC profile of drug were also recorded and given in figure (Fig.2).

Results and Discussion

The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of 'Marham Kafoor'. Total ash (8.33%), Acid insoluble ash (0.82%), Water soluble ash (0.15%), Alcohol soluble matter (5.84%), Water soluble matter (1.00%), Pet. ether soluble matter (36.23 %), Water content (4.22%), Loss on drying (5.5%), Total Zinc oxide (0.8-0.9%) and Congealing point (62 - 65^0C), are the parameters considered as tools of checking the quality, identity, purity and strength of the ointment. The HPLC profile of the drug was recorded as the obtained graph can be compared with the batches in future. The HPLC pattern shows 29 peaks and peak number 9 is the major peak. The percentage composition of this compound is 86.154%. This peak is followed by peak number 6 (3.217%) and peak number 5 (2.327%). The total percentage composition of these three compounds is 91.698%. Peak number 7,8,10 and 14 are comparatively smaller peak and the percentage compositions of compounds are 1.817%, 1.867%, 1.406% and 1.139% respectively. Other peaks show non significant concentration, so for checking the quality of future batch peak number 5, 6 and 9 should be compared. The change in the profile of any batch will be an indication for low quality or adulteration. Thin Layer Chromatography (TLC) profile (Table 4) and Rf value obtained alongwith photographs of the TLC plate (Fig. 3) was also recorded for future refernce. The heavy metals, aflotoxins, pesticidal residue and microbial load were also studied and reported (Table 3a, 3b, 3c, 3d) No growth of any Fungi or Bacteria were observed in the cultural media and no aflotoxines (B1,B2,G1,G2) were detected. Pb, Hg, and As are detected in the formulation but present with in the limit. The HPLC analysis shows no commonly used pesticide as in HPLC profile of drug and there is no any peak correspond to peak number 2,4,5,7, and 8 of soft ware driven HPLC graph of the mixture of

different pesticides on the same instrument and under the same conditions (Fig.1; Table 5). The presence of heavy metals is due to the presence of these metals in the Zinc oxide used for prepration of ointment but all are with in limits. No aflotoxin, and microb were detected, hence passing all the test for its clinical use. As a topical applicant it is safe and reported effective for minor infections. This semisolid white ointment has campharous odour and during preperation of one batch 5% loss is expected.

Table 1: Ingredients of Marham Kafoor

S. No.	Unani Name	Botanical/ English Name	Part Used	Reference	Quantity
1	Safeda Kashgari	Zinc Oxide	Zinc Oxide	IP; 1978, pp 550*	60 g
2	Kafoor	Cinnamomum camphora Linn.	Crystals from oil	IP, 1978, p 99*	15 g
3	Roghaan kunjad	Sesamum indicum Linn.	Oil of seed	IP, 1978, p. 442*	450 ml
4	Mom Asli	Beeswax	Wax from honey comb	IP, 1978, p. 62*	150 g
5	Alcohol Khabshi	Methyl Alcohol	Methyl Alcohol	IP, 1978, p. 589*	20 ml
6	Egg	Egg albumin	White albumin	—	5 pieces

*IP=Indian Pharmacopoeia

Table 2: Physicochemical Properties of Marham Kafoor

Parameter*	Marham Kafoor
Total ash	Not more than 8.33%
Acid insoluble ash	Not more than 0.82%
Water soluble ash	Not more than 0.15%
Alcohol soluble matter	Not less than 5.84%
Water soluble matter	Not less than 1.00%
Pet. ether soluble matter	Not less than 36.23 %
Water Content	Not more than 4.22%
Loss on dry	Not more than 5.5%
Total Zinc oxide	0.8-0.9%
Congealing point	62 - 65 ^{0}C

***Each parameter is mean of three experiments**

Table 3: Heavy Metals (a), Microbial Load (b), Aflatoxin (c) and Pesticide residue (d) of 'Marham Kafoor'

(a) Qualitative Analysis for Heavy Metals

S. No.	Test Parameters	Results*	Limits
1	Lead as Pb	1.818 ppm	Not more than 10 ppm
2	Mercury as Hg	0.726 ppm	Not more than 10 ppm
3	Arsenic as As	0.134 ppm	Not more than 3.0 ppm
4	Cadmium as Cd	Not Detected	Not more than 0.3 ppm

(b) Microbial Load (for three samples)

S. No.	Microbs	Result*	Limit
1	Total Bacterial Count	Nil	Not more than 105 /g
2	Total Fungal Count	Nil	Not more than 103/g
3	*Enterobacteriaceae*	Nil	Nil
4	*Salmonella*	Nil	Nil
5	*Staphylococcus aureus*	Nil	Nil

(c) Aflatoxin (for three samples)

S. No.	Aflatoxin	Result*	Limit
1	B1	Not detected	Not more than 0.50 ppm
2	B2	Not Detected	Not more than 0.10 ppm
3	G1	Not Detected	Not more than 0.15 ppm
4	G2	Not Detected	Not more than 0.10 ppm

(d) Pesticide residue (for three samples)

S. No.	Pesticide	Result*	Limit
1	Chloropyriphos	Not detected	Not more than 0.2 mg/kg
2	DDT	Not detected	Not more than 1.0 mg/kg
3	Endosulphan	Not detected	Not more than 3.0 mg/kg
4	Malathion:	Not detected	Not more than 1.0 mg/kg
5	Parathion	Not detected	Not more than 0.5 mg/kg

Note. *All result based on three experiments

Table 4: Thin Layer Chromatography Profile of Marham Kafoor

Drugs	Extract	Mobile Phase	Spraying Reagent	Observation
Marham Kafoor	Methanolic	Chloroform: Toluene: Ethyl acetate (1:1:1)	Vanillin H2SO4 Iodine vapors	After spray of Vanalne Sulphuric acid seven spots appears; Rf.10, 0.20, 0.30, 0.40, 0.45, 0.56, 0.90 After exposure in Iodine Vapour Six spots appears; Rf. 0.10, 0.20, 0.30, 0.4, 0.60, 0.90

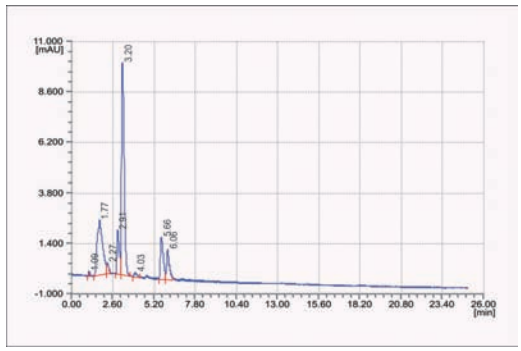

Fig. 1. HPLC of the Mixture of different pesticides

Table 5: HPLC Obtained Peaks of Pesticides

Peak	Retain. Time	Height	Area	Concentration
1	1.092	23	82.9	1.1967
2	1.768	261	6405.7	13.5796
3	2.268	54	378.2	2.8096
4	2.912	210	2042.2	10.9261
5	3.203	1009	11936.6	52.4974
6	4.030	21	294.6	1.0926
7	5.665	199	2523.1	10.3538
8	6.058	145	1701.5	7.5442

Note: Peak 2, 4, 5, 7 and 8 are the major pesticides

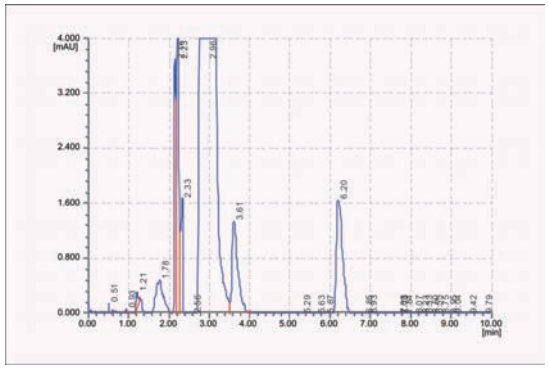

Fig. 2. HPLC profile of Marham Kafoor

Table 6: HPLC Obtained Peaks of Marham Kafoor

Peak	Retain. Time	Height	Area	Concentration
1	0.510	19	26.8	0.118
2	0.927	13	28.1	0.081
3	1.214	23	73.8	0.143
4	1.780	53	723.1	0.330
5	2.115	374	1056.5	2.327
6	2.225	517	1944.9	3.217
7	2.325	292	1319.1	1.817
8	2.559	300	2542.1	1.867
9	2.958	13845	202295.7	86.154
10	3.612	226	3043.2	1.406
11	5.285	13	19.1	0.081
12	5.627	12	70.3	0.075
13	5.868	15	105.7	0.093
14	6.202	183	2219.5	1.139
15	6.856	13	58.1	0.081
16	6.930	14	33.2	0.087
17	7.675	10	19.7	0.062
18	7.725	10	18.9	0.062
19	7.842	10	20.3	0.062
20	8.067	11	17.8	0.068
21	8.233	14	51.6	0.087
22	8.325	11	25.9	0.068
23	8.495	24	47.0	0.149
24	8.553	11	20.4	0.068
25	8.745	13	77.8	0.081
26	8.954	11	27.1	0.068
27	9.037	10	28.8	0.062
28	9.415	11	15.2	0.068
29	9.790	12	94.4	0.075

Vanillin sulfuric acid **Iodine vapour**

Fig. 3. Thin Layer Chromatography of Marham Kafoor

Acknowledgement

Authors are thankful to the Unani Pharmacopoeia Committee and Department of AYUSH, New Delhi for the financial support.

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Antibacterial and Pharmaco-poeial Studies of Itrifal Usthukhuddus Formulation

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Abstract

Unani formulation Itrifal Usthukhuddus was prepared in laboratory scale as per the guidelines of NFUM (part I) in three batches and was tested against the gram negative eight isolates of *Klebsiella pneumoniae.* The efficacy of drug over the test organisms was studied using the cup plate method. The study revealed the inhibition capacity of the prepared drug on all tested organism irrespective of the strain variation. The activity of the drug was expressed in terms of zone of inhibition and exhibited the MIC value as 6.25mg/ml for most of the organism. The antibacterial study is also supported with the developed pharmacopoeial data and quality control parameters like microbial content, heavy metals, aflatoxin and pesticidial residues laid down by the WHO in usage of traditional medicine.

Keywords: Antibacterial activity, Pharmacopoeial parameters, Quality control parameters.

Introduction

Antimicrobial agents are the miracle drugs in the treatment of infectious diseases. Antimicrobial resistance is the ability of certain microorganisms to withstand the attack by antimicrobials. Development of this uncontrolled rise in resistant microorganism threatens the lives and also urges many pharmaceuticals companies, global health institutions in search of newer potential drugs without implementing side effects and relies on plant based medicines.

In natural products the raw materials like plants, animals and minerals are the vital source in discovery of many novel valuable potential drugs. Presently classical Unani medicines impart a great revolution among the public in treating various diseases. Though the scenario falls like this, it becomes necessary to establish scientific validation in usage of such folklore medicine to achieve the global acceptance. In Unani system of medicine Itrifal Usthukhuddus is a semisolid formulation being prescribed by the Unani physicians for the treatment of various ailments like Muzmin (Sinusitis), Sara (Epilepsy) and Laqwa (Facial Paralysis).

Klebsiella pneumoniae is a gram negative, non motile, encapsulated, lactose fermenting facultative anaerobic, rod shaped bacterium found as a normal flora in mouth, skin and intestine. In general *Klebsiella* infections are seen in patients with weak immune system. The clinical manifestations falls

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like pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia and septicemia. New multidrug resistance strains of *Klebsiella pneumoniae* are increasingly emerging and posing a major global health care issue (Vinod Kumar *et al.,* 2011).

Keeping this in view the present study was planned to explore the therapeutic efficacy of the prepared drug Itrifal Usthukhuddus against various strains of *Klebsiella pneumoniae,* to evaluate the pharmacopoeial standards of drug on WHO parameters.

Materials and Methods

Collection and preparation of drug

The drug Itrifal Usthukhuddus used in the study was prepared at the Drug Standardisation Research Unit (DSRU), Chennai. The authenticated good quality raw drugs procured from the local market, Chennai were used in the preparation. The drug was prepared in three batches using the raw drugs namely Halela Siyah, Post-e-Halela Zard, Post-e-Halela Kabuli (*Terminalia chebula* Retz.), Post-e-Balela (*Terminalia bellirica* Roxb.), Aamla (*Emblica officinalis* Gaertn.), Gul-e-Surkh (*Rosa damascena* Mill.), Usthukhuddus (*Lavandula stoechas* Linn.), Bisfayej (*Polypodium vulgare* Linn.), Aftimoon (*Cuscuta reflexa* Linn.), Kishmish (*Vitis vinifera* Linn.) and Raughan-e-Badam (*Prunus amygdalus* Batsch var. Dulcis.), using the guidelines of National formulary of Unani Medicine Part I (Anonymous, 2006).

Collection of microorganism

The microorganisms used in the study were all clinical strains of *Klebsiella pneumoniae,* collected from the diagnostic centers and hospitals of Chennai. The collected isolates of *Klebsiella pneumoniae* were subjected to biochemical reactions in the Department of Microbiology, RRIUM Chennai for further confirmation of the organism (Mackie & McCartney, 1996).

Physico-chemical analysis

Physico-chemical parameters like total ash, acid insoluble ash, solubility in alcohol and water, loss on drying at 105º were performed as per the developed protocol. The bulk density, sugar estimation and *pH* values for 1% and 10% aqueous solution were also carried out (Flow Chart - 1) (Anonymous, 1987).

Thin layer chromatography

Chloroform and alcohol extracts of the drug were developed on precoated silica gel 60 F254 TLC plates (E.Merck) using solvent systems, toluene : ethyl acetate 9:1 and 1: 1 respectively and observed the colour spots at UV-254, UV-366 nm and vanillin-sulphuric acid spraying reagent (Wagner *et al.,* 1984).

Quality control parameters

The microbial load and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxin and pesticide residues were carried out by standard methods (Anonymous, 2000).

Antibacterial activity

In-vitro antimicrobial susceptibility test was performed using the cup plate method (Anonymous, 1996). Required numbers of Muller Hinton agar plates were prepared and swabbed with eight isolates of the *Klebsiella pneumoniae* and were allowed to stand for few minutes and wells of 6mm diameter were made using the agar gel borer. The wells were loaded with 50µl of the drug at the concentration of 100mg/ml. Dimethylsulphoxide (DMSO) (Howard C.Ansel *et al.* 1969) was used as the solvent. The disc Ampicillin (10mcg/disc) was used as standard for comparison (Anonymous, 1982) and the plates were kept at 37 °C for 18-24 hours. Zone of inhibition was measured using the calipers.

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of the drug required to inhibit the microorganism was determined by the agar diffusion method (Anonymous, 198210). Petri dishes containing 20ml of Muller Hinton agar media were prepared and swabbed with eight isolates of *Klebsiella pneumoniae*. Required number wells of 6mm diameter were made using the agar gel borer and the drug was added with increasing concentration (50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) and was allowed to solidify. The lowest concentration of the drug (MIC) that completely inhibits the growth was determined after overnight incubation at 37°C.

Results and Discussion

Physico-chemical parameters

The results of Physico-chemical parameters are summarized (Table 1).

Thin layer chromatography

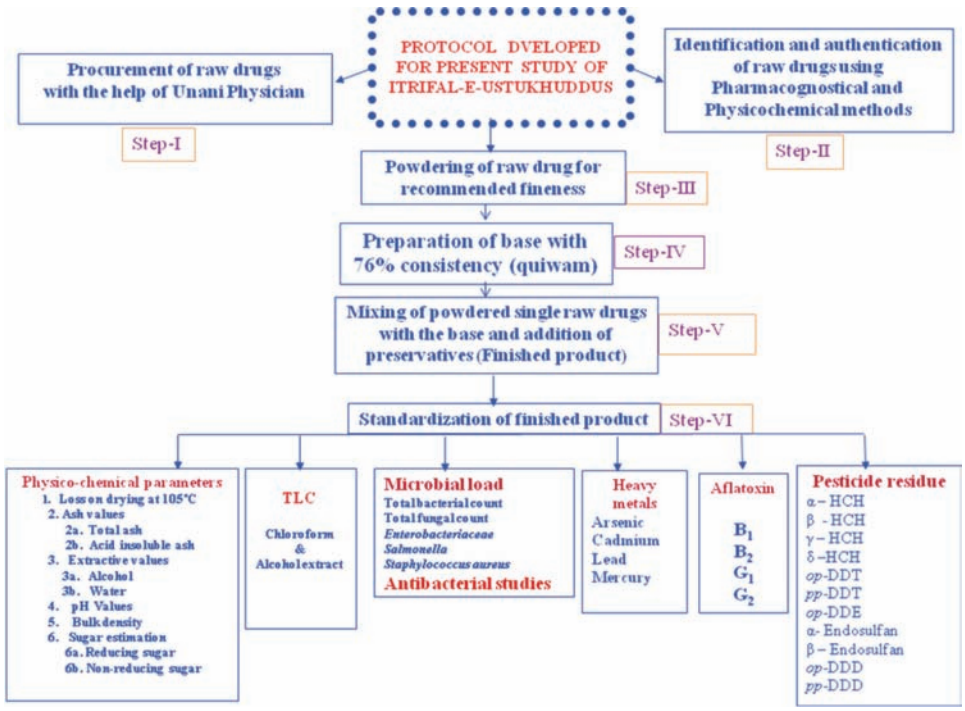
Chloroform and alcohol extract of all the three batch samples shows identical spots in various detector ranges. The R_f values of the chloroform and alcohol extracts are shown (Table 2, 3 & Fig.1, 2).

Quality control parameters

Heavy metals such as lead, mercury, cadmium and arsenic are found below the permissible limit (Table 4). The microbial load also is present within the permissible limit (Table 5). Studies of other parameters like estimation of aflatoxins such as B_1, B_2, G_1 and G_2 and pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion are not detected from the drug (Table 6 & Table 7) .

Antimicrobial activity and MIC

The drug exhibited good antimicrobial activity against all the tested clinical strains of *Klebsiella pneumoniae* at the drug concentration of 100mg/ml with slight variation in zone diameter. The MIC value of the drug also differs and ranged between 6.25mg/ml to 3.125mg/ml against the eight tested strains of *Klebsiella pneumoniae* (Table 8, Chart 1 & Fig 3).



Flow Chart: 1 Protocol for Study

Table 1: Physico-chemical parameters

Parameters Analyzed	Batch Number (n = 3)		
	I	II	III
Extractives			
Alcohol soluble matter	56.81%	57.13%	56.86%
Water soluble matter	67.25%	67.64%	68.04%
Ash			
Total ash	0.87%	0.77%	0.87%
Acid insoluble ash	0.06%	0.11%	0.05%
pH values			
1% Aqueous solution	5.87%	5.73%	5.66%
10% Aqueous solution	4.32%	4.56%	4.46%
Sugar estimation			
Reducing sugar	37.68%	37.92%	37.81%
Non-reducing sugar	12.93%	13.04%	12.98%
Moisture	12.29%	13.17%	12.42%
Bulk Density	1.5342%	1.5496%	1.538%

Table 2: R_f Values of chloroform extract

Solvent system Toluene: Ethyl acetate (9 : 1)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
	0.93 Light pink 0.74 Light pink 0.66 Pink 0.41 Pink 0.28 Light pink 0.21 Pink 0.14 Pink	0.76 Light blue 0.41 fluorescent blue 0.28Violet	0.92 Blue 0.85 Pink 0.73 Grey 0.65 Dark green 0.58 Dark green 0.40 Dark green 0.28 Grey 0.21 Grey 0.12 Grey

Fig. 1. V. S. Reagent

Table 3: R_f Values of alcohol extract

Solvent system Toluene: Ethyl acetate (1 : 1)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
	0.94 Light pink 0.66 Light pink 0.41Pink 0.34Pink 0.14 yellowish green 0.10 Pink	0.89 Blue 0.66 Light Blue 0.20 Orange 0.10 Violet	0.86 Grey 0.76 Violet 0.56 Violet 0.46 Grey 0.41 Grey 0.37 Blue 0.20 Grey

Fig. 2. V. S. Reagent

Table 4: Estimation of Heavy Metals

Parameters	Results	WHO/API Limits
Lead	0.1087ppm	10ppm
Cadmium	Nil	0.3ppm
Mercury	0.0104ppm	1ppm
Arsenic	Nil	3ppm

Table 5: Estimation of Microbial load

Parameters	Results	WHO Limits for internal use
Total Bacterial Count (TBC)	$2x10^2$cfu/g	$1x10^5$cfu/g
Total Fungal Count (TFC)	Absent	$1x10^3$cfu/g
Enterobacteriaceae	Absent	$1x10^3$cfu/g
Escherichia coli	Absent	$1x10^1$cfu/g
Salmonella spp	Absent	Absent
Staphylococcus aureus	Absent	Absent

Table 6: Estimation of Aflatoxin

Aflatoxin	Results
B1	Nil
B2	Nil
G1	Nil
G2	Nil

Table 7: Estimation of Pesticidal residue

S. No.	Pesticide residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfon	ND
9	Ethion	ND
10	Endosufon sulphate	ND

11	Fenthion	ND
12	Heptachlor	ND
13	Lindane	ND
14	Methoxychlor	ND
15	Phorate sulfoxide	ND
16	Phorate sulfone	ND
ND – Not detected		

Table 8: Determination of Minimum Inhibitory Concentration

S. No.	Microorganisms	Zone diameter in mm (n=2)						Std (amp) 10mcg/ml
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	
1	*Klebsiella pneumoniae* KP-I	17	15	10	9	-	-	S (15mm)
2	*Klebsiella pneumoniae* KP-II	16	15	14	12	11	-	S (22mm)
3	*Klebsiella pneumoniae* KP-III	15	13	12	11	9	-	S (17mm)
4	*Klebsiella pneumoniae* KP-IV	16	14	12	10	8	-	S (17mm)
5	*Klebsiella pneumoniae* KP-V	18	15	11	9	-	-	S (18mm)
6	*Klebsiella pneumoniae* KP-VI	17	16	14	11	10	-	R
7	*Klebsiella pneumoniae* KP-VII	18	16	13	12	10	-	S (20mm)
8	*Klebsiella pneumoniae* KP-VIII	15	13	12	10	8	-	S (19mm)
S – Sensitive; R- Resistant; Amp - Ampicillin								

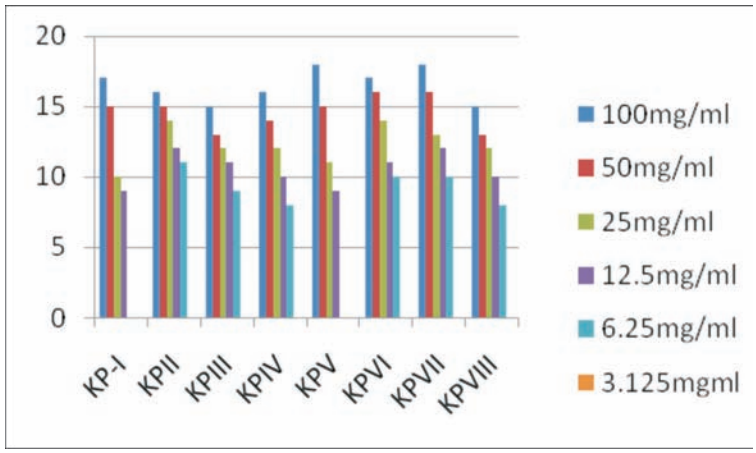
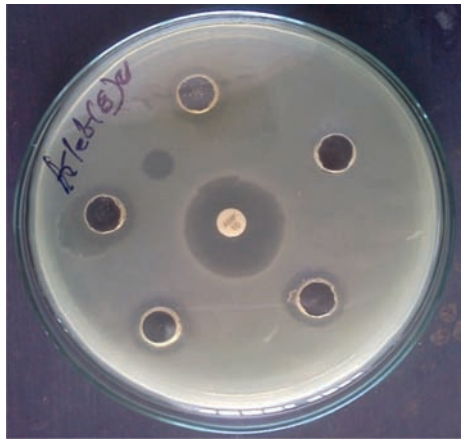
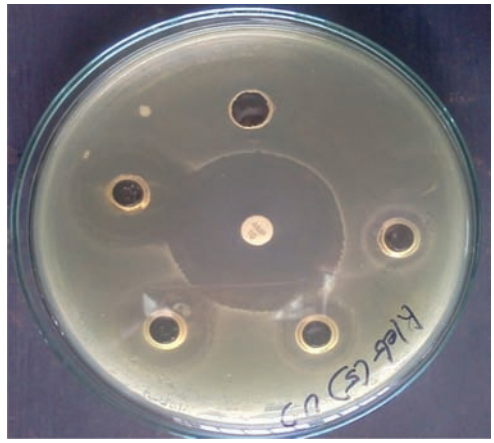


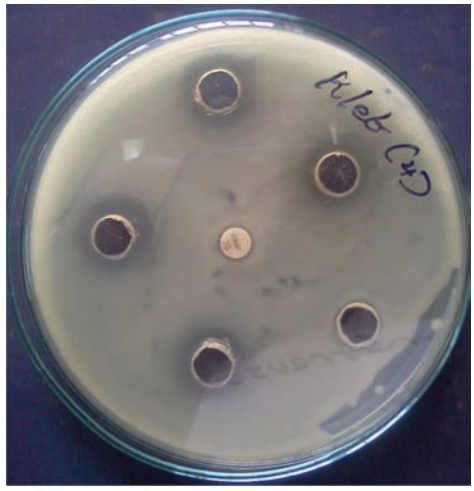
Chart 1. Antibacterial activity



Klebsiella pneumoniae - I



Klebsiella pneumoniae - II



Klebsiella pneumoniae - VI

Drug Conc.	
1.	50mg/ml
2.	25mg/ml
3.	12.5mg/ml
4.	6.25mg/ml
5.	3.125mg/ml
6.	Std (Amp-10µg/ml)

Fig. 3: Plates showing antibacterial activity

Conclusion

Results of present investigations reveal that formulation prepared is free from Microbial growth and Heavy metals, Aflatoxins, Pesticidial residues are within permissible limits. The antibacterial study revealed that Itrifal usthukhuddus is effective herbal formulation to treat infections caused by organism *Klebsiella pneumoniae*.

Acknowledgement

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Pharmacognostic Evaluation of Stem of Gumma (*Leucas cephalotes* Spreng)

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Abstract

The study has been carried out on the stem of Gumma (*Leucas cephalotes* Spreng, family Lamiaceae), primarily a folklore medicine also used in Unani medicine and Ayurveda, in order to generate sufficient pharmacognostical data for proper identification of the plant. Anatomy, observation of isolated elements, physicochemical evaluation, and micrometry and fluorescence analysis of the stem were performed by using the standard methods usually applied in pharmacognostical studies. HPLC of aqueous extract was run. Spectrum scan curves of aqueous and methanol extracts were also obtained. Detailed results are shown in figures and tables. The findings can serve as the source of information to ascertain the authenticity and standardization of the available sample of the plant.

Keywords: Gumma, *Leucas cephalotes* Spreng, Stem Anatomy, Standardization, HPLC, Spectrophotometery.

Introduction

Herbal drugs can be used safely only when they are authentic, but unfortunately due attention has not been paid towards making these drugs up to the mark, hence, a number of crude drugs used in traditional systems of medicine need extensive screening for standardization. Botany and traditional systems of medicine have been indissolubly linked as plants have been indispensable for human life for basic needs, medicinal plants sustained human health for centuries making these plants the back bone of almost all traditional systems of medicine. Authentic samples of crude drugs to be used as therapeutic agents are concerned with the safety of consumers. Reproducibility of the effectiveness of herbal formulations is also a major health concern for which homogenous starting material is inevitable. Increasing interest of people in herbal drugs has called for scientific appraisal of these drugs (Patra et al., 2010). The fast expansion of various aspects of crude drugs has necessitated a systemic approach to study these drugs with a methodical approach by means of appropriate methods of standardization as per WHO guidelines (Shinde et al., 2007).

Sophisticated analytical instruments play a significant role in the evaluation of new products. The use of these instruments, in present days, is an enthralling part of chemical analysis. Though, it is necessary to use several instrumental techniques to obtain the information required solving analytical problems,

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significance of classical methods of standardization can't be underestimated. Therefore, combination of classical physical and chemical operations on the samples of crude drugs substantiated with modern analytical tools will be better choice for checking the genuineness of crude drug samples.

Leucas cephalotes Spreng commonly known as *'Gumma'* in India, belonging to the family Lamiaceae (Khanam, 2005) is an annual herb and an upland rainy season weed (Rajan, 2004), usually found along roadsides, in meadows, waste lands and cultivated grounds throughout the greater part of India (Khare, 2007). This plant as a whole and its different parts are used in Indian Systems of Medicine (ISM) as stimulant and laxative (Pullaiah, 2000), diaphoretic (Kirtikar, 2003), antiseptic, (Anonymous, 2000), anthelminthic (Anonymous, 2003), insecticidal, (Dymock, 2005), germicidal (Chpora, 2002), fungicidal, (Anonymous, 1997), emmenagogue (Chatterjee, 2003), expectorant and antipyretic (Rastogi, 1999) drug. Though, the plant especially fruit and seed have been evaluated for some pharmacological actions (Bhukya, 2010; Sharma, 1978; Sailor, 2010; Singh, 1978) and chemical constituents (Miyaichi, 2006), but the plant has not been evaluated on pharmacognostical basis. The present study, therefore, was taken up to evaluate the stem of the plant on pharmacognostical parameters for the purpose of identification.

Material and Methods

(i) Collection and authentication of the plant

Fresh plant was collected from the forest of Satpura range of Burhanpur (M P), India, in the month of July. The plant was authenticated by botanists vide authentication No. Drug Authentication / SMPU/ NADRI/ BNG/2009-10/ 896. Fresh material was used for anatomical studies whereas the material was dried well in shade and powdered in electric grinder for other studies.

(ii) Preparation of extract

A calculated amount of the coarse powder of air dried drug was subjected to Soxhlet apparatus for 8 h for hot extraction with distilled water, methanol, acetone, diethyl ether, petroleum ether and chloroform, separately. The extracts were filtered and the filtrate was evaporated to dryness. The percentage yield of each solvent was calculated with reference to the air dried drug and expressed in g % $\pm$SEM for calculating extractive values and was used for further studies.

(iii) Microscopic studies

Transverse sections of the stem were cut according to the method described by Johnson (Johnson, 1940). The sections were stained, mounted and observed under microscope. Photographs were taken by digital camera (Sony10.1MP). Micrometry of various cells and study of isolated elements were carried out by the method reported (Trease and Evans, 2008).

(iv) Physicochemical studies

For estimation of ash and extractive values, standard methods described in British pharmacopoeia (Anonymous, 1980) were applied. Moisture content was determined by the reported method (Jenkin et al, 1957). Florescence analysis of powdered drug was carried out by reported the method (Kokoshi et al, 1958).

(v) Preliminary Phytochemical studies

Preliminary phytochemical screening for detection of various phytochemicals was carried out following the method (Bhattacharji and Das, 1969).

(vi) High performance Liquid Chromatography (HPLC)

HPLC of aqueous extract was run on an ultra fast liquid chromatography (UFLC) system (Shimadzu, Japan) with a LC-20AD pump and 20A auto-sampler, Phenomenex Luna C_{18} (2) column (250 x 4.6 mm id) 5 micron was maintained at 40^{o}C. Mobile phase solvents were filtered through 0.45 µ membrane Millipore, PVDF under vacuum. The sample for analysis was filtered through the 0.22 µ membrane. The mobile phase A, solvent was double distilled water. The mobile phase B, solvent was HPLC analytical grade methanol. The flow rate was 0.5 ml/minutes using methanol: water (7:3) as mobile phase solvent, under a pressure of 100 f/sq.cm, run time of 10 minute and an injection volume of 20µL. at 240, 205, 254, and 238 nm. Analyst 1.4 software was used to control all the parameters.

(vii) Spectrum scan

Spectrum scan curves of aqueous and methanol extracts were obtained by using UV-Vis spectrophotometer 3000 (Labindia). After preheat time, spectrophotometer was assessed to spectrum scanning mode. The parameters were set, the photometric mode was assessed to Abs, scanning speed was set as middle with the wave length range 190 – 660 nm. Base line correction was performed with the blank cell, and then samples of extracts of drug were scanned.

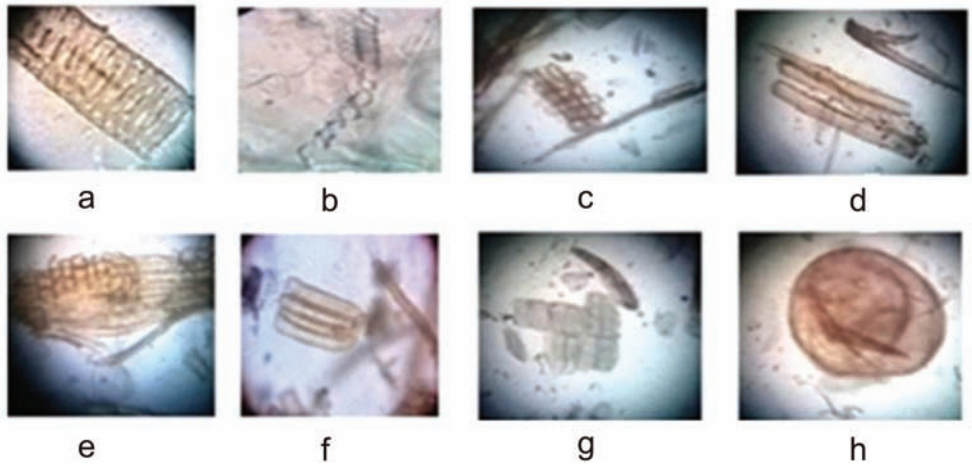
Results

Detailed micrometry is shown in Table 1. Transverse section of stem was quadrangular in shape, covered with thick cuticle and numerous uniseriate, multicellular, 1-3 celled, of sharp tip covering trichomes. Occasional trichomes were sessile, glandular with broad base. The epidermis was composed of single layer of compactly arranged cells. The cortex underlying the epidermis was constituted a hypodermis layer, the outer most layer of the cortex composed of oval to circular collenchymatous cells. The pericycle made up of perenchymatous cells measured 11.53 – 18.44 – 23.06 µ in thickness. The vascular bundle was well developed, bicollateral with lignified xylem arranged radially, consisted of the vessel of protoxylem and metaxylem, phloem small. Pith portion was very clear and covered most of the part of the stem and made up of parenchymatous cells. Macerated stem showed the presence of reticulate vessel, tracheid, compound vessel, pith cells, fibers, annular vessel and trichomes (Fig.3a-3f). Isolated elements are shown in Fig.2.

The mean percentage values of total ash, acid insoluble ash, water soluble ash and water insoluble ash were found to be 8.14 + 0.10, 1.41 + 0.07, 3.80 + 0.07, and 4.61 + 0.07. The extractive values in petroleum ether, diethyl ether, chloroform, acetone, methanol, and distilled water were found to be 2.56 + 0.02, 3.60 + 0.12, 3.35 + 0.08, 3.55+ 0.06, 10.24 + 0.13, and 18.52 + 0.51. The percentage of moisture content was found to be 4 + 0.44, respectively (Table 1, 2). The result of fluorescence analysis of powder is shown in table 2. Preliminary phytochemical screening demonstrated the presence of glycoside, carbohydrate, phenol compounds, tannin, protein and amino acids.

HPLC analysis of the aqueous extract showed two peaks. Spectrum scanning of the aqueous extract exhibited five peaks and two valleys whereas that of methanol displayed three peaks and one valley (Fig.4, 5a, 5 b).

Fig. 1. *Leucas cephalotes* Spreng : A twig with stem, fruit and flower



(2a) Reticulate vessel (10 x 10), (2b) Annular vessel (10 x 45), (2c) Compound vessel (10 x 45), (2d)Vascular bundles (10 x10), (2e) Vascular bundles (10 x10), (2f) Compound vessel (10 x10), (2g) Parenchymatous cells (10x10), (2h) Pith cell (10x10).

Fig. 2. Study of isolated elements of stem of Leucas cephalotes Spreng

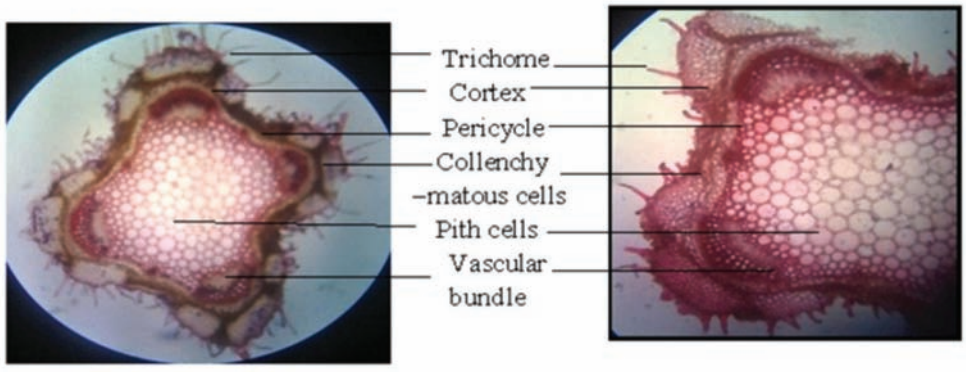


Fig. 3a. T.S of Stem (10 x 5)

Fig. 3b. T.S of Stem portion (10x5)

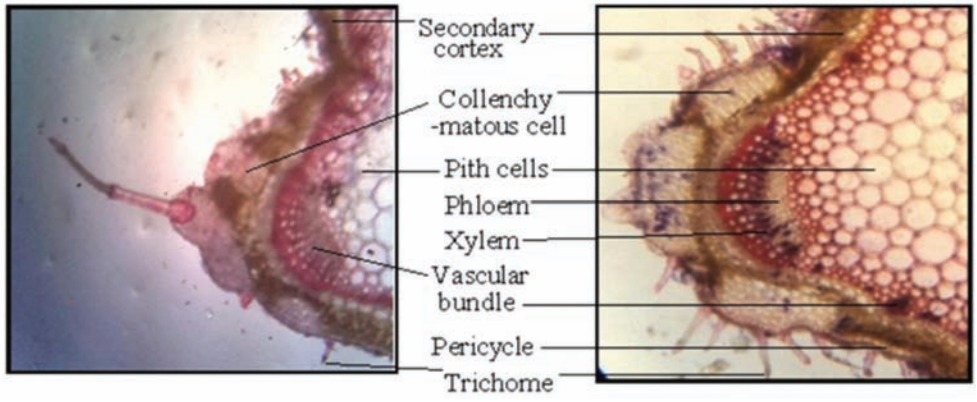
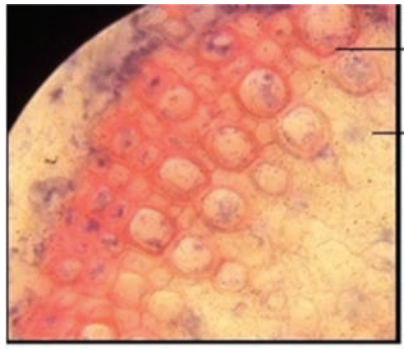


Fig. 3c. T.S. stem showing large Trichome (10 x 10)

Fig. 3d.T.S of Stem portion (10 x10)



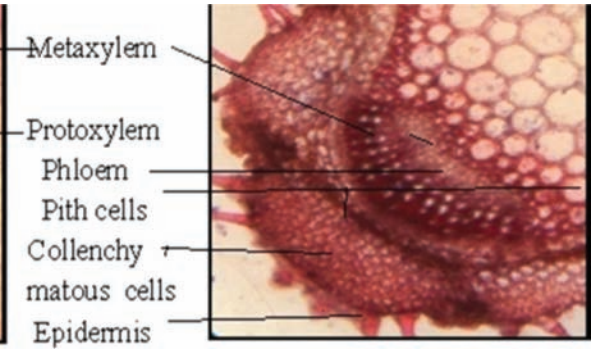


Fig. 3e. T.S. stem showing Vascular

Fig. 3f. T.S. of stem portion (10 x 10)

Fig.3. Microscopy of stem of *Leucas cephalotes* Spreng

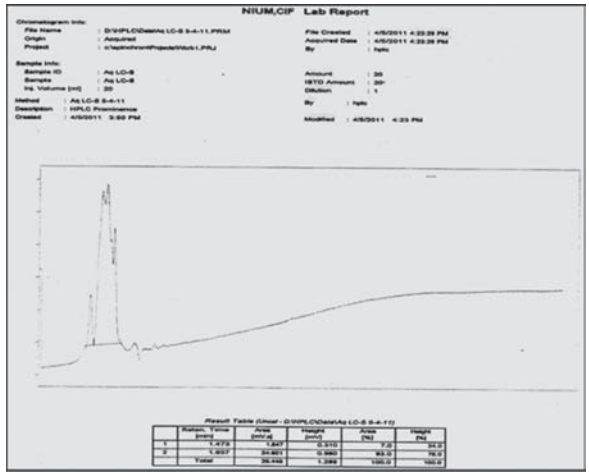
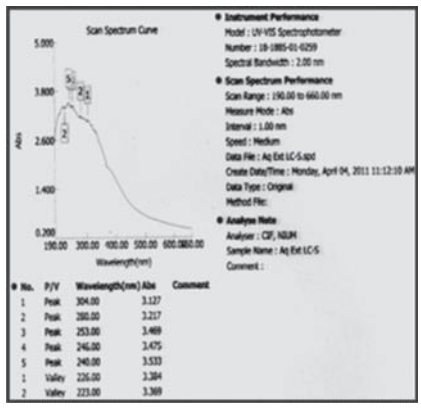
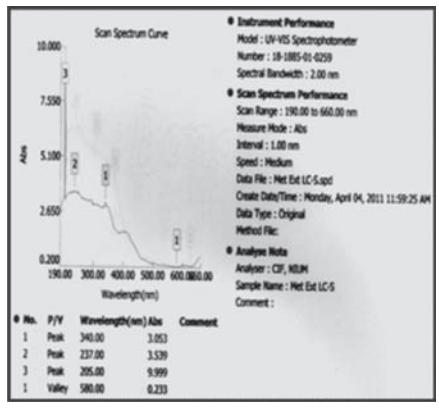


Fig. 4. HPLC of aqueous extract of stem of *Leucas cephalotes* Spreng





Aqueous extract

Methanol extract

Fig. 5. UV/VIS Spectrophotometery of extract of stem of *Leucas cephalotes* Spreng

Table 1: Measurements of cells (Micrometry) of stem of *Leucas cephalotes* Spreng

S. No.	Cells	Measurement (µm)
		Length X Breadth (Range)
1.	Trichome	80.71 – 392.02 – 1360.54 x 11.53 – 18.44 – 23.06
2.	Outer cortex	69.18 – 115.3 – 138.36 x 11.53 – 13.83 – 23.06
3.	Inner cortex	23.06 – 32.28 – 46.12 x 11.53 – 16.14 – 23.06
4.	xylem	8.12 – 15.46 – 23.06 x 8.12 – 14.77 – 23.06
5.	Phloem	8.12 – 8.80 – 11.53 x 8.12 – 8.80 – 11.53
6.	Pith	34.59 – 85.32 –126.83 x 34.59 –87.67–126.83

Table 2: Physicochemical values of stem of *Leucas cephalotes* Spreng

S. No.	Particulars	Values					
1.	Extractive values	Petroleum ether	Diethyl ether	Chloroform	Acetone	Methanol	Distilled water
		2.56 + 0.02	3.60 + 0.12	3.35 + 0.08	3.55+ 0.06	3. 10.24 + 0.13	18.52 + 0.51
2.	Ash values	Total ash	Acid insoluble ash	Water soluble ash	Water insoluble ash	x	x
		8.14 + 0.10	1.41 + 0.07	3.80 + 0.07	4.61 + 0.07	x	x
3.	Moisture content	4 + 0.44	x	x	x	x	x

Table 3: Fluorescence analysis of powder of stem of *Leucas cephalotes* Spreng

S. No.	Tests	Observations	
		Day light	U/V light
1.	Powder	Dark golden red	Golden red
2.	Powder + 1NHCl	Yellowish green	Light yellow
3.	Powder +1NNaOH + Methanol	Orange red	Yellowish green
4.	Powder + 50%KOH	Dark golden red	Yellowish green
5.	Powder + 50%H2SO4	Dark golden red	Lime
6.	Powder + Conc.H2SO4	Dark golden red	Spring green
7.	Powder + 50%HNO3	Scarlet Dark	Slate grey
8.	Powder + Conc.HNO3	Light orange	Yellowish green
9.	Powder + Acetic acid	Brilliant orange	Yellowish green
10.	Powder + Iodine solution	Golden red	Spring green
11.	Powder + Distilled water	Dark olive green	Yellowish green
12.	Powder + Chloroform	Golden red	Medium spring green
13.	Powder + Acetone	Dark golden red	Lime
14.	Powder + Picric acid	Golden red	Yellowish green

Discussion

Quality of raw materials plays vital role in guaranteeing purity, safety, efficacy and stability of herbal preparations which is often challenging but can be triumphed over by making appropriate strategies for standardization. The approach includes a range of classical and analytical methods such as macroscopic, microscopic, physic chemical, phyto chemical and analytical studies.

Microscopic characters of a plant material such as types and arrangements of different cells, typical shape of trichomes, stomata, vascular bundle and other cells, micrometry and quantitative microscopy are not only helpful for identification but are also indispensible, specially for those parts of the plants which are available in pieces (Lux, 2005).

Physicochemical standards such as ash values, extractive values, moisture content, fluorescence analysis of powdered drug, and qualitative and quantitative analysis of chemical constituents are widely accepted parameters. Ash value is an important parameter for detection of adulteration in herbal drugs (Anonymous, 1992). Another valuable parameter is the extractive value taken in different solvents. A specific solvent extract is specific phytochemical in specific amount. Any adulteration or substitution may cause change in extractive value. The amount of extract in a particular substance plays an important role in establishing the index of the purity (Lux, 2005). Estimation of moisture content is important for the material which deteriorates quickly in the presence of water. Thus, estimation of moisture content may be a good parameter for checking the purity of the drug (Wallis, 2005). Herbal drugs are generally used in powder form which is more susceptible for adulteration. This problem can be solved by observing the powder of the drug under day light and U/V light after treating the powder with different chemicals because the fluorescence characters are diagnostic.

Phytochemicals present in plants are mainly alkaloids, glycosides, essential oil, tannins, resins, and flavonoids. Analysis of these constituents is a receptive parameter for standardization. These phytochemicals not only vary from species to species but also differ in different samples of the same drug; therefore it can be used as an approachable parameter in the quality control of drugs (Do, 2005).

Recently, it has been possible to use sophisticated analytical methods such as HPLC, HPTLC, and UV/VIS spectrophotometery for isolation and identification of phytochemicals with high end results. HPLC is a fast, sensitive and most

preferred chromatographic technique for routine assay of new drug as well as for determination of adulterant of established drugs. In the present study HPLC of aqueous extract and UV/VIS spectrophotometery of aqueous and methanol extracts were carried out. These two studies performed were of preliminary type, hence could not be interpreted with the reported phytochmicals, however, in combination with other methods may be considered as method development. It was also not possible to compare our findings with any other data as no such study has been reported earlier, hence our findings may be considered as an addition to the existing reserve of knowledge.

Conclusion

In the light of the present study it can be concluded that the findings can serve as the source of information to ascertain the authenticity and standardization of the available sample of the drug.

Acknowledgment

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Ethnoveterinary Plants of Uttarakhand State of India including those with Galactogogue Properties

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Abstract

The Central Himalayas have the great diversity of plants and natural resources. Himalayan people have close relationship with nature for their basic needs like food, fuel, fodder, medicine, etc. Animal husbandry is the prime occupation of Himalayan society and the major source of their income. In health care system of their cattle, they use their own traditional medicine system, which is based on the ancient cultural traditions. Naturally nearby available resources like plants, minerals etc. are the primary source of medicine for the treatment of various diseases and disorders. Present paper deals with the 45 medicinal plants species used in ethnoveterinary medicines in the study area besides many of these practiced as galactogogue.

Keywords: Ethoveterinary medicines, Galactogogue, Central Himalaya.

Introduction

McCorkle (1986) defined the systematic concept of ethno-veterinary medicine as dealing with the folk beliefs, knowledge, skills, methods and practices pertaining to the health care of animals. Subsequently McCorkle *et al.* (1996) gave a description of ethno-veterinary research as the holistic interdisciplinary study of the local knowledge and the socio-cultural structures and the environment associated with animal health care and husbandry.

In India, domestication of dogs, buffaloes, elephants, and fowls occurred between 6000 and 4500 BC. According to Somvanshi (2006), "cattle husbandry was well developed during the Rigvedic period (1500-1000 BC) and the cow (Kamdhenu) was adored and considered the 'best wealth' of mankind.

The Central Himalayan Region covers the new state of Uttarakhand where livestock occupy a very important place in human life and play an integral part of agriculture-based economy. More than 70% of the rural population of Uttarakhand Himalaya depends upon animals for their economical needs. In this region, every land cultivating family, attempts to maintain a pair a cow and a buffalo for milk. Dairy is the main component of income of Himalayan people. For enhancing the production of milk, they use locally available natural resources like plant and plant products. The present communication deals with the traditional uses of plants to treat various veterinary diseases and disorders practiced by the ethnic tribes and people of Uttarakhand Himalaya.

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The present study updates the earlier reports on ethnoveterinary plants of the study area (Gaur *et al*., 1992, Samal *et al*., 2002 and 2003, Tiwari and Pande, 2004; Bisht *et al*., 2004, Tiwari and Pande, 2005; Pande *et al*., 2006, Tiwari and Pande, 2006; Tiwari and Pande, 2006a,b; Shah *et al*., 2007 ; Tiwari *et al*., 2007 ; Pande *et al*., 2007; Shah *et al*., 2008 ; Tiwari and Pande, 2009, Tiwari and Pande, 2010; Tiwari and Pande, 2011; Tiwari *et al*., 2011 and Agnihotri *et al*., 2012).

Methodology

Based on medicinal plants explorations in the study area between 2008-2011, special attention was paid to record their ethnoveterinary uses, particularly those widely employed for galactogogue application. The data were collected through interviewing local folk medicine men and old-aged people well reputed in the area (Fig. 1-18). Botanical specimens of all such folk drug plants were collected with the help of local people and identified at the camp. In most of the cases, information recorded on their folk medicinal claims have been cross-checked in other localities as well. Voucher specimens of all folk drugs duly identified have been deposited in the herbarium of botany department of Kumaon University, S.S.J. Campus, Almora (Uttarakhand), for future reference and study.

1. Uttarkashi **2.** Tehri **3.** DehraDun **4.** Rudraprayag
5. Haridwar **6.** Pauri **7.** Chamoli **8.** Pithoragarh
9. Bageshwar **10.** Almora **11.** Champawat **12.** Nainital
13. Udham Singh Nagar

Study Area : Location map of Uttarakhand State of India

Table 1: Ethnoveterinary plants of Uttarakhand State of India

S. No.	Plant species	Family	Local name	Part used	Galactogogue and Other ethnoveterinary medicinal uses
1.	*Achyranthes aspera* L.	Amaranthaceae	Latjeera, Saji-basi	Root	Dog bite
2.	*Amaranthus caudatus* L.	Amaranthaceae	Marsha	Leaf	Cough, skin diseases, dysentery, haemachuria
3.	*Ampelocissus rugosa* (Wall.) Planch.	Vitaceae	Chhipari	Leaf	Galactogogue
4.	*Asparagus curillus* Buch.-Ham. ex Roxb	Liliaceae	Karua, Karu	Root	Gastric troubles
5.	*Asparagus racemosus* Willd.	Liliaceae	Jirani, Kaunta	Root	Haemachuria, gastric troubles, cuts, wounds, indigestion, skin diseases
6.	*Bergenia ciliata* (Royle) Raizada	Saxifragaceae	Pathar-chat, Silpara	Whole plant	Lactation, mastitis, intestinal worm, haemachuria, hydrophobia
7.	*Cicer arietinum* L.	Fabaceae	Chana	Seed	Internal parasites, skin diseases, eczema, scabies, strength
8.	*Cirsium wallichii* DC.	Asteraceae	Kendeiya, Kandra, Bungsee, Kateri, Kanyakan	Whole plant	Eye diseases, sun stroke, haemachuria
9.	*Clematis nepalensis* DC.	Ranunculaceae	Kanjul	Leaf	Galactogogue
10.	*Cryptolepis buchanani* Roem. & Schult.	Asclepiadaceae	Dudil	Leaf	Galactogogue
11.	*Curcuma domestica* Vallars	Zingiberaceae	Haldi	Rhizome	Injury, eye diseases, mastitis, mouth blisters, neck sore, heat strokes, wounds, sprains, haematuria, skin diseases, cracked nipple, external parasites, burn, bone fracture, hoof diseases, indigestion
12.	*Debregeasia longifolia* (Burm. f.) Wedd.	Urticaceae	Tusyaru	Leaf	Bone fracture
13.	*Dendrophthoe falcata* (L. f.) Etting.	Loranthaceae	Banda, Ban	Leaf	Galactogogue
14.	*Echinochloa crusgalli* (L.) P. Beauv.	Poaceae	----------	Seed	Skin irritation
15.	*Echinochloa frumentacea* (Roxb.) Link	Poaceae	Madira	Seed	Bone fracture, diarrhoea, infertility
16.	*Euphorbia heterophylla* L.	Euphorbiaceae	Dudil-ghas	Whole plant	Galactogogue
17.	*Fagopyrum esculentum* (L.) Moench.	Polygonaceae	Ugal	Seed	Mouth diseases, hoof diseases
18.	*Ficus auriculata* Lour.	Moraceae	Timul, Timla	Leaf	Prolapse of uterus

19.	*Ficus palmata* Forssk.	Moraceae	Beru, Fedu	Leaf	Wounds
20.	*Ficus sarmentosa* Buch.-Ham. ex Sm.	Moraceae	Dhyar-lagul	Leaf	Bone fracture
21.	*Glycine max* (L.) Merr.	Fabaceae	Bhatt	Seed	Mouth disease, dysentery, diarrhoea, mastitis, skin diseases, indigestion, gastric troubles
22.	*Glycine soja* Sieb.	Fabaceae	Kao-bhatt	Seed	Strength, scabies
23.	*Grewia optiva* J.R. Drumm. ex Burrett	Tiliaceae	Bhimal, Bhekua	Leaf	Indigestion, throat infection, constipation, sprains, dysentery
24.	*Hordeum vulgare* L.	Poaceae	Jau	Seed	Post-calving care, itching, haematuria, strength, dysentery, skin diseases
25.	*Musa paradisiaca* L.	Musaceae	Kela	Fruit	Indigestion, gastric troubles, mastitis, haematuria, flatulence, diarrhoea
26.	*Opuntia stricta* (Haw.) Haw.	Cactaceae	Nagfani	Stem	Galactogogue
27.	*Oryza sativa* L.	Poaceae	Dhan	Seed	Post-calving care, strength, neck sore, bone fracture
28.	*Parthenocissus semicordata* (Wall.) Planch.	Vitaceae	Laduli, Laderi	Whole plant	Bone fracture, eye disorders
29.	*Phoenix humilis* Royle ex Becc.	Arecaceae	Khajoor	Leaf	Galactogogue
30.	*Pimpinella diversifolia* DC.	Apiaceae	Teroi, Phoree	Seed	Indigestion, stomachic
31.	*Quercus floribunda* Rehder	Fagaceae	----------	Leaf	Galactogogue
32.	*Quercus leucotrichophora* A. Camus	Fagaceae	Banj	Leaf	Mastitis, bone fracture, increase food poisoning, constipation, foot & mouth disease
33.	*Quercus semecarpifolia* Sm.	Fagaceae	Banj	Leaf	Yolk sore, carbuncles, food poisoning, pimples, constipation
34.	*Quercus glauca* Thunb.	Fagaceae	Lattu-banj	Leaf	Galactogogue
35.	*Rubia manjith* Roxb. ex Fleming	Rubiaceae	Manjeeth	Whole plant	Sunstroke, skin diseases
36.	*Saccharum officinarum* L.	Poaceae	Gud (jaggery)	Stem	Diarrhoea, fever, strength, sunstroke, stomachic, pimples, chickenpox, cough, wounds
37.	*Sonchus oleraceus* L.	Asteraceae	Dudiya	Root	Galactogogue
38.	*Tinospora cordifolia* (Willd.) Miers ex Hook. f. Thoms.	Menispermaceae	Gurg	Stem	Stomach disorders, fever, hoof diseases, strength, diarrhoea, skin diseases, sunstroke, haematuria, tympany, heat stroke

39.	*Trachyspermum ammi* (L.) Sprague	Apiaceae	Ajwain	Seed	Diarrhoea, gastric troubles, mouth blisters, anorexia, hoof diseases, constipation
40.	*Trifolium alexandrium* L.	Fabaceae	Barseem	Whole plant	Galactogogue
41.	*Triticum aestivum* L.	Poaceae	Gehun	Seed	Skin infection, stomachache, indigestion, anaemia, diarrhoea, flatulence, strength, burn
42.	*Urtica ardens* Link	Urticaceae	Sisun	Leaf	Bone fracture, haematuria, neck sore, yolk sore
43.	*Urtica dioica* L.	Urticaceae	Sisona	Whole plant	Haematuria, rheumatism, neck sore, wounds, internal injury
44.	*Vigna mungo* (L.) Hepper.	Fabaceae	Mash	Seed	Bone fracture, regulate fertility, food poisoning
45.	*Vigna radiata* (L.) R. Wilczek	Fabaceae	Moong	Seed	Bone fracture, sprains, wounds, broken horn, constipation

Fig. 1: A view of Malari village in Niti valley (Chamoli district) of Central Himalaya

Fig. 2: A view of Niti village (Chamoli district) in Central Himalaya

Fig. 3: A view of Laspa village in Johar valley (Pithoragarh district) of Central Himalaya

Fig. 4 : A view of Upali-Pau village in Rawain area (Uttarkashi district) of Central Himalaya

Fig. 5: Survey / Collection of ethnoveterinary plants in progress

Fig. 6: A native lady in process of milking the buffalo

Fig. 7: *Achyranthes aspera* L.

Fig. 8: *Bergenia ciliata* (Royle) Raizada

Fig. 9: *Dendrophthoe falcata* (L. f.) Etting.

Fig. 10: *Ficus palmata* Forssk.

Fig. 11: *Parthenocissus semicordata* (Wall.) Planch.

Fig. 12: *Quercus glauca* Thunb.

Fig. 13: *Cryptolepis buchanani* Roem. & Schult.

Fig. 14: *Debregeasia longifolia* (Burm. f.) Wedd.

Fig. 15: *Fagopyrum esculentum* (L.) Moench.

Fig. 16: *Oryza sativa* L.

Fig. 17: *Quercus floribunda* Rehder

Fig. 18: *Quercus leucotrichophora* A. Camus

Results and Discussion

Present study deals with a total of 45 ethnoveterinary medicinal plants species widely used by the people of Central Himalaya. Of these, 34 are for treatment of various animal diseases and disorders like stomach disorders, fever, hoof diseases, to gain strength, diarrhoea, skin diseases, sunstroke, haematuria, tympany, heat stroke, foot and mouth diseases etc., and 16 viz. *Amaranthus caudatus* L., *Cicer arietinum* L., *Curcuma domestica* Vallais, *Echinochloa crusgalli* (L.) P. Beauv., *Echinochloa frumentacea* (Roxb.) Link, *Fagopyrum esculentum* (L.) Moench., *Glycine max* (L.) Merr., *Glycine soja* Sieb., *Hordeum vulgare* L., *Musa paradisiaca* L., *Oryza sativa* L., *Saccharum officinarum* L., *Trachyspermum ammi* (L.) Sprague, *Triticum aestivum* L., *Vigna mungo* (L.) Hepper., *Vigna radiata* (L.) R. Wilczek are commonly cultivated in the study area and practiced very frequently in daily food habits of people of Uttarakhand. It is evident from the tabel-1 that the leaves of 16 species; seeds of 13 species; whole plant of 7 species; roots of 4 species; stems of 3 species and fruits and rhizomes of 1 species, each are used as the galactagogue.

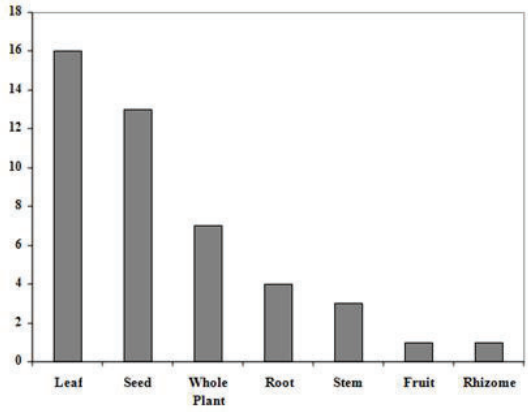

Table 1: Plant and plant parts used as galactagogue

However, this important ethno-veterinary knowledge of plants is in danger of being lost due to rapid modernization of the area and has so far survived only by being passed on orally from one generation to next. It is, therefore,

very important to undertake ethno-veterinary explorations of plants in such uninvestigated areas and record this valuable field data before this knowledge goes in eternity. Nevertheless, detailed chemical and pharmacological investigations of all plants used in ethnoveterinary medicines including those as galactogogue are suggested with a view to develop new drugs of natural origin.

Acknowledgements

The co-operation extended by local healers in recording the information presented in this study is gratefully acknowledged.

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Antibacterial Activity of Itrifal Mundi Against *Staphylococcus aureus*

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Abstract

The study of medicinal properties of plant based products is as old as medicine itself. Curative properties of herbal products have been exploited from the ancient past against various diseases. The recent trend of antibacterial research has revealed interest in search of new antibacterial agents of herbal origin that can have acceptability usually being nontoxic and inexpensive. The present study was undertaken to identify the strains of *Staphylococcus aureus* from the pus samples by using PCR technique. 16S rRNA gene was amplified using Universal primers. The amplified product was sequenced and the sequence was confirmed for *Staphylococcus aureus* after BLAST analysis with the NCBI database. Identified microorganisms were checked for their susceptibility to the Unani drug Itrifal Mundi. The microorganisms were found to be sensitive to the drug with the zone diameters ranging from 15mm to 22mm at the concentration of 10mg/ml. The MIC value of the drug was found to be in the range of 0.3125mg/ml to 0.625mg/ml for most of the tested strains of *S.aureus.*

Keywords: Itrifal Mundi, *Staphylococcus aureus,* Antibacterial activity.

Introduction

The microbial pathogens are responsible for more than 40 million human deaths per annum. Control and prophylactic measures for most of the diseases are far from expectation due to non-availability of effective medicines (Sharma, *et al.,* 2006). Antibiotics are being advised for either treatment or prophylaxis of infection. The first and the foremost criteria is the efficient identification and culture of relevant pathogens. The sequence analysis of genes encoding small sub-unit ribosomal RNA (16S rRNA) is currently the most promising approach in identification of organism, which involves the usage of Polymerase Chain Reaction (PCR) technology and BLAST analysis with NCBI database to identify the organism. *Staphylococcus aureus* is widely found in nature and is responsible for superficial, deep pyogenic infection and for toxin mediated illness. The *Staphylococcus aureus* acts as predominant bacteria in causing secondary infection in scabies (Brook, 2002; Walton *et al.,* 2007).

The study was aimed at the identification and isolation (genomic level) of Gram positive *Staphylococcus aureus* (coagulase positive) from the pus samples and their susceptibility pattern to the Unani drug Itrifal Mundi which is being prescribed by the Unani physicians for the treatment of scabies and itching (Anonymous, 2008).

Author for correspondence

Materials and Methods

a. Collection of raw drugs

The formulation Itrifal Mundi (Anonymous, 2008) was prepared using twelve ingredients namely Post-e-Halela Zard (*Terminalia chebula* Retz. (Pericarp) DSM64), Halela Siyah (*Terminalia chebula* Retz. (Pericarp) DSM31), Post-e-Halela Kabuli (*Terminalia chebula* Retz. (Pericarp) DSM60), Post-e-Balela (*Terminalia bellirica* Roxb. (Pericarp) DSM56), Aamla Khushk (*Emblica officinalis* Gaertn. (Fruit) DSM07), Tukhm-e-Kishneez (*Coriandrum sativum* Linn. (Fruit) DSM76), Berg-e-Shahtara (*Fumaria parviflora* Lam. (Leaves) DSM11), Asl-us-Soos (*Glycyrrhiza glabra* Linn. (Root) DSM09), Ustukhuddus (*Lavandula stoechas* Linn. (Inflorescence) DSM85), Gul-e-Mundi (*Sphaeranthus indicus* Linn. (Inflorescence) DSM22), Qand Safaid (Sugar) and Raughan-e-Zard (Ghee) at laboratory scale in Drug Standardisation Research Unit (DSRU), Chennai, for the development of Standard Operating Procedures (SOPs) and to evaluate the pharmacopoeial standards. The raw drugs were procured from R.N. RAJAN & CO. local raw drug dealer, Chennai, identified and authenticated using pharmacognostical and physicochemical methods (Kokate *et al.,* 2000).

*DSM – Drug Standardisation Museum

b. Collection of microorganism

The pus samples were collected from Excellent Laboratory Chennai - 99 and Diagnostic Centre, Chennai-17. The samples were processed to phenotypic identification of various strains of *Staphylococcus aureus* using the existing conventional microscopical and biochemical tests (Mackie & McCartney, 1996). The eight *Staphylococcus aureus* isolates showing positive for coagulase test were isolated in pure form and were stored in the nutrient agar slants for further studies. The molecular level identification was carried out using FD1 and RP2 primers targeting 16S rRNA gene.

c. Molecular identification of micro-organism

i) Extraction of DNA

A loopful of isolated *Staphylococcus aureus* strains were suspended in 250 - 400 ml of Luria Bertani broth and their genomic DNA were extracted according to the standard protocol (Sambrook *et al.,* 1989). The DNA obtained was stored at -20°C until further use.

ii) Polymerase chain reaction (PCR) for amplification and Sequencing

The PCR was carried out in a Gradient Thermal Cycler (AG22331, Eppendorff, Germany) with the profile of initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 59°C for 1 min annealing and 72°C for 1 min extension and final extension of 72°C for 7min. The commercial primers and the PCR reagents were used. The FD1 and RP2 universal primers coding for 16S rRNA gene were used for the analysis. The obtained 1460 bp of PCR product were purified and commercially sequenced (Banglore Genie). The sequences were further BLAST analysed using NCBI Database for molecular level identification.

d. Antibacterial activity

The in-vitro antibacterial activity of the drug Itrifal Mundi was performed using the cup plate method (Rai *et al*., 2011). The required number of Muller Hinton agar plates were prepared and swabbed with the molecular level identified *Staphylococcus aureus* isolates after confirmation using NCBI database. The plates were allowed to stand for few minutes. Wells of 10mm diameter were made using the agar gel borer and 100µl drug solution of 10mg/ml dissolved in the solvent DMSO was added into the wells. Commercially available drug norfloxacin (10mcg/disc) was used as positive control. Plain disc with100µl loaded solvent DMSO was placed as the vehicle control and the plates were incubated at 37°C for 24 hours.

e. Determination of Minimum Inhibitory Concentration (MIC)

MIC, the lowest concentration of the drug required to inhibit the microorganism was also determined by the agar diffusion method (Anonymous, 1982). Petridishes containing 20ml of Muller Hinton agar media were prepared and swabbed with different strains of *Staphylococcus aureus* isolates. The plates were allowed to stand for few minutes. Required numbers of 10mm diameter wells were made using the agar gel borer and 100µl of increasing concentration of the drug 0.3125mg/ml, 0.625mg/ml, 1.25mg/ml, 2.5mg/ml and 5mg/ml DMSO were added. The plates were incubated at 37°C for 24 hours. The lowest concentration of the drug that completely inhibits the growth was determined after overnight incubation at 37°C.

Results and Discussion

The Polymerase Chain Reaction amplified 1460bp, 16S rRNA gene from the various isolates of *Staphylococcus aureus* are shown in Fig. 1. The drug Itrifal

Mundi shows significant activity on all the tested strains of *Staphylococcus aureus* at the concentration of 10mg/ml. The drug has exhibited various degrees of inhibition against the tested bacterial strains with zone diameter ranging from 15mm to 22mm. The MIC values of three tested strains are found in the range of 0.3125mg/ml to 0.625mg/ml Fig. 2 (A, B & C). No inhibition was found below the concentration of 0.3125mg/ml in any of the tested strains\

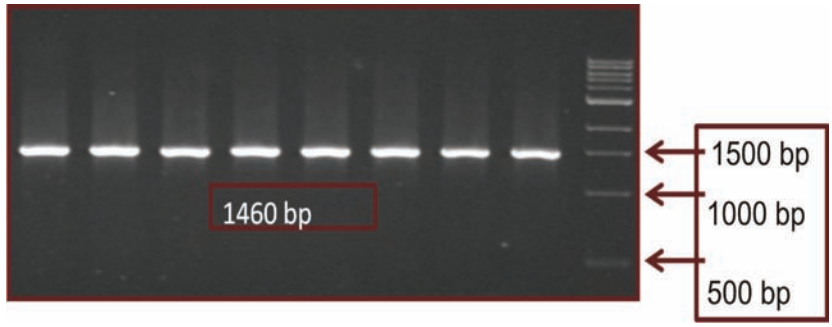

Fig. 1: PCR amplified 16S rRNA gene of Staphylococcus aureus Isolates

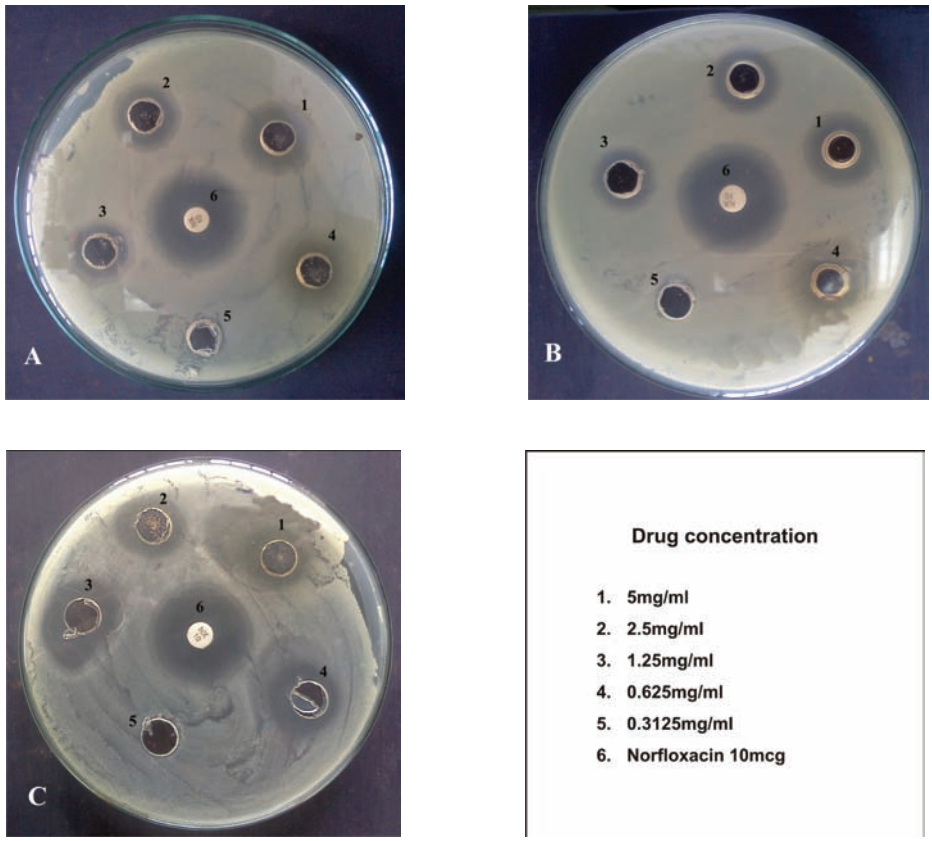

Fig. 2: Antimicrobial activity of Itrifal Mundi against different strains of Staphylococcus aureus

Conclusion

The study reveals the antibacterial activity of Itrifal Mundi against the pathogen *Staphylococcus aureus* responsible for skin infection. The use of PCR based technology has enabled easier and accurate identification of *Staphylococcus aureus* upto species level.

Acknowledgement

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Diagnostic Morpho-Anatomical Characteristics of *Arctostaphylos uva-ursi* (L. Spreng. and Its Adulterants

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Abstract

rctostaphylos uva-ursi (L.) Spreng. is well known medicinal plant for the treatment of cystitis (bladder inflammation) and kidney catarrh (mucous) and the official drug of Homoeopathic System of Medicine. The leaves of Uva-ursi have high trade value in the national and international markets in the herbal drug industry. The leaves of Cowberry (Vaccinium vitis-idaea L.) and the Box (Buxus sempervirens L.) are commonly adulterated with the leaves of *Arctostaphylos uva-ursi* (L.) Spreng. Present communication deals with the morphological and anatomical characteristic features of the leaf of *Arctostaphylos uva-ursi* (L.) Spreng. and also the comparative characters of its possible adulterants Vaccinium vitis-idaea L. and Buxus sempervirens L. with a view to check adulteration in the genuine drug to ensure manufacture of quality medicines.

Keywords: Morpho-anatomy, Uva-ursi, *Vaccinium vitis-idaea* L. and *Buxus sempervirens* L.

Introduction

Arctostaphylos uva-ursi (L.) Spreng., commonly known as 'bearberry' (English) and 'Uva-ursi' in herbal trade, belongs to Ericaceae family. It is an evergreen shrub with short creeping reddish-brown branches bearing pink or white bell shaped flowers that bloom in the summer, followed by clusters of berries (Figure 1). It is distributed throughout northern hemisphere alpine including North America, Europe, the Iberian Peninsula and Siberia (Bailey, 1961; Gleason, 1968 and Polunin, 1969).

Leaves of Uva-ursi have a long-traced history of medicinal use since 2nd Centaury and were used very commonly until the discovery of sulfa drugs and antibiotics in the treatment of urinary bladder and urinary tract infection. Till date, the drug is used to treat urinary tract infections and cystitis (bladder inflammation) and also listed in many official Pharmacopoeias of various countries.

The leaves of Uva-ursi contain a compound called arbutin that is metabolized into the antibacterial compounds hydroquinone glucuronide and hydroquinone sulphate. Uva ursi leaf tea is also listed in the German Pharmacopoeia as a urinary disinfectant for the treatment of bladder and kidney catarrh (mucous) and inflammation. One of the important mechanisms of action of bearberry leaves is their ability to influence the surface characteristics of microbial cells

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and thereby their putative virulence properties. Uva-ursi extract and arbutin are also therapeutic against immuno-inflammation (such as that sometimes related to painful bone and joint inflammations) (Hering, 1879; Allen, 1874; Clarke, 1900, Chevallier, 1996 and Matsuda *et al*., 1992).

In India, the herb is imported by the Homoeopathic drug manufacturers to prepare the mother tincture named 'UVA URSI'. The leaves of Uva-ursi have the high trade value in the national and international market in the herbal drug industry. The leaves of other plants have been mistaken for uva ursi leaves, notably those of the Cowberry (*Vaccinium vitis-idaea* L.) and the Box (*Buxus sempervirens* L.), and have occasionally been used to adulterate the drug, but uva ursi leaves are readily distinguished by the characteristics given, viz. the spatulate outline, entire margin and rounded apex (Wallis, 1985). Present study deals with the pharmacognostical study of uva ursi leaf which will be very helpful to check the adulteration.

Material and methodology

Fresh leaves were arranged from various sources. Vertical sections of lamina were cut with the help of sharp razor. The fine sections were double stained with safranin and light green and mounted in Canada balsam. Organoleptic characters and histological data were studied as per methods described in Youngken (1951) and Trease & Evans (1983). Photomicrographs of anatomical details of section and powder were obtained through Motic digital microscope

Results and Discussion

The observations made in the study on *Arctostaphylos uva-ursi* (L.) Spreng., *Buxus sempervirens* L. and *Vaccinium vitis-idaea* L. are given in table 1 elaborating diagnostic characteristics in respect of taxonomical, morphological/ organoleptic and anatomical features.

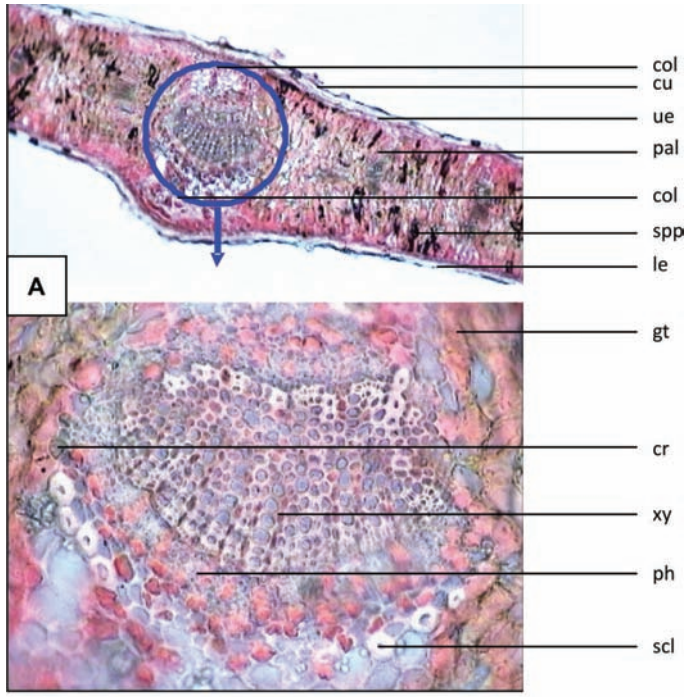
Table 1: Dignostic characteristic

Dignostic characteristics	Arctostaphylos uva-ursi (L.) Spreng.	Buxus sempervirens L.	Vaccinium vitis-idaea L.
Taxonomical			
i. Family	Ericaceae	Buxaceae	Ericaceae
ii. Common name	Bearberry	Box, Boxwood	Blue berry
iii. Life form	Evergreen trailing shrub with ascending branches.	Evergreen shrubs or small tree.	Small glabrous shrub with sharply angled branches.

iv. Distribution	Europe, United States, northern Asia	Europe, USA, north Africa.	
Morphological / Organoleptic			
i. Colour	Dark green to brownish-green above and paler beneath.	Dark glossy green above and paler beneath.	Dark green above, paler and dotted with dark glands beneath.
ii. Shape	Obovate to oblanceolate or spathulate, entire; apex obtuse to emerginate and margin entire; short petioled; usually pubescent on midrib and margin	Ovoid, oblong or elliptical; shortly peotioled; apex emerginate margin somewhat revolute; short petioled.	Elliptical to oblong or obovate; apex abtuse, margin entire, revolute.
iii. Size	12-30 X 4-12mm	15-30 X 7-15mm	10-12 X 6-15mm
iv. Texture	Brittle, coriaceous	Coriaceous	Coriaceous
v. Taste	Astringent, bitter	Bitter	Slightly bitter
vi. Odour	Odourless	Odourless	Odourless
Anatomical			
i. Cuticle	Thick	Thick	Thick
ii. Epidermis	Single layered	Single layered	Single layered
iii. Stomata	Aomocytic, only on lower epidermis; more grouped in patches on midrib, venation fine reticulate	Aomocytic, confined on lower epidermis, each stomata is surrounded by rosette of clearly defined subsidiary cells, guard cells strongly crusted	Aomocytic, only on lower epidermis.
iv. Hairs	Hairs few, unicellular, conical, thick-walled, present on petiole and margin of young leaves; a few glandular hairs with 2-celled uniseriate stalk and multicellular secretory head also present.	Simple, unicellular and multicellular.	Both, simple multicellular, short and long-stalked glandular hairs.
v. Crystals	Crystals common in petiole and in midrib ground tissue subepidermal collenchyma as single prism or irregular crystals, sometime associated with numerous small crystals.	Clusters or solitary crystals of calcium oxalate present; coarse crystal sands with corroided appearance also present.	Crystals present in petiole.

vi. Mesophyll	3 to 5 layered, having droplets of oils.	Differentiated in palisade and spongy parenchyma; secretory ducts prominent in palisade region.	Differentiated in single layered (somewhere double layered) palisade and spongy parenchyma; tanniniferous contents present.
vii. Stone cells	Few thick-walled fibrous sclerotic patches cells present.	Not seen	Not seen

On the basis of diagnostic characteristics commercial samples sold under the name of uva ursi can be authenticated for genuineness prior to use in a various herbal formulations.



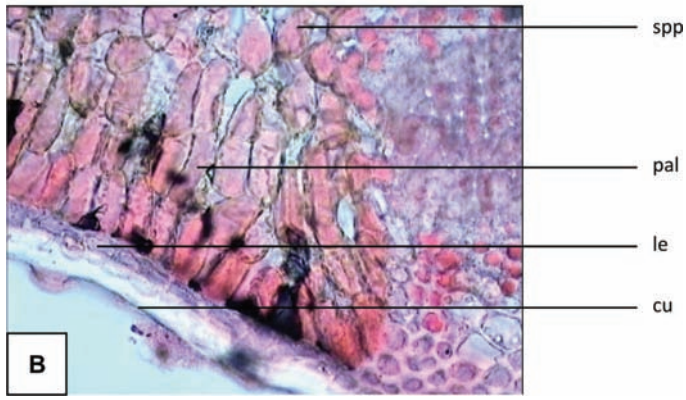


Fig. 2. Anatomical structure of lamina of Arctostaphylos uva-ursi (L.) Spreng.

A. Mid-rib region

B. Vertical section

Abbreviations: col, collenchyma; **cr,** rosette crystals of calcium oxalate; **cu,** cuticle; **gt,** ground tissue; **le,** lower epidermis; **pal,** palisade; **par,** parenchyma, **ph,** phloem; **scl,** sclerids; **spp,** spongy parenchyma; **ue,** upper epidermis; **xy,** xylem.

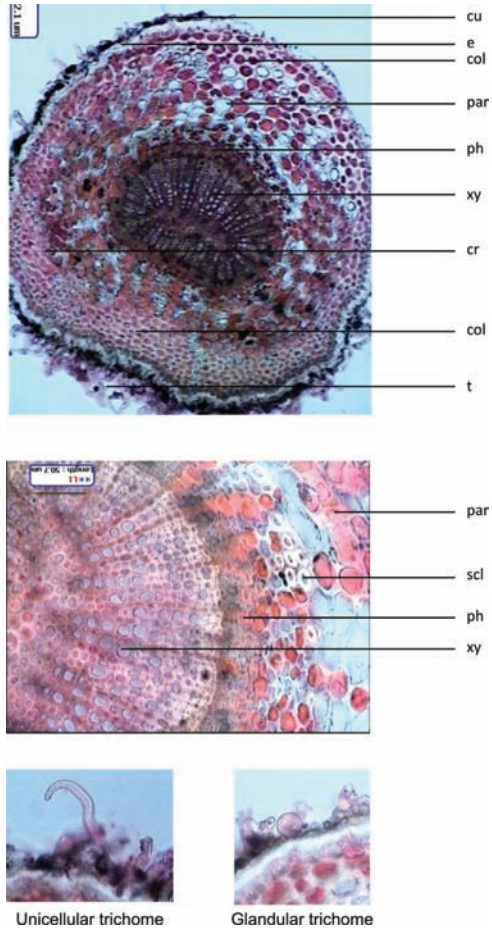


Fig. 3. Anatomical structure of petiole of Arctostaphylos uva-ursi (L.) Spreng

Abbreviations: col, collenchyma; **cr,** rosette crystals of calcium oxalate; **cu,** cuticle; **e,** epidermis; **par,** parenchyma, **ph,** phloem; **t,** trichome; **scl,** sclerids; **xy,** xylem.

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Development of HPTLC Fingerprint of *Eclipta alba* L. for Quality Evaluation

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Abstract

The present study was designed to determine the HPTLC profile of the medicinally important plant *Eclipta alba* L. The chloroform : methanol (7:3) was employed as mobile phase for phyto-constituents. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The developed plate was seen under UV light 254 nm and 366 nm. The methanolic extract of whole parts of *Eclipta alba* L showed the presence of 10 different types of phyto-constituents with different Rf. values. The developed HPTLC fingerprints will help the herbal drug industry to distinguish the adulterant and standardization of herbal formulations. Such chemo finger printing will act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies

Keywords: *Eclipta alba* L., Weadelolactone, HPTLC fingerprint, Phytochemistry

Introduction

Eclipta alba L., 'Bhringaraj' in Hindi and 'King of the Hair' and 'False Daisy' in English, belongs to family Asteraceae. Plant is native to the tropical and sub tropical regions and grows as common weeds throughout India, ascending upto 1800m in the Himalayas, common in areas of upper Gangetic plains, in pasture lands, roadsides in Utter Pradesh, all districts of Bihar, Madhya Pradesh, Uttar Pradesh, Orissa and Punjab. It is an annual herbaceous plant, erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate (Sharma *et al.,* 2001; Roy *et al.,* 2008). The genus name comes from the Greek word meaning "Deficient," with reference to the absence of the bristles and awns on the fruits. The specific *Eclipta alba* L. means white which refers to the color of the flowers (Mehra and Handa, 1968; and Kapoor, 2001).

The major chemical constitutes of plants are wedelolactone, desmethylwedelolactone, furanocoumarins, eclalbatin oleanane and taraxastane glycosides (Sikroria *et al*., 1968; Sarg and Khafagi, 1981; Jain & Singh 1988; Singh, 1988; Singh & Bhargava, 1992). The plant is commonly used in hair oil for healthy black and nourishment (Khare, 2004). The fresh juice of leaves is used for increasing appetite, improving digestion and as a

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mild bowel regulator and in viral hepatitis to promote bile flow and protect the parenchyma and enhance memory (Singh *et al.,* 2001). It is also used as a chologuague and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder. Charaka advises taking the juice of plant with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies. This plant is well documented and several *in vitro* and *in vivo* studies describe its antiageing agent, hepatoprotective, anti inflammatory diuretic, hypertensive, immune stimulant, anti hyperglycaemic acid, and analgesic, antivenom, anticancer, antioxdent, antiviral, antibacterial, antifungal, spasmogenic property ,inflammations, gastric disorders, anorexia, worm infection, skin diseases, ulcers, ophthalmic disorders, headache, hypertension leprosy, fever, and jaundice (Wanger *et al*., 1986; Wagner and Fessler, 1986; Jayaram *et al*., 1987; Chandra *et al.,* 1987; Sharma *et al*., 1989; Saxena, 1993; Singh, 1993; Zhang and Chen, 1996; Pandey *et al*., 1997; Kirtikar & Basu, 1999; Leal *et al.,* 2000; Upadhyay, 2001; Syed, 2003; Jayatirtha and Mishra, 2004; Joshi, 2004 and Sawant *et al.,* 2004). It is also used in catarrhal jaundice and for skin diseases (Dixit and Achar, 1981; Sankaran, 1984; Ananthi *et al*., 2003; Thakur and Mengi 2005).

Present study deals with the HPTLC finger print profiling of *Eclipta alba* L. which will help the herbal drug industry to distinguish the adulterant and standardization of herbal formulations.

Material and Methods

a) Plant material and extraction

Fresh plant materials were collected from Ghaziabad and Haridwar, India in the month of August 2011. The collected plant materials were authenticated with the help of standard floras and pharmacopoeial reference (Anonymous, 2010). The whole plant was shade dried and powdered then extracted with 500 ml methanol for 8 to 12 hours by using Soxhlet apparatus. Extracts was filtered through Whatman paper no. 42 and were concentrated under reduced pressure and finally vacuum dried. The yield of the methanolic extract was 11.2 % w/w. The protocol for preparing sample solutions was optimized for high quality fingerprinting and also to extract the marker compounds efficiently. Since the marker compounds were soluble in methanol, therefore methanol was used for extraction. For the experimental work pre-coated silica gel 60 F_{254} HPTLC plates, reference standard 'wedelolactone' (Purity: 90 % w/w) and analytical reagent (AR) grade chemicals were used.

b) Physico-chemical studies

Physico-chemical characters were determined as per standard methods described in Indian Pharmacopoeia (IP), (1996), Lala (1993) and Kokate *et al*. (2005).

c) HPTLC analysis

A densitometric HPTLC analysis was carried out to develop characteristic fingerprint profile of methanol extract of both the sample with reference 'weadelolactone' for standardization purpose; 5 mml sample solution of each of methanol extract with reference were applied on silica gel 60 F254 precoated (E-Merck, India) plate of uniform thickness of 0.2 mm using CAMAG Linomet-5 automated TLC applicator with the nitrogen pressure 4kg/cm² from applicator syringe. Condition of work kept constant throughout the analysis. Following sample application bands were developed in a twin trough glass chamber that had been pre saturated with the mobile phase of chloroform : methanol (7:3 v/v) till proper separation of bands up to 8cm height. After development bands were scanned using CAMAG TLC scanner 3.

Observations

Diverse compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The separation was achieved using Chloroform : Methanol (7:3) as the mobile phase. The methanol extract of whole aerial parts of Eclipta alba L. showed the presence of 10 different types of phyto-constituents (at 254 nm) and 8 (at 366 nm) with different Rf. values (Figure 1 & 2 and Table 4 & 6). Reference standard 'weadelolactone' showed peak at Rf. 0.41 at 254 nm and Rf. 0.41 at 366 nm.

Table 1: Preliminary phytochemical tests

Nature Product	Test Performed	Result
Steriod	Liber mann's Reagent	+ ve
Flavonoid	Shinoda Test	+ ve
Tannin	Neutral FeCl3	+ ve
Carbohydrate	Molesch Test	+ ve
Starch	Iodine Solution	+ ve
Protein	Million's Solution	– ve
Saponin	NaOH Solution	+ve
Mucilage	Swelling in Water	– ve

Alkaloid	Mayer's Test, Dregandroff's Test, Wanger Test, Hager's Test	+ ve
Amino Acid	Ninhydine	– ve
Coumaine Glycoside	Alkaline Solution	+ ve
Fat and Oil	Pt. ether ext.	Trace
Diterpenoids	Picric acid (alkaline)	- ve

Table 2: Phytochemical screening of different extractions

Constituents	Dichloromethane extract	Methanolic extract	Aqueous extract
Alkaloids	+	+	+
Amino Acids	–	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Steroids	+	+	–
Tannins	–	+	+
Terpenoids	+	+	+

Table 3: Peak display of weadelolactone at 254 nm

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.41	2.0	0.89	77.7	100.0	0.91	0.00	1279.0	100.0

Table 4: Peak display at different Rf values of *Eclipta alba* methanol extract at 254 nm

Peak	Start rf	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1	0.00	6.2	0.02	696.1	68.68	0.07	50.4	13799.6	60.93
2	0.08	50.7	0.10	69.7	6.87	0.13	18.4	2011.7	8.88
3	0.14	18.2	0.17	30.0	2.96	0.19	21.1	1024.1	4.52
4	0.19	21.2	0.22	38.8	3.83	0.23	35.7	936.1	4.13
5	0.24	37.2	0.27	56.3	5.55	0.29	6.8	1487.1	6.57
6	0.30	6.9	0.31	21.6	2.13	0.39	0.2	455.3	2.01
7	0.37	0.8	0.40	55.0	5.43	0.45	1.1	1399.8	6.18
8	0.41	4.4	0.49	11.2	1.10	0.52	3.5	283.0	1.25
9	0.55	2.0	0.61	18.6	1.84	0.64	4.7	809.1	3.57
10	0.72	0.1	0.75	16.2	1.60	0.78	1.2	444.2	1.96

Table 5: Peak display of weadelolactone at 366 nm

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.41	3.6	0.93	15.4	100.0	0.95	0.7	305.8	100.0

Table 6: Peak display at different Rf values of *Eclipta alba* at 366 nm

Peak	Start rf	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1	0.0	15.1	0.02	719.6	46.23	0.07	83.0	16411.9	39.84
2	0.07	83.1	0.1	127.0	8.24	0.14	66.1	4905.3	11.91
3	0.15	67.8	0.18	79.3	5.01	0.19	76.8	2271.6	5.51
4	0.22	89.7	0.27	150.2	9.75	0.29	15.9	5308.6	12.87
5	0.30	16.0	0.31	62.7	4.07	0.36	0.1	1347.9	3.27
6	0.41	1.5	0.4	191.0	12.4	0.45	1.5	3921.6	9.52
7	0.62	4.5	0.61	73.8	4.79	0.66	8.0	2788.1	6.77
8	0.74	0.0	0.75	137.8	8.95	0.79	0.2	4246.4	10.31

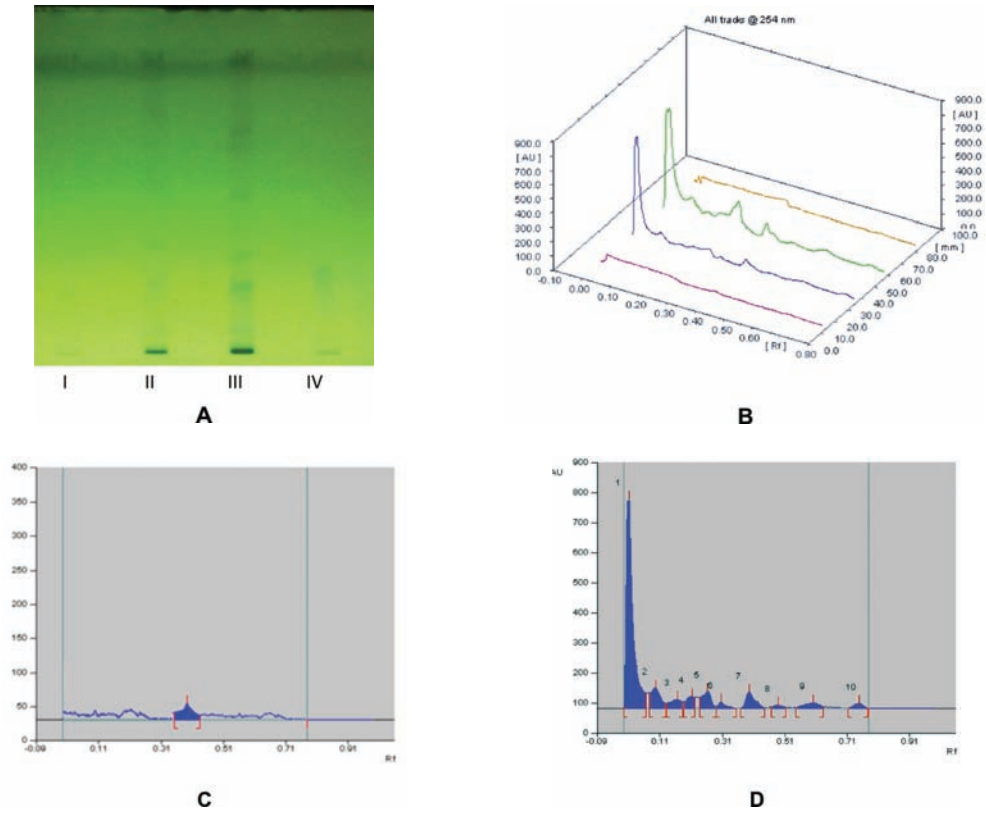

Fig. 1. HPTLC fingerprinting profile under UV 254 nm

A. HPTLC fingerprints profile of methanol extract and reference standard 'weadelolactone' (**I.** 3µg/ml of wedelolactone; **II.** 5µg/ml of methanol extract of sample; **III.** 10µg/ml of methanol extract of sample; **IV.** 6µg/ml of wedelolactone)

B. Overlay Chromatogram

C. Chromatogram of weadelolactone

D. Chromatogram of methanol extract

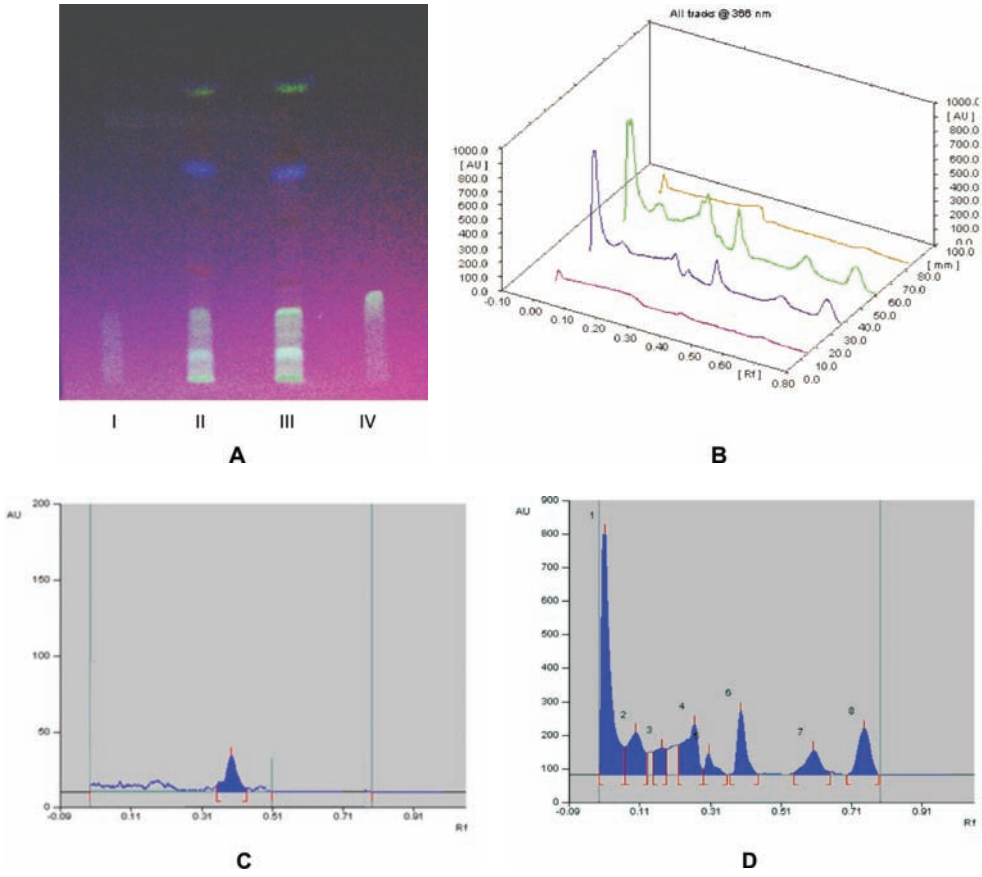

Fig. 2. HPTLC fingerprinting profile under UV 366 nm

A. HPTLC fingerprints profile of methanol extract and reference standard 'weadelolactone' (**I.** 3µg/ml of wedelolactone; **II.** 5µg/ml of methanol extract of sample; **III.** 10µg/ml of methanol extract of sample; **IV.** 6µg/ml of wedelolactone)

B. Overlay Chromatogram

C. Chromatogram of weadelolactone

D. Chromatogram of methanol extract

Results and Discussions

The results of the preliminary phytochemical studies confirm the presence of flavoniods, steroids, alkaloids, tannin, glycosides, carbohydrates, and saponins (Table-1 & 2). The HPTLC finger print analysis of methanol extracts of *Eclipta alba* L. showed the presence of various phytoconstitutents. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. In recent times during this molecule era in addition to morphological characters in plant taxonomy anatomical, cytological, biochemical and molecular markers are also being used to classify the plants. HPTLC finger printing profile is useful

as phytochemical marker and also a good estimation of genetic variability in plant populations. The data generated from the present study would help in the authentication and quality control for *Eclipta alba* L. Such chemo finger printing will also act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

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Pharmacognostic evaluation of Herbal Drugs of Stem Origin Resourced from Market

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Abstract

arket samples of three stem origin herbal drugs viz. *Cinnamomum v*erum Presl., *Terminalia arjuna* (Roxb. ex DC) W. & A. and *Piper longum* L. were studied to assess their quality in respect of identity, purity and strength. The samples were procured from herbal markets of Delhi, Hardwar and Cochin/Trichur. Study is based on specific parameters and limits developed by standardising authentic quality specification.

Keywords: Pharmacognostic evaluation, Commercial herbal drugs, Quality assessment.

Introduction

Herbs are staging a comeback and herbal 'renaissance' is in place all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes. The drugs of herbal origin have been used in traditional systems of medicines such as *Ayurveda*, *Siddha* and *Unani*. Even the modern system of medicine has also adopted a number of plant-derived drugs. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants.

The World Health Organization (WHO) has estimated that the present demand for medicinal plants is approximately US $14 billion per year (Sharma, 2004). The demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% annually, and according to an estimate of WHO, the demand for medicinal plants is likely to increase more than US $5 trillion by 2050. In India, the medicinal plant related trade is estimated to be approximately US $1 billion per year (Joshi et al., 2004). India's potential in market for medicinal and aromatic plants (MAPs) is evident with the facts that the MAPs required to prepare 50 per cent of the drugs mentioned in British Pharmacopoeia are reported to be present in Western Himalayan region alone. Further, this region caters to about 80 per cent of *Ayurvedic*, 46 per cent of *Unani* and 33 per cent of allopathic system of medicines and contributes a major share to the economy of the rural farmers and tribals (Singh, 2006).

Herbal drugs used by the industries are collected from the wild resources. It is estimated that about 800 species are used in production by the pharmaceutical industry, whereas less than 40 species of plants are resourced through

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commercial cultivation. Over 70% of the plant collection involves destructive harvesting. This poses a definite threat to the genetic stocks and to the diversity of medicinal plants. Adulterants/substitutes are being traded/used with at times with full knowledge of the sellers/buyers and are very common in the herb trade especially when the trade is involved. In many cases, substitutes have taken over the original plants. In some cases, substitutes have become popular, manufacturers have forgotten about the original plant and they only use substitutes available in the market. It is very much doubtful if such substitution is made after testing or as recommended by any authority. Sometimes different morphological parts of same plant species are used in place of the prescribed part. At times mere look alike species are used as a substitute, which may not even contain the active ingredients available through the main plants nor the effects of the end product is the same as that obtained from that of original plant (Sharma, 1987; Rai *et al.,* 2011 and Padmakumar *et al.,* 2012). Based on this rational the present study has been taken and deals with three important herbal drugs widely used in Indian systems of medicine for centuries.

Materials and Methods

The stem and stem bark origin herbal drugs under study were collected from natural habitats and authenticated with references to pharmacopoeial standards and other literature. The commercial samples sold under the trade names purported to be prescribed species were obtained from different market sources (Hardwar, Delhi and Cochin/Trichur) (Fig. 1-3). Standard protocols/ methods prescribed in pharmacopoeia were followed for pharmacognostical, physico-chemical and phytochemical values prescribed in Ayurvedic, Unani and Siddha Pharmacopoeia of India were taken as standards values (Anonymous, 1986, 1998, 1999, 2007a,b and 2008).

Table 1: Commercial Herbal Drugs under study

Botanical Name	Official Name	Trade Name	Morphological Part
Cinnamomum verum Presl.	Twak	Dalchini	Stem bark
Terminalia arjuna (Roxb. ex DC) W. & A.	Arjuna	Arjun chal	Stem bark
Piper longum L.	Pippalimula	Pippali	Stem

Observations and Results

All the commercial samples of the drugs were evaluated as per the specifications laid in Pharmacopoeia and other literature. Observations made are given in Table 2 to 4 -

Table 2: Pharmacognostical evaluation of commercial crude drug samples of *Cinnamomum verum* Presl.

Sl. No.	Specifications	Market Sample		
		Delhi	Haridwar	Cochin
A.	Entire Drug 1. Macromorphological characteristics	Conforms	Conforms	Conforms
	2. Micromorphological characteristics			
B.	Powdered drug	Conforms	Conforms	Conforms
C.	Major organic groups			
	(i) Alkaloids	-	-	-
	(ii) Tannins	√	√	√
	(iii) Glycosides	-	-	-
	(iv) Sterols	-	-	-
	(v) Volatile Oil	√	-	√
	(vi) Essential Oils	√	-	√
	(vii) Flavonoids	-	-	-
	(viii) Anthraquinone	-	-	-
	(ix) Resins	-	-	-
	(x) Fixed oil	-	-	-
	(xi) Poly phenolic compounds	-	-	-
D.	Physico-Chemical Characteristics			
	(i) Moisture Content %	4.60	3.95	5.25
	(ii) Total ash %	2.50	3.28	2.20
	(iii) Acid insoluble ash %	1.60	1.20	0.90
	(iv) Water soluble extractives	4.10	4.50	4.80
	(v) Alcohol soluble extractives %	3.20	5.42	5.90
E.	Foreign Matter %	0.55	1.20	1.60

Table 3: Pharmacognostical evaluation of commercial crude drug samples of *Terminalia arjuna* (Roxb. ex DC) W. & A.

Sl. No.	Specifications	Market Sample		
		Delhi	Haridwar	Cochin
A.	Entire Drug 1. Macromorphological characteristics	Conforms	Conforms	Varies slightly
	2. Micromorphological characteristics	Conforms	Conforms	Conforms
B.	Powdered drug	Conforms	Conforms	Conforms
C.	Major organic groups			
	(i) Alkaloids	-	-	-
	(ii) Tannins	√	√	√
	(iii) Glycosides	-	-	√
	(iv) Sterols	-	-	-

	(v) Volatile Oil	-	-	-
	(vi) Flavonoids	-	-	-
	(vii) Anthraquinone	-	-	-
	(viii)Resins	-	-	-
	(ix) Fixed oil	-	-	-
	(x) Poly phenolic compounds	√	√	√
D.	Physico-Chemical Characteristics			
	(i) Moisture Content %	6.50	4.56	4.25
	(ii) Total ash %	12.50	11.90	21.30
	(iii) Acid insoluble ash %	0.80	0.21	0.54
	(iv) Water soluble extractives	22.10	20.80	23.20
	(v) Alcohol soluble extractives %	20.13	19.20	27.80
E.	Foreign Matter %	1.08	1.20	0.95

Table 4: Pharmacognostical evaluation of commercial crude drug samples of *Piper longum* L.

Sl. No.	Specifications	Market Sample		
		Delhi	Haridwar	Cochin
A.	Entire Drug 1. Macromorphological characteristics	Conforms	Conforms	Conforms
	2. Micromorphological characteristics	Conforms	Conforms	Conforms
B.	Powdered drug	Conforms	conforms	Conforms
C.	Major organic groups			
	(i) Alkaloids	√	√	√
	(ii) Tannins	-	-	-
	(iii) Glycosides	-	-	-
	(iv) Sterols	-	-	-
	(v) Volatile Oil	-	-	-
	(vi) Flavonoids	-	-	-
	(vii) Anthraquinone	-	-	-
	(viii) Resins	-	-	-
	(ix) Fixed oil	-	-	-
	(x) Poly phenolic compounds	-	-	√
D.	Physico-Chemical Characteristics			
	(i) Moisture Content %			
	(ii) Total ash %			
	(iii) Acid insoluble ash %			
	(iv) Water soluble extractives%			
	(v) Alcohol soluble extractives%			
E.	Foreign Matter %	1.15	0.80	0.40

Fig. 1: *Cinnamomum* verum Presl.

Fig. 2: *Terminalia arjuna* (Roxb. ex DC) W. & A.

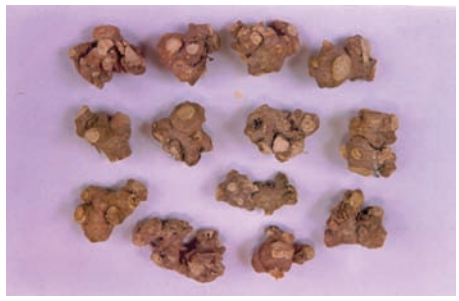

Fig. 3: *Piper longum* L.

Discussion and Conclusion

Dried stem bark of *Cinnamomum v*erum Presl. is sold in the market under the trade name of 'Dalchini' and used in the preparation of various Unani preparations and also used in spices. Bark is brittle and dull yellowish-brown in colour with occasional small scars. It also contains minute acicular crystals of calcium oxalate. Active chemical constituents are Tannin, Essential oil and mucilage. Moisture content varies from 3.95% to 5.25%, alcohol soluble extractives from 3.2% to 5.9% and total ash 2.2% to 3.28%. Foreign matter content varies from 0.55% to 1.60%. All the commercial samples conform to that of authentic drug specimen. Drug available as dried cut stem pieces of *Piper longum* L. which are reddish brown to grey in colour with distinct internodes and swollen nodes with small rootlets and root scars. Starch grains simple and compound having 2 to 7 components, round to oval, present abundantly. Active chemical constituents are alkaloids. All the commercial samples of Delhi, Haridwar and Cochin conforms to the properties of the authentic sample. *Terminalia arjuna* (Roxb. ex DC) W. & A. available as pieces of curved bark, recurved, channelled to half quilled with smooth and grey outer surface and inner surface somewhat fibrous and pinkish with the trade name of 'Arjun chal'. Outer layers of cells filled with brown colouring matter. Phloem

parenchyma contains rosette crystals of calcium oxalate. Starch grains simple and compound of 2 to 3 components round to oval found throughout the tissue. Powder of *Terminalia arjuna* (Roxb. ex DC) W. & A. is reddish-brown in colour with rosette crystals of calcium oxalate, a few rhomboidal crystals and simple and compound starch grains. Active chemical constituents are tannins. All the collected commercial samples conform to the values of authenticated samples. However, the macro-morphological characteristic of Cochin sample slightly varies. Foreign matter content varies from 0.95% to 1.2%.

The present study demonstrates that market samples should always be subjected to quality evalution to ensure identity, purity and strength as per pharmacopoeial specifications and other quality standards of drugs before their use in the formulations.

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Ethnopharmacological survey of Rampur district forests in Rohilkhand region of Uttar Pradesh

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Abstract

This report deals with the results of an ethnopharmacological survey recently conducted in Rampur district of Rohailkhand, one of the important regions of Uttar Pradesh. It lists 30 plant species belonging to 21 families of angiosperms that are commonly used by the indigenous communities of the area as folk drugs for treatment of various diseases and conditions of humans and cattle. For each plant species the scientific and local names, part used, claimed medicinal use(s), mode of administration are given. The study enriches our existing knowledge on ethnopharmacopoea of this region of northern India.

Keywords: Ethnopharmacological survey, folk medicines, Rampur, Rohilkhand, Uttar Pradesh.

Introduction

Rohilkhand region, lying in the north-western part of Uttar Pradesh, a northern province of India, includes districts of Bareilly, Moradabad, Rampur, Bijnor, Pilibhit, Shahjahanpur, etc. It is inhabited by various indigenous castes and cultural groups who claim to be the descendant of pastoral races. They still have knowledge of plants and their healing properties which they have inherited orally from many generations. Folk medicines of different parts of this region have been described by many ethnobotanists and other investigators (Ali, 1999; Ali and Ahmad, 2007; Ali et al., 2011a, 2011b; Ali et al., 2003; Khan, 2002; Khan and Siddiqui, 1987; Maheshwari and Singh, 1984; Sharma, 1985, 1991, 1996; Sharma and Gautam, 1992; Sharma et al., 1989). No list, however, exists of the plants which are in therapeutic use among the inhabitants of Rampur district. Hence, an ethnopharmacological survey was conducted in this part of Rohilkhand. In this contribution, the results of this field study are reported.

Rampur district of Rohilkhand from which data were gathered is situated between 28° 25′ - 29° 10′ N latitude and 78° 52′ - 79° 26′ E longitude. In configuration, it is almost heart-shaped bounded on the north by Udham Singh Nagar district, on the east by Bareilly and Udham Singh Nagar districts, on the south by Badaun and Bareilly districts and on the west by Moradabad district (Fig. 1). There are four forest ranges (namely: Bilaspur, Suwar, Milak and Rampur) with reserve forests covering an area of 6610.80 hectare. The forests of this area are generally of northern tropical dry deciduous type. These are found only in the Tarai of Bilaspur and Suwar forest ranges. Sal (Shorea

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robusta Gaertn.f.) is entirely absent in the district. However, some of its usual associates are commonly found here. There are some scattered settlements of 'Vangujjars' (a nomadic forest dwelling tribe). Some other ethnic and cultural groups like 'Kamboj', 'Boxas', 'Jatsikh' and 'Raisikh' are also found around these forests. The agriculture and horticulture are their main occupations. The forest areas surveyed include Pipli Ban, Ambarpur, Aryanagar, Dandiya, Rawana and Ehrula.

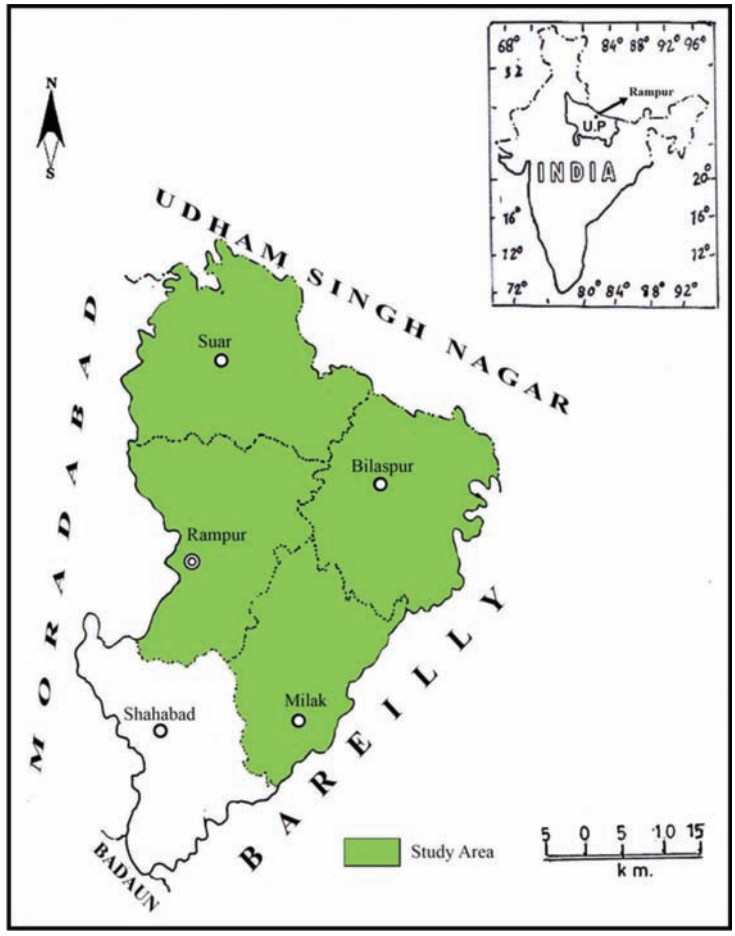

Fig. 1: Map showing the areas surveyed in Rampur district (U.P.), India

Methodology

The present investigation was carried out in March 2012. During the fieldwork a number of tribal settlements and villages were visited. Information on folk medicinal uses of local plants was obtained through direct field interviews with reliable informants who were local medicine men and other knowledgeable village elders. Data on common name of the plant or the crude drug, medicinal use(s), part used, other ingredients added (if any), method of drug preparation

and mode of administration were recorded for each claim. Plant specimens were collected with the help of informants and later identified by the senior author with the help of related floras (Duthie, 1903-1922; Hooker, 1872-1897; Kanjilal, 1982; Raizada, 1976). Voucher herbarium specimens of all the species were prepared and deposited in the Herbarium of the Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India.

Observations

In the following listing medicinal plants are arranged in alphabetical order by their scientific names. Each entry provides information on correct botanical name, family between parentheses, prevalent local name in inverted commas, locality from which a particular use was recorded, voucher specimen number and folk use(s). As far as possible, the probable dosage and duration of these crude drugs are also given.

Acacia nilotica (L.) Willd. ex Del. ssp. *indica* (Benth.) Brenan (Mimosaceae), 'Babool', Milak (*ZAA9425)*. Lukewarm decoction of the stem bark is poured on dhobie itch. The stem bark of the plant is also used to treat dysentery. It is mixed with vegetative buds of 'jaman' (*Syzygium cumini* (L.) Skeels) and 'amrud' (*Psidium guajava* L.) and boiled; the liquid is strained and given orally two to three times a day for 5-7 days.

Achyranthes aspera L. (Amaranthaceae), 'Chirchita', Pipli (*ZAA9281*). Plant paste is applied locally for bruise.

Ageratum conyzoides L. (Asteraceae), 'Podinajari', Dandiya (*ZAA9352*). A freshly made paste of the leaves, obtained by crushing, is applied on cuts and wounds to stop the bleeding.

Alstonia scholaris (L.) R. Br. (Apocynaceae), 'Ajan', Dandiya (*ZAA9359*). Stem bark decoction is used as cough sedative in cases of children.

Blumea lacera (Burm. f.) DC. (Asteraceae), 'Karonda', Pipli (*ZAA9286)*. Fresh leaf juice mixed with powder of few black peppers and common salt is given orally for dog-bite.

Bombax ceiba L. (Bombacaceae), 'Semal', Rawana (*ZAA9399*). The tap root of the young plant, locally known as 'semal musli', is cut into small pieces, dried and ground to make a fine powder. One spoon of this powder is given with milk once daily as aphrodisiac.

Butea monosperma (Lam.) Taub. (Fabaceae), 'Dhak', Ambarpur (*ZAA9317*). A fine paste of the stem bark is applied in the mouth of children to treat stomatitis. Leaf decoction is given to cattle for mild fever.

Calotropis procera (Ait.) R. Br. (Asclepiadaceae), 'Akawa', Dandiya (*ZAA9424*). Latex is mixed with the latex of 'bargad' (*Ficus benghalensis* L.) and put on aching tooth.

Cassia fistula L. (Caesalpiniaceae), 'Karangal' and 'Amaltas', Dandiya (*ZAA9379*). Young root is rubbed on the stone with water and applied externally for fungal infection.

Centella asiatica (L.) Urban (Apiaceae), Dhandai (*ZAA9332*). Fresh leaves are crushed and taken with water as refrigerant.

Cissampelos pariera L. (Menispermaceae), 'Jaljamni', Pipli (*ZAA9278*). Leaf juice coagulates on being allowed to stand in a cup for 4-5 hours. It is given three times a day for 5-7 days to treat amaebiasis.

Clerodendrum cordatum D. Don (Verbenaceae), 'Bhat', Pipli (*ZAA9784*). Young vegetative buds are chewed and taken daily to control diabetes.

Cuscuta reflexa Roxb. (Cuscutaceae), 'Agasbel', Pipli (*ZAA9304*). Paste of the plant is applied on boil as poultice for suppuration and healing.

Dicliptera roxburghiana Nees (Acanthaceae), 'Hadjor', Pipli (*ZAA9276)*. About 25g of the paste, obtained by grinding the whole plant in water, are given two times a day to hasten the healing process of bone fracture.

Euphorbia helioscopia L. (Euphorbiaceae), 'Dudhi', Pipli (*ZAA9285*). Fresh latex obtained from the plant is applied externally in leucoderma.

Euphorbia thymifolia L. (Euphorbiaceae), 'Chunia ghans' Pipli (*ZAA9279*). One tea spoonful of the leaf powder mixed with 'khand' (crude sugar) is given two times a day for 5 days to treat dysentery.

Ficus benghalensis L. (Moraceae), 'Bargad', Dandiya (*ZAA9365*). Paste of the tender aerial root is applied locally for prolapsed rectum.

Glycosmis arborea (Roxb.) DC. (Rutaceae), 'Elu', Pipli (*ZAA9307*). Tender twig is used as toothbrush for dental care.

Gomphrena serrata L. (Amaranthaceae), 'Kana', Ehrula (*ZAA9388)*. Fresh plants are fed to buffaloes and cows for increasing lactation.

Holarrhena pubescens (Buch.-Ham.) Wall. ex G. Don (Apocynaceae), 'Kokar', Aryanagar (*ZAA9309)*. Decoction of the seeds is given for malaria fever. Stem bark is mixed with leaves of 'ajan' (*Alstonia scholaris*) and boiled in milk. It is administered through pipe in the nose of buffaloes and cows for treating mastitis.

Ixeris polycephala Cass. (Asteraceae), 'Dudhi ghans', Pipli (*ZAA9280*). Crushed leaves are boiled in seed-oil of 'alsi' (*Linum usitatissimum* L.) and cooled. It is lightly massaged on affected side of the body in hemiplegia.

Justicia adhatoda L. (Acanthaceae), 'Basooti', Aryanagar (*ZAA9299)*. Leaves are mixed with leaves of 'barna' (*Cratevea adansonii* DC.) and boiled in water. It is given to cattle for treating bronchitis with fever.

Kalanchoe pinnata (Lam.) Pers. (Crassulaceae), '*Patthar Chata*', Pipli (*ZAA9282*). Leaf paste (20g) is given orally two to three times a day for one month to dissolve and expel small kidney stones.

Launaea procumbens (Roxb.) Ramayya & Rajagopal (Asteraceae), 'Jangli Gobhi', Pipli (*ZAA9283*). Crushed leaves are fried in ghee, cooled and given orally in the dose of 20g twice daily for 1-2 month to treat haemorrhoids.

Malvastrum coromandelianum (L.) Garcke (Malvaceae), 'Khurenti', Pipli (*ZAA9277*). Leaf paste is given with water for palpitation.

Pogostemon benghalenses (Burm.f.) Kuntze (Lamiaceae), 'Maspindi', Aryanagar (*ZAA9300*). Leaf juice is applied on sharp cuts to stop the bleeding.

Pongamia pinnata (L.) Pierre (Fabaceae),'Kanju', Ambarpur (*ZAA9369*). Tender twig is used daily as toothbrush for dental care.

Quirivelia frutescens (L.) M.R. & S.M. Almeida (Apocynaceae), 'Keef Bel', Dandiya (*ZAA9353*). Whole plants are cut into pieces and fed to buffaloes and cows as galactagogue.

Terminalia arjuna (Roxb. ex DC.) Wight (Combretaceae), 'Arjun', Ambarpur (*ZAA9323*). Powdered stem bark is boiled in water, cooled and liquid is strained. It is used daily as cardiac tonic.

Zizyphus mauritiana Lam. (Rhamnaceae), 'Beri', Aryanagar (*ZAA9373*). Fresh leaf paste is applied on forehead to treat headache.

Discussion

This pioneer ethnopharmacological study on Rampur forests of Rohilkhand region, Uttar Pradesh has brought into light information on folk medicinal utility

of some 30 plant species belonging to 21 different families of angiosperms. The data were obtained from reliable informants who were usually elder people. They have long been using these plants in their day-to-day health related problems despite the fact that government primary healthcare centres and dispensaries are now accessible to rural populace. These medicinal uses were analyzed and compared with the available literature on medicinal and economic plants of the country (Anonymous, 1948-1976, 2001; Chopra et al., 1956; Jain, 1991; Kirtikar and Basu, 1935; Nadkarni, 1954; Watt, 1889-1892) and it was found that majority of the claims reported herein seem to be new or less-known and enrich our existing traditional knowledge.

In the course of fieldwork it was observed that extent of natural forests which are the main habitats of the medicinal plants has been reducing especially in Tarai of this region due to the heavy pressure of an agricultural population with a constantly increasing demand for land for cultivation. This ancestral knowledge of medicinal plants in the region is in danger of being lost because of expansion of agriculture, increasing access of allopathic system of medicine as well as acculturation and above all the apathy of younger generation who does not show much interest in traditional medicine. It is, therefore, desirable to conduct extensive field surveys of other ethnobotanically unexplored or under explored areas of this part in particular and in other areas of Uttar Pradesh in general. Such investigations could bring some more new ethnomedicinal information which can be a source of significant drug leads. As many potent drugs of today have their origin in Indian traditional medicine and ethnopharmacology (Mukherjee et al., 2007; Patwardhan, 2005).

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Physicochemical and Phytochemical evaluation of Cocoon of *Bombyx mori* Linn. (Abresham)

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Abstract

De-wormed cocoon of *Bombyx mori* Linn. (Silkworm) of Bombycidae family, plays a foremost role in Unani system of medicine. Its decoction is used in the preparation of Unani compound formulations for the treatment of different diseases like cardiovascular and cerebro-vascular disorders. Because of these prominent medicinal properties, the drug was standardized according to WHO guidelines. The main aspects included in the study were organoleptic characters, morphological features, physico-chemical & phytochemical parameters, fluorescence analysis of decoction and infusion of the drug extracts and HPTLC profile. The study also included safety evaluation measures, such as heavy metal analysis, microbial load, aflatoxins which provide scientific means regarding the qualitative and quantitative aspects. They are widely accepted in the quality assessment of herbal drugs and also to lay down the standard for the genuine drug. Phytochemical screening was also carried out in the drug extracts. The present study is aimed to standardize and provide the scientific evidences for the decoction and infusion of the drug for its safe and effective therapeutic potential.

Keywords: *Bombyx mori,* Physicochemical, Phytochemical screening, HPTLC fingerprint, Standardization.

Introduction

Bombyx mori Linn. Syn. *Phalaena mori* Linn. is an economic insect whose silk is emerging as a source for solving a broad range of biological problems (Mondal, 2007). Its dietary flavonoids are metabolized and accumulate in cocoons, thereby causing green coloration. Flavonoids increase the UV-shielding activity of cocoons and thus could confer an increased survival advantage to insects contained in these cocoons (Daimona *et al*., 2010). Cocoon-making behavior is the most highly developed in Lepidoptera (moths and butterflies). The silkworm, *Bombyx mori*, is a monophagous insect whose only food is mulberry leaves. The cocoon shell of the silkworm consists mainly of proteins such as fibroin and sericin (Tamura *et al*., 2002). Silkworm cocoon colors are determined by two main pigments, carotenoids (Harizuka, 1953) and flavonoids (Tazima, 1978), which are derived from mulberry leaves (Fujimoto, 1959).

Flavonoids modified by *B. mori* may be useful as medicinal or cosmetic materials. The ethanolic extracts of yellow-green colored cocoon shells of a range of strains of *B. mori* have potent antibacterial activity (Kurioka *et al*.,

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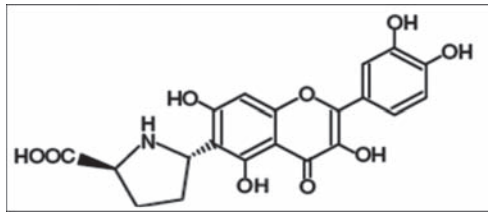
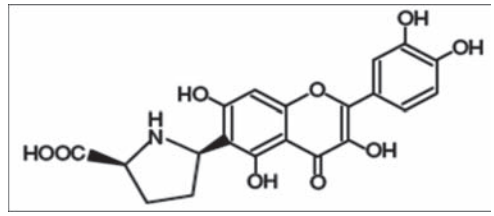
1999) and strong antioxidant activity (Yamazaki *et al*., 1999). Two flavonoids containing the L-proline moiety, 6-*C*-[(2*S*,5*S*)-prolin-5-yl] quercetin (prolinalin A) and 6-*C*-[(2*S*,5*R*)-prolin-5-yl] quercetin (prolinalin B), were isolated from the cocoon shell of the silkworm of *Bombyx mori* by Hirayama *et al*., 2006. Further research among silkworm strains for novel flavonoids towards possible biological activity reveal that the aqueous MeOH extract of the yellow cocoon shell of a Chinese race of Daizo contain novel flavonoids with an amino acid moiety (Hirayama *et al*., 2006).



Prolinalin A



Prolinalin B

Bombyx mori in Unani context

In Unani Medicine *B. mori* cocoon is known as 'Abresham' and popularly known as Abresham Muqriz; Muqriz means cut (Kabeeruddin, undated). It is used in various formulations as one of the ingredients like 'Khamira-e-Abresham Sada', 'Khamira-e-Abresham Hakeem Arshad Wala' etc. for many cardiac and neurological disorders (Khan et al., 2006). Therapeutic actions attributed to the drug are Muffareh (Exhillerent), Munaffis-e-Balgham (Expectorant), Jali (Detergent) and Muqawwi-e-Qalb (Cardiac tonic). Crude extract of *Bombyx mori* cocoons along with two other drugs also acts as protective remedy in hyperlipidemia. National Formulary of Unani Medicine (Anonymous, 2007) includes several formulations with large or small number of ingredients for the treatment of cardiovascular and cerebrovascular disorders. Some of the formulations are being used in Unani medicine with good results and efficacy (Goswami, 1977). But there is no definite data regarding dose and effect relationship for the extract of cocoons of *Bombyx mori*. This led to the logical study for evaluating the role of aqueous extract i.e., infusion and decoction of silk cocoons of *Bombyx mori* as a single drug for its safe therapeutic potential.

Properties of Bombyx mori silk (Abresham Muqriz)

Bombyx mori silk is attributed the qualities viz., 'Hot and dry' (Garm-o-Khushk) in its temperament. It is a cardiac tonic and a nervous stimulant. It is an

expectorant and removes excess 'Kapha' (Phlegm) from the blood (Hamdani, 1980). Recent advancement have shown that it is being used to treat palpitation, hypertension and heart diseases, which occur due to hardening of arteries.

Materials and methods

Collection of material

Dewarmed cocoons were procured from the pharmacy of Central Research Institute of Unani Medicine (CRIUM), Hyderabad. The present investigation on the drug include parameters such as morphological studies, physico-chemical parameters, Phytochemical screening and HPTLC fingerprint of infusion and decoction (Aqueous extracts), fluorescence studies and safety evaluation.

Chemical analysis

Physico-Chemical parameters of the cocoons were studied as shown in table 1, such as total ash, water and alcohol soluble matter, PH value and loss on drying at 1050C. Physico-chemical parameters were determined according to the methods described in 'The Unani Pharmacopoeia of India' (Anonymous, 2009). Fluorescence analysis was carried out as per the method described by Trease and Evans (1972) and GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of heavy metals. Microbial load and aflatoxins contamination were analyzed as per the methods described in WHO guidelines (Anonymous, 1998).

Phytochemical screening was carried out in the aqueous extracts i.e., infusion and decoction as per the methods described by Trease and Evans (1972) to observe the nature of phyto-constituents present in the drug. Phyto-constituents such as flavanoids and proteins were detected as the major constituents in the cocoons.

HPTLC fingerprint profile

Preparation of extract of the sample drug

Cocoons in a quantity of 10 g were macerated in 100 ml of methanol and water separately in stoppered conical flasks and kept for 2 hours while shaking at regular intervals. Later the contents were filtered through whatman No. 41 filter paper and evaporated the solution to 10 ml. The solutions thus obtained, were used as samples for separation of components.

Infusion preparation

Infusion was prepared by macerating 10 g of Cocoons for a short period of time with either cold or boiling water.

Decoction Preparation

To obtain the decoction, 10 g of Cocoons were boiled in specified volume of water for defined time, cooled and strained. This procedure was carried out for extracting water soluble or heat stable constituents.

Development and determination of the solvent system

The samples were spotted as 6mm band on Pre-coated Aluminum sheets of Silica Gel 60 F_{254} (Merck). After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated in table 2 was selected in its proportional ratio, and developed in the Twin through TLC chamber to the maximum height of the plate.

Detection system

After developing, the TLC plate was dried completely and detected by spraying Ninhydrin reagent on the plate heated at 110^0C for 5 minutes and then observed in visible region and photographed as shown in figure 5.

Results and Discussion

Organoleptic Characters

The crude drug consists of de-wormed cocoons of *Bombyx mori* (Silkworm) were yellow in colour.

Identification

Macroscopy: Cocoons yellowish, oblong-ovate, 3-4.5 cm long and 2-3 cm in diameter; with very fine silk threads. Outer surface rough, fibrous, shining; inner surface smooth, shining; taste and odour indistinct. Macroscopical features of Cocoon of *Bombyx mori* as observed under visible light and UV at 254nm & 366nm are shown in figure 1.

Microscopy: Microscopical study reveals that Cocoon wall is made up of silk fibres of 35-50µ thickness. The fibres are translucent, silk threads solid, cylindrical or slightly flattened; highly refractive threads from 10-25µ (Fig. 2, 3 & 4).

Powder characters: The Powder is golden yellowish and contains pieces of silk fibres.

Physico-Chemical Standards

The Physico-chemical parameters data in the study shows the mean values of three readings and depicted in table 1.

Chemical Analysis: (TLC analysis, Heavy metals, microbial load, Aflatoxins, fluorescence behavior).

Infusion and decoction of Cocoons of aqueous extract of the drug whose chromatogram was developed using the solvent n-butanol: Glacial Acetic Acid: Water (4:1:1) and detected by spraying with ninhydrin reagent and heating the plate at 110^0C was observed as given in table 2. Under visible region it showed various spots with Rf values as given in table 3-5. Densitogram obtained from the HPTLC system for infusion, decoction and methanolic extract of cocoon at 580 nm were observed as shown in figures 6-8. Fluorescence study of infusion and decoction pertaining to their colour in daylight i.e, visible region and under ultra-violet light were noticed as presented in table-6. The preliminary phytochemical screening for nature of compounds present were carried out in infusion and decoction of cocoon of *Bombyx mori,* revealed the presence of alkaloids, carbohydrates, flavonoids, proteins. In aqueous and alcoholic extracts it revealed the presence of Glycosides, flavanoids and proteins as shown in the table 7 and 8. For safety evaluation studies of the drug, estimation of heavy metals such as cadmium, lead, mercury and arsenic were carried out and found to be absent except the presence of mercury which was within the permissible limits as given in table 9. Similarly, Aflatoxins were analyzed and found to be absent as given in the table 10. Microbial load were analyzed and found to be within the permissible limits as given in the table 11, inferring the drug to be safe and non toxic.

Table 1: Physico-chemical parameters of the de-wormed Cocoon

Parameters	Results in average (n=3)	Limits
Total ash (% w/w)	1.20%	(Not more than 1.5%)
Acid insoluble ash (%w/w)	0.25%	(Not more than 1.0%)
Alcohol sol. Matter (%w/w)	0.85%	(Not less than 0.5%)
Water sol. matter (% w/w)	5.23%	(Not less than 5%)
Loss of weight on drying at 1050C	6.38%	(Not more than 7.0%)
P^H of Infusion	6.7	
P^H of Decoction	6.3	

Table 2: TLC profile of infusion and decoction of cocoon of Bombyx mori along with Rf values and detection system

S. No.	Name of the extract	Solvent system	Detection	No. of spots	Rf values
1.	Infusion	n-butanol: Glacial acetic acid: water =4:1:1	Ninhydrin reagent	5	0.01, 0.31, 0.36, 0.56, 0.67
2.	Decoction	n-butanol: Glacial acetic acid: water =4:1:1	Ninhydrin reagent	3	0.01, 0.30, 0.37

Table 3: Peak list of Infusion of cocoon of Abresham at 580 nm

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1	10.8	20.90	3.0	11.95	0.01
2	26.8	142.31	20.6	33.60	0.31
3	29.5	148.99	21.6	34.34	0.36
4	40.0	77.57	11.2	19.57	0.56
5	46.1	299.84	43.5	70.05	0.67

Table 4: Peak list of Decoction of cocoon of Abresham at 580 nm.

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1	10.8	40.85	28.2	20.12	0.01
2	26.2	42.12	29.1	14.38	0.30
3	30.2	61.83	42.7	15.64	0.37

Table 5: Peak list of methanolic extract of cocoon of Abresham at 580 nm

Peak No.	Y-Pos	Area	Area (%)	Height	Rf value
1	26.2	78.15	33.0	24.55	0.30
2	29.8	54.45	23.0	17.19	0.37
3	44.3	104.11	44.0	27.58	0.64

Table 6: Fluorescence analysis of powdered drug

S. No	UV 254 nm	UV 366nm	Visible region
Infusion	Black	Blue	Pale yellow
Decoction	Blue	Blue	Pale yellow

Table 7: Phytochemical screening of the nature of compounds present in the infusion and decoction of cocoon of *Bombyx mori.*

S. No.	Phyto constituents	Infusion	Decoction
1.	Alkaloids	-	+
2.	Carbohydrates	+	+
3.	Flavonoids	+	+
4.	Glycosides	-	-
5.	Phenols	-	-
6.	Proteins	+	++
7.	Saponins	-	-
8.	Steroids	-	-
9.	Tannins	-	-

Table 8: Phytochemical screening of the nature of compounds present in the aqueous and alcohol of cocoon of *Bombyx mori.*

S. No.	Phyto constituents	Aqueous	Alc
1.	Glycosides	-	+
2.	Flavonoids	+	+
3.	Proteins	+	+
4.	Alkaloids	-	-
5.	Tannins	-	-

Table 9: Heavy Metal Analysis

S. No.	Parameter analyzed	Results	Permissible limits as per WHO
1	Arsenic	Nil	Not more than 3.0 ppm
2	Cadmium	Nil	Not more than 0.3 ppm
3	Lead	Nil	Not more than 10.0 ppm
4	Mercury	0.206 ppm	Not more than 1.0 ppm

Table 10: Aflatoxin Contamination

S. No.	Parameter analyzed	Results	Permissible limits as per WHO
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm

Table 11: Safety evaluation

S. No.	Parameter analyzed	Results	Permissible limits as per WHO
1	Total Bacterial Load	8 x 102	Not more than 105/g
2	Total Fungal count	Nil	Not more than 103/g
3	E.Coli	Nil	Nil
4	Salmonella Spp	Nil	Nil

Conclusion

The drug under study was subjected to Physico - chemical analysis, which is supportive in establishing the standards along with other parameters such as macroscopic, microscopic and fluorescence behavior as reported in the present investigation. The study on heavy metals, microbial and aflatoxins found within the permissible limits, indicating the drug is safe. Phytochemical screening also reveals the presence of nature of compounds which play the prominent role in the therapeutic efficacy of the drug as reported in the literature. Consequently the drug was brought up in determining and ascertaining its quality standard and also developed HPTLC fingerprint profile which helps in identification and assessment of the quality of drug. Therefore, it may be concluded that the study is an attempt to lay down quality parameters of the drug used in Unani System of Medicine.

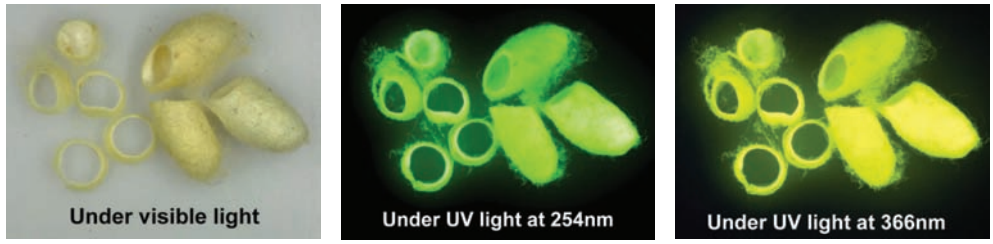


Fig. 1. Macroscopical feature of Cocoon of Bombyx mori under visible light and UV at 254nm & 366nm

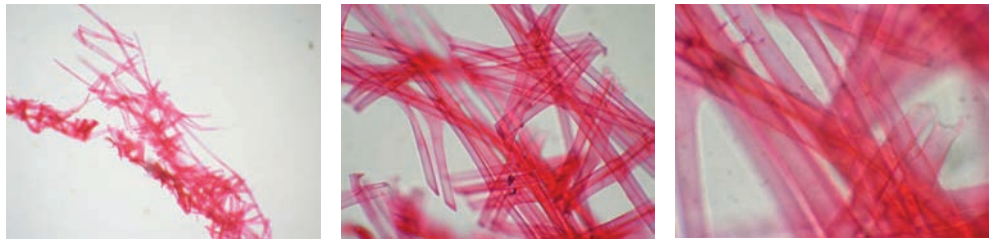


Fig. 2. Microscopical feature of shell of Bombyx mor showing the fibres of silk

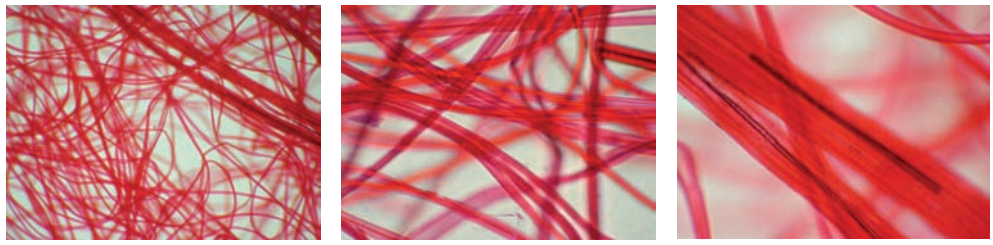


Fig. 3. Microscopical feature of silk fibres of cocoon of Bombyx mori with staining of fibres

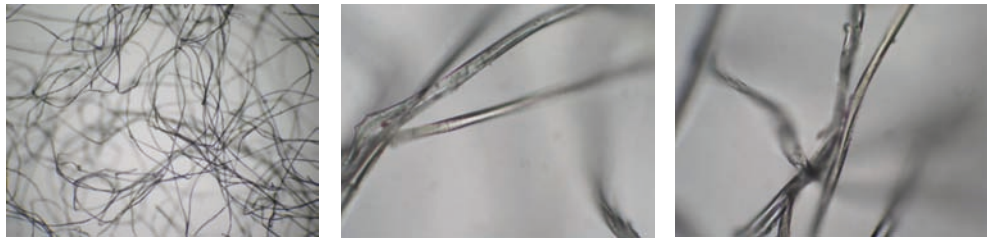
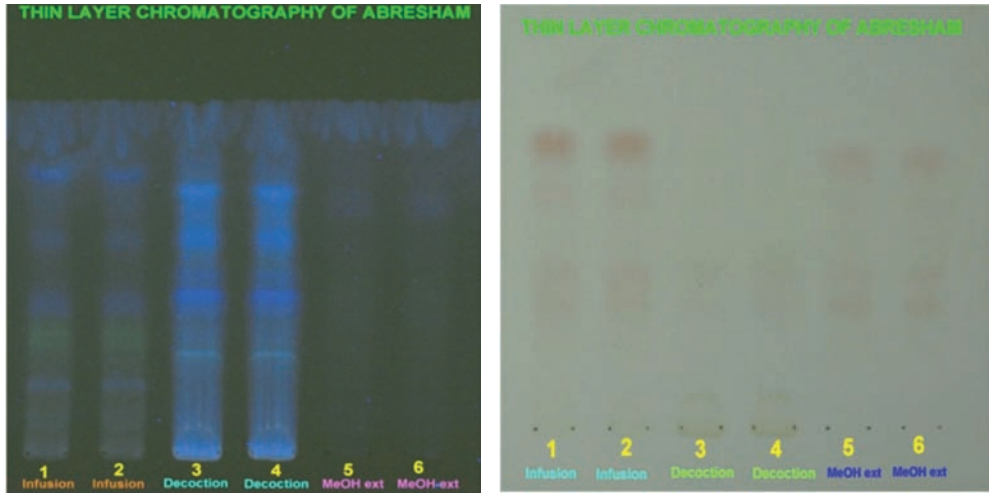


Fig. 4. Microscopical feature of silk fibres of cocoon of Bombyx mori with out staining of fibres



Before spraying at UV 366nm

After spraying under visible region

Fig. 5. TLC plates shown for the Infusion, decoction and methanolic extract of Cocoon of Bombyx mori (Abresham) before observed at UV 366nm and after spraying with ninhdyrin reagent and observed under visible

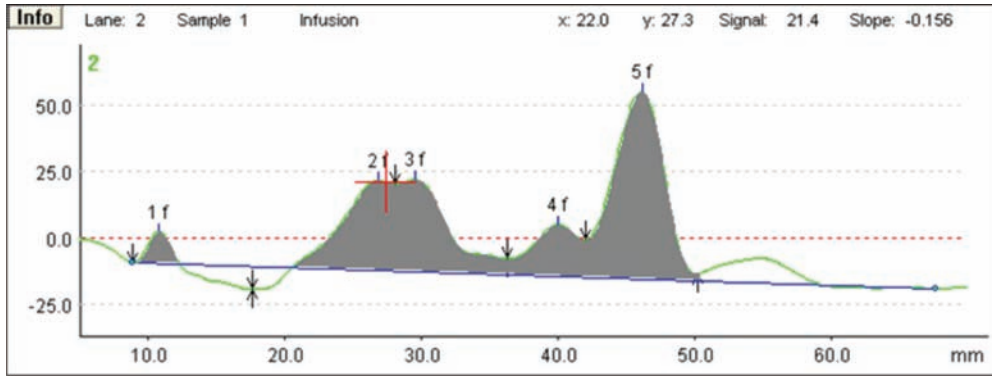


Fig. 6. Densitogram of Infusion of cocoon at 580 nm

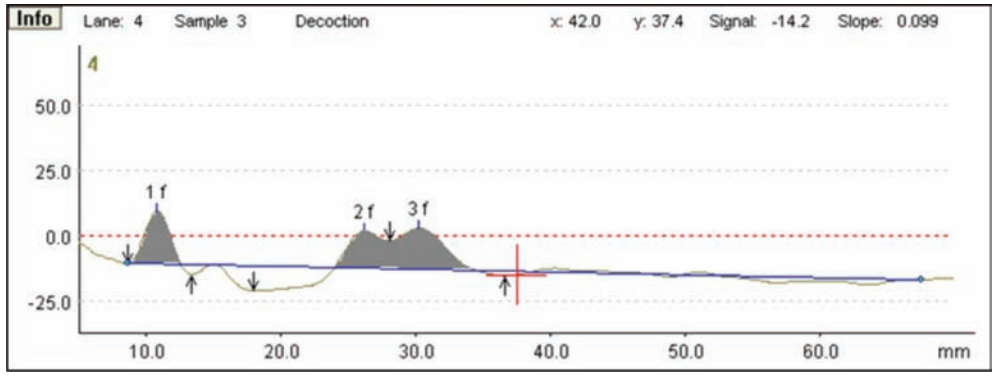


Fig. 7. Densitogram of Decoction of cocoon at 580 nm

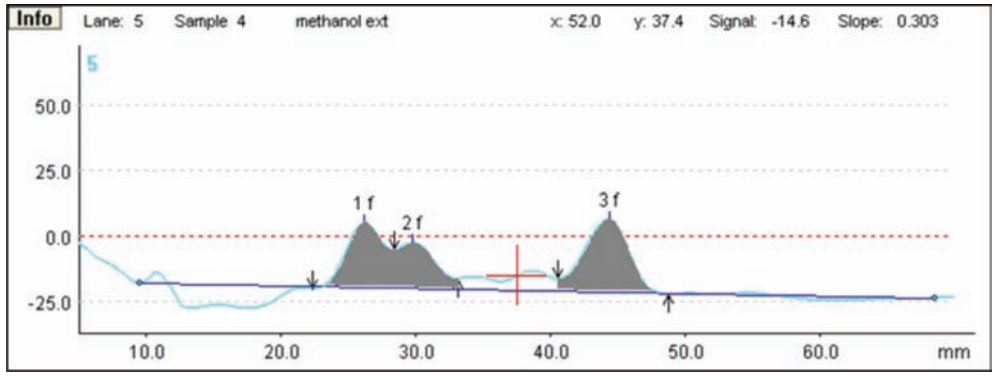


Fig. 8. Densitogram of methanolic extract of cocoon at 580 nm

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