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Contents

EDITORIAL

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

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

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

The current issue of this journal provides 13 original research and review papers in the areas of clinical research, drug standardization, vermitechnology, agronomy, pharmacology and ethnobotanical surveys contributed by eminent scholars in their respective fields. Council acknowledges the authors for their contributions included in this issue and hope for their continued support in this endeavor. We wish to ensure the readers to bring out the future issues of the journal on time.

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

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

Effect of *Hijamat Bila Shurt* in the Management of *Waja-ul-Mafasil* – A Clinical Study

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Abstract

Waja-ul-Mafasil is a broad term encompasses almost all the painful conditions of the joints including Osteoarthritis. *Hijamat* is a Unani mode of treatment, which is carried out by producing partial vacuum in the cupping glasses, placed on the body surface by mean of heat or suction, in order to evacuate morbid material or to divert the material from the deeper tissues of diseased part, or to encourage the blood flow to the site of *Hijamat.* Since, this therapy is not still scientifically validated. Therefore, after reviewing Unani literature, a clinical study on *Hijamat-bila-Shurt* in the management of *Waja-ul-Mafasil* was carried out. It the present study thirty patients of 18 to 60 years of age, who were clinically diagnosed, were enrolled in the study for 15 days from OPD/IPD of NIUM Hospital. The duration of application of cups was 30 minutes in each sitting. There was significant improvement in the subjective parameters ($p<0.05$) except in muscular weakness. Based on the results of the study, it has been concluded that daily *Hijamat-bila-Shurt* should be advised along with the drug treatment for the management of *Waza-ul-Mazasil.*

Key Words: *Waja-ul-Mafasil, Hijamat-bila-Shurt,* Osteoarthritis*, Akhlât, Tadabeer,* Cupping, Regimenal Therapy

Introduction

Waja-ul-Mafasil is the most common joint disorder with a world wide distribution. *Waja-ul-Mafasil* is a wide term which encompasses all types of painful conditions of the joint.[13] It includes inflammatory joint diseases, degenerative joint diseases, infectious joint disorders and immunological joint problems. There are so many types of *Waja-ul-Mafasil*, described by the Unani eminent physicians according to their causative factors. According to Unani System of Medicine, the diseases are due to the disproportionate distribution of *Akhlat* or Humours (*Dam* or Blood, *Balgham* or Phelgm, *Safra* or Bile, *Sauda* or Black Bile) inside the body. The most commonly found *Waja-ul-Mafasil* is *Waja-ul-Mafasil Barid* especially due to the dominance of *Balgham* (Phelgm).[5, 7] The clinical presentation of *Waja-ul-Mafasil Balghami* is very similar to Osteoarthritis. The prevalence of OA is very high in elderly people and it is detectable radiographically in 80% of patients above the age of 50 years.[3] Knee OA is a condition that is much more common in India and accounts for as much disability as by any of the other chronic condition.[2] The common risk factors for OA include being female, increasing age, obesity, family history, trauma and certain occupational exposures.[3] Usual complaints of OA are joint pain, morning stiffness, joint swelling, restriction of movements, tenderness and muscular weakness.[8] The major complications of OA are genu varus, genu valgus, Backer's Cyst etc.[12]

In Unani system of Medicine, there are 3 modes of treatments – *Ilaj-bil-Tadabeer* (regimenal therapy) and *Taghzia* (Nutrition), *Ilaj-bil-Dawa* (Pharmacotherapy), *Ilaj-*

bil-Yad (surgery).[11] *Ilaj-bil-Tadabeer* can definitely be regarded safer than the other two modes of treatments as nothing goes inside the body encountering the body viscera especially liver and kidney during metabolism and excretion. Therefore, if *Ilaj-bil-Tadabeer* is carried out under the recommended rules and regulations it will not cause adverse effects. Unani Physicians have recommended *Ilaj-bil-Tadabeer* or Regimenal therapies which include *Hijamat* or Cupping, *Kai* or Cauterization, *Irsal-e-Alaq* or Leeching, *Fasd* or Venesection etc., which were commonly practiced and were the main stays of surgical practice in ancient and medieval times.[1,10]

Hijamat is one of the *Tadabeer* which is being practiced for many disease conditions since ancient times. Hijamat is of two types, *Hijamat-bil-Shurt* (Cupping with blood letting) and *Hijamat-bila-Shurt* (Cupping without blood letting). Either one can be performed in 2 ways, Vacuum is created by using fire i.e. *Hijamat-bil-Naar* and vacuum is created without using fire i.e. *Hijamat-bila-Naar*, where suction pumps or manual suction is used to create a vacuum that helps in the adhesion of the cups.[4,6,14]

The principles for each type of *Hijamat* (Cupping) is different. *Hijamat-bil-Shurt* works on the basis of the principle of *Tanqia-e-Mawad* i.e. evacuation of morbid matter from the affected areas, whereas, *Hijamat-bila-Shurt* works according to the principle of *Imala-e-Mawad* i.e. diversion of the morbid matter from the diseased parts. This diversion may be to the near *(Imala-e-Qareeb*) or distant (*Imala-e-Baeed*) regions.[6,9]

Material and Methods

Thirty patients of 18 to 60 years of age, who were clinically diagnosed, were enrolled in the study for 15 days from OPD/IPD of NIUM Hospital. The duration of application of cups was 30 minutes in each sitting.

Methodology

The study was conducted in Regimenal Therapy Unit of NIUM Hospital, Bangalore on 30 clinically diagnosed patients. The design for the study was open single group uncontrolled clinical study. The duration of therapy was 15 days and total duration of the study was 3 months.

Criteria for selection of cases:

Inclusion Criteria

Age between 18-60 years, Either Sex, Clinically diagnosed patients of *Waja-ul-Mafâsil,* Patient agree to follow the protocol

Exclusion Criteria

Patients below the age of 18 and above the age of 60 years, pregnancy and lactation, Diabetes mellitus, Anemia (Hb% less than 10gm %), Patients having past history of bleeding disorders, Liver diseases, Renal failure, Ischaemic heart disease, Other types of arthritis (Rheumatoid arthritis, Infective arthritis, Psoriatic arthritis etc.)

Schedule of the therapy

In all patients *Hijamat-bila-Shurt* was performed. Schedule of the procedure was daily for 30 minutes in each sitting up to 15 days.

Assessment of the efficacy

The assessment of patients was done according to the subjective parameters (Joint Pain, Morning Stiffness, Joint Swelling, Restriction of Movements, Tenderness and Muscular Weakness). As the subjective parameters differ in severity from patients to patients, an arbitrary grading of 0-4 was improvised for appropriate assessment and statistical evaluation. The subjective parameters were recorded before starting the treatment and after completing the treatment.

Statistical analysis was restricted to those patients who completed the full duration of the study. Non parametric test (Man Whitney U test) was used to analyze the efficacy of the procedure. The confidence level was set to be at $p<0.05$ for significant results of the treatment.

Results and Observations

There was significant improvement in the subjective parameters ($p<0.05$) except in muscular weakness.

The findings of demographic and subjective parameters were as follows:

Table-1. Distribution of Patients according to Age

Age Group	No of Patients	Percentage
20-30	2	6.67%
30-40	3	10%
40-50	10	33.33
50-60	15	50%
Total	30	100%

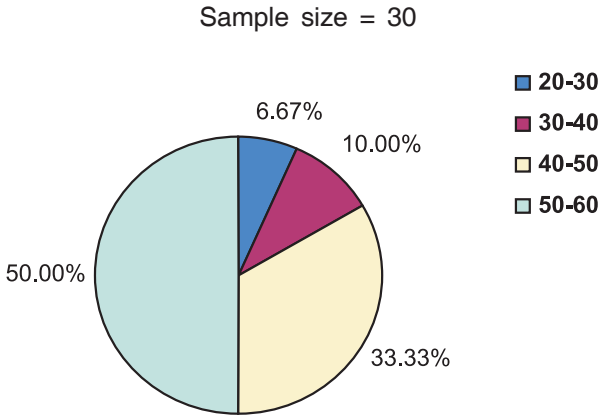

Fig. 1. Distribution of Patients according to Age

As shown in table no 1, out of the total patients, 2 (6.67%) patients were found in age group 20-30 years, 3 (10%) patients in age group 30-40years, 10 (33.33%) patients in age group 40-50 years and 15 patients in age group 50-60 years.

Table-2. Distribution of Patients according to Sex

Sex	No. of Patients	Percentage
Male	9	30%
Female	21	70%
Total	30	100%

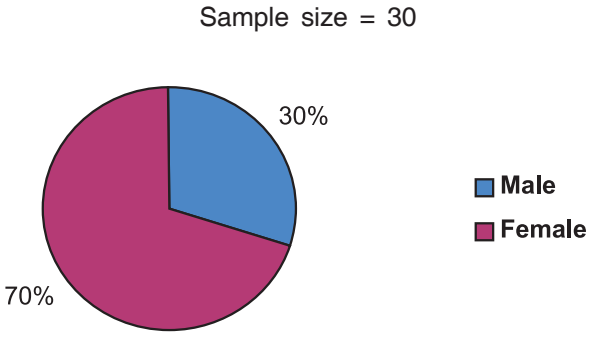

Fig. 2. Distribution of Patients according to Sex

As shown in Table-2, out of total patients, 9 (30%) patients were males and 21 (70%) patients were females.

As shown in Table-3, the mean score of the joint pain was 2.7 before starting the treatment while it was 1.4 at the end of the treatment. The improvement in joint pain at the end of the treatment was 48.15% which was statistically significant at $p < 0.05$. The mean score of the morning stiffness was 3.0 before starting the treatment

Table-3. Effect of the therapy on Subjective Parameters

Parameters	Before Treatment Mean + SE	After Treatment Mean + SE	% of Improvement	P Value
Joint Pain	2.7 + .108	1.4 +.114	48.15%	<0.05
Morning Stiffness	3 + .048	1.4 +.196	51%	<0.05
Joint Swelling	2.6 + .088	1.3 + .204	50%	<0.05
Restriction of Movements	2.63 + .102	1.27 + .151	51.9%	<0.05
Tenderness	2.5 + .124	1.2 + .172	49.6%	<0.05
Muscular Weakness	2.07 + .214	1.9 + .216	8.07%	>0.05

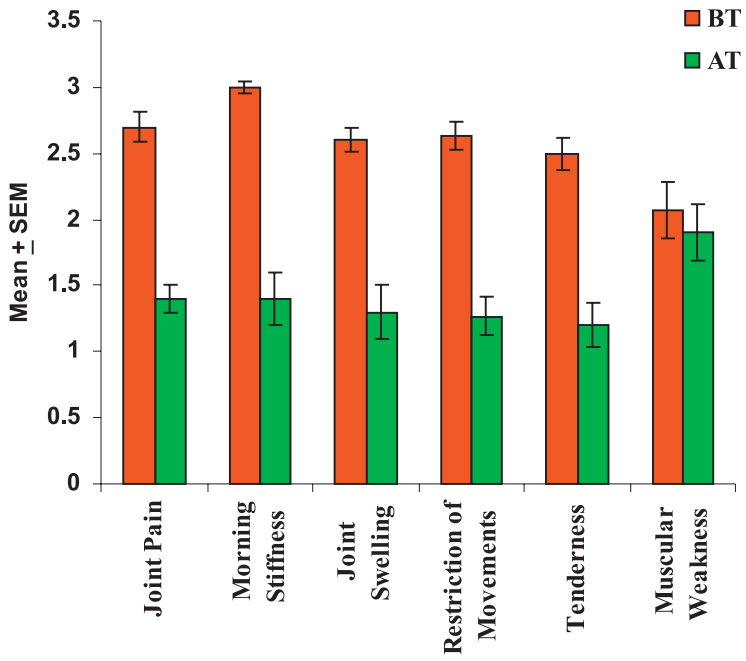

Fig. 3. Effect of the therapy on Subjective Parameters

while it was 1.4 at the end of the treatment. The improvement in morning stiffness at the end of the treatment was 51% which was statistically significant at p <0.05. The mean score of the joint swelling was 2.6 before starting the treatment while it was 1.3 at the end of the treatment. The improvement in joint swelling at the end of the treatment was 50% which was statistically significant at p <0.05. The mean score of the restriction of movements before starting the treatment was 2.63 while it was 1.27 at the end of the treatment. The improvement in restriction of movement at the end of the treatment was 51.9% which was statistically significant at p <0.05.

The mean score of the tenderness before starting the treatment was 2.5 while it was 1.2 at the end of the treatment. The improvement in tenderness at the end of the treatment was 49.6% which was statistically significant at $p < 0.05$. The mean score of the muscular weakness before starting the treatment was 2.07 while it was 1.9 at the end of the treatment which was not statistically significant.

Discussion

In the present study, the efficacy of *Hijamat-bila-Shurt* was evaluated over a period of 15 days on the basis of improvement in the subjective parameters. The improvement in joint pain, morning stiffness, joint swelling, restriction of movement, tenderness and muscular weakness was 48.15%, 51%, 50%, 51.9%, 49.6% and 8.07%, respectively.

In *Waja-ul-Mafasil* there is accumulation of morbid humours especially synovial fluid, in the joint space, exerting pressure on the capsule giving rise to the pressure symptoms. *Hijamat-bila-Shurt* diverts the morbid humours from the diseased tissues relieving the pressure symptoms.

The reason behind the morning stiffness is spasm of the synovial membrane and related tendons due to the lack of oxygen, tissue nourishment and it is the coldness which causes spasm in synovial membrane. *Hijamat-bila-Shurt* increases the blood circulation at the site of cupping, fulfilling the requirement of oxygen and other nutrients. When cups are applied at the affected site, blood circulation increases at the site and kinetic energy of blood changes into thermal energy improving the local temperature. Once the local temperature is maintained the spastic condition gets rectified and the stiffness disappears or subsides.

Local swelling and effusion is generally due to the extravasations of fluid and cells from the blood stream into the intercellular space. *Hijamat-bila-Shurt* directly affects the joint swelling by *Imala-e- Akhlat-e-Fasida* (Diversion of Morbid Humours) from the deeper tissues towards the superficial tissues by reducing the swelling.

Restriction of movement is directly related with pain and swelling. As discussed above, *Hijamat-bila-Shurt* reduces pain and swelling so ultimately reduces the restriction of the movements.

Tenderness is due to the synovitis and synovitis is due to the accumulation of some pro-inflammatory mediators in the articular space or may be due to raised intraarticular pressure. Intraarticular pressure is raised by the accumulation of *Akhlat-e-Fasida* (Morbid Humours) in the joint cavities. The relief in joint tenderness may be due to the *Imala-e-Akhlat-e-Fasida* (Diversion of Morbid Humours) from the diseased parts.

Muscular Weakness is basically due to the poor nourishment of the area and immobilization of the part due to restriction of the movement. As *Hijamat-bila-Shurt* increases the nourishment of the affected muscular area and decreases the restriction of the movement so it increases the muscular strength.

Conclusion

On the basis of the results it can be concluded that *Hijamat-bila-Shurt* is effective in the management of *Waja-ul-Mafasil*. However, larger controlled studies are needed to arrive at a conclusion.

Acknowledgement

I am grateful to the Director, National Institute of Unani Medicine for facilitating the study. I am also thankful to Dr. Mohammad Anwar for his cooperation during the study.

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Assessment of Mizaj (Temperament) in the Cases of Chronic Stable Angina (Muzmin Zubeh Sadariya) According to Unani Parameters – A Clinical Study*

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Abstract

Sixty four patients suffering from Chronic Stable Angina were examined clinically and the temperament of each patient in different age and either sex was assessed according to qualitative and quantitative state on the basis of standard Unani parameters. The clinical data was analysed statistically and showed the incidence of disease is significantly high in the cases having Balghami Mizaj (Phlegmatic temperament) i.e. 56.25% followed by Damavi Mizaj (Sanguine temperament) i.e. 39.7%. Very few cases were assessed in Safravi Mizaj (Choleretic temperament) and Saudavi mizaj (Melancholic temperament). The same has been discussed and analyzed statistically in the present paper.

Key Words: Chronic Stable Angina, Damavi, Balghami, Safravi, Mizaj (Temperament).

Introduction

The incidence of cardiovascular disease (C.V.D.) is more prominent. A WHO statistical survey shows that the cardiovascular diseases constitute the leading cause of death in many parts of the world, particularly in the more developed countries. Cardiovascular disease (C.V.D.) which are in forefront of these new threats to public health, already constitute to at least 2.5 millions lives last each year in India and many of these deaths occurs early.

The heart is the most absolute, sovereign and noblest of all vital organs. It is superior to all other organs and is the life source of the spirit. Life depends on it, because it controls and regulates all the actions and affairs of the body and in the source of life and the animal sprit which actuates and equipoises all the elements of the body and its action . Aristotle says that the heart is the first organ in the body which comes into motion and it is also the last that causes motion and stop culminating in death. (Azam Khan, 1983).

The literal meaning of Mizaj according to Burhanuddin Nafise (1934) is intermixture. He says Mizaj indicates the properties of Unsur (Atom), a molecule, a cell, a tissue, an organ and the organism as a whole. Each and every atom, molecules (murakkab), khilt (humour), cell, organ and body as a whole is furnished with a mizaj (equilibrium) upon which their properties, functions and life depends. (Kabiruddin, 1935).

Mizaj (temperament) as defined by Ibn-e-Sinna (980-1037 AD) as the new state of a matter, having quality different from that present in the elements or compound before coming into imtizaj (intermixture or chemical combinations) which results from the action and reaction among the contrary qualities and powers present in the minute particles (atoms) of different elements (or molecules of different

*Paper presented in National Seminar on Mizaj (Temperament), Organized by P.G. department of Kulliyat, A.K. Tibbiya Collge, AMU, Aligarh (30-31 October, 2000).

compounds). When they are combined together the resultant new quality, a uniform state or the state of equilibrium emerging after the combination of more than one elements is called mizaj (temperament).

The unani physicians have classified the temperament according to the dominating sign and symptoms as follows.

1. Damavi Mizaj (Sanguine temperament)

They have hot and moist temperament. They are obese and average built. They are very active and tense, and are moderately hypersexual. They have a good appetite and a full strong pulse. They have a slight feeling of heaviness in their body. Wide chest and curly hairs. They pass fiery (reddish concentrated) urine. (S.I. Ahmed,1980)[4]

2. Balghami Mizaj (Phlegmatic temperament)

They have cold and wet temperament. They are flaccid and obese with white and pasty skin. They have thin and soft hair. Their blood vessels are not prominent. Their movements and activities are sluggish. Their intelligence is dull. They do not get angry (even tempered) and overcome with drowsiness. They are sexually frigid. They have lack of thirst. They experience excessive heaviness of the body. Their digestion is sluggish. Their urine is White (Colourless).

3. Safravi Mizaj (Choleretic or bilious)

They have hot and dry temperament. They get angry quickly. They have a sallow complexion, and lean hairy body. They are energetic and intelligent with a strong inclination to indulge in sexual pleasures. They are fastidious about food and have a strong rapid pulse. Blood vessels are prominent. In some cases sensation of pains and pricks is felt by these individuals over their body. They pass fiery and yellow urine.

4. Saudavi Mizaj (Melancholic temperament)

Their temperament is cold and dry. They are thin and dark. Their blood vessels are narrow and pulse slow and hard (sulb) they show sluggish inclinations towards sexual activity and suffer from insomnia their urine may be black, reddish black or of greenish tinge.

Method of Diagnosis of Mizaj by Ten Principles

Besides diagnosing normal or abnormal temperaments (su-al-mizaj) by chemical analysis of different Akhlath (humors) of the body the physicians have devised

some other ways and means to find out mizaj. There are ten parameters upon which a person is tested. There the sign and symptoms by which the temperament is diagnosed is classified into the following ten division. (Ahmed, 1980).

1.	Malmus	(Tactile sensations)
2.	Lahm-wo-shahm	(Muscles and fats)
3.	Ashar	(Hairs of the body)
4.	Laun	(Colour of the body)
5.	Hayat-al-aza	(Stature)
6.	Kafiat-al-infial	(Quality of passiveness of the organs)
7.	Naum-wo-yaqzah	(Sleep and wakefulness)
8.	Afal-al-aza	(Bodily functions)
9.	Fdhlat-al-badan	(Excreta of the body)
10.	Infialat nafsaniyah	(Psychic reaction)

Material and Methods

Total sixty-four cases of chronic stable angina (Muzmin Zubeh Sadariya) were registered for clinical trial in the age group of 30-70 years in either sex. During the year from 1996 to 2000 in the Department of Cardiology (Amraz-e-Qalb) CRIUM, Hyderabad. These cases are diagnosis clinically on the basis of presenting clinical sign and symptoms. Further the diagnosis is confirmed by cardiac stress test (TMT). Beside this all selected cases for study referred to Akhlath Unit, CRIUM, Hyderabad for temperament assessment. The assessment of temperament was done on the basis of fixed unani parameters by the Physicians of Akhlath Unit and reported on a prescribed proforma and recorded on a case sheet.

Observations and Discussion

Total 64 cases of Chronic Stable Angina (Muzmin Zubeh Sadariya) were registered for clinical study in the age group of 30-70 years in either sex. Out of 64 cases assessed for temperament of which 57 (89.07%) were males and 7 (10.93%) were females. All the cases were analyzed statistically according to age and temperament. It is found that the incidence of chronic stable angina significantly high in patient having Balghami mizaj (Phlegmatic temperament) i.e. 36 (56.26%) followed by 25 (29.06%) Damavi mizaj (Sanguine temperament) and the remaining 3 (4.86%) patients were belongs to Safravi Mizaj (Choleretic temperament) Table-1. Similarly the incidence of disease is found high in the age group of 41-60 years in the patients having Balghami mizaj and Damavi Mizaj i.e. 52(81.2%) Table-2. In all 64 patients registered for study systolic blood pressure and pulse rate was recorded on admission and analyzed statistically. It was found that the mean systolic B.P. is 140.8 ± 22.34 and the mean pulse rate is 80.52 ± 4.65 in 25 patients of Damavi Mizaj (Sanguine temperament). And mean systolic B.P. is 139.16 ± 20.47 and the mean pulse rate is 76.22 ± 6.58 in 36 patients of Balghami Mizaj (Phlegmatic

Table-1. Classification of Angina cases according to temperament.

S.No.	Temperament	No.of cases	%
1	Damavi	25	39.06
2	Balghami	36	56.26
3	Safravi	03	04.68
4	Saudavi	—	—
	Total	64	100.0

Table-2. Classification of Angina cases according to temperament in different age groups

S. No.	Age Groups (Years)	Damavi	Balgami	Safravi	Saudavi	Total cases	%
1	30-40	01	01	02	—	04	6.3
2	41-50	16	10	—	—	26	40.6
3	51-60	07	18	01	—	26	40.6
4	61-70	01	07	—	—	08	12.5
5	71 & Above	—	—	—	—	—	—
	Total	25	36	03	—	64	100.0

temperament). And the mean systolic B.P. is 130.0 ± 17.32 and the mean pulse rate is 81.00 ± 3.60 in 3 patients of Safravi Mizaj (Choleretic temperament) Table-3. It is quite clear with the above Table-3 that the systolic B.P. and pulse rate in patients of damavi mizaj, balghami mizaj were found in normal range. Pulse rate is slight increased in safravi mizaj.

Similarly the character of pulse was also assessed in the patients of different temperament. Out of 25 Damavi Mizaj patients in 23 patients the pulse was found strong, regular, large and soft. Whereas, in 2 patients the pulse was slow, deep small and soft. Out of 36 balghami mizaj patients of which 35 patients, the pulse was found slow, deep, small and soft and in 1 patient the pulse was found strong, regular, large and soft. The remaining 3 patients of safravi mizaj the pulse was found superficial, rapid and equal. Table-4. It is remarkable to note that the above mentioned pulse findings is cent percent matching with the findings of Avicenna 980-1037 AD as described in Al-Qanoon Fit Tibb in the chapter of temperament and pulse about the character of pulse in different temperament.

In 64 patients of chronic stable angina of different temperament the Quwa (power) and Afaal (functions) of different symptoms of the body was also studied. Out of 25

Table-3. Showing Mean ± SD of Systolic Blood pressure and pulse rate in Angina cases of different temperament.

S. No.	Temperament	No.of cases	± S.D. Mean Systolic Blood Pressure	± S.D. Mean Pulse Rate
1	Damavi	25	140.8 ± 22.34	80.52 ± 4.65
2	Balghami	36	139.16 ± 20.47	76.22 ± 6.65
3	Safravi	03	130 ± 17.32	81.00 ± 3.60
4	Saudavi	—	—	—
	Total	64	—	—

Table-4. Character of pulse of angina cases in different temperament assessed.

S.No.	Temperament	Damavi	Balgami	Safravi	Saudavi
1	Strong, regular large and soft	23*	01	—	—
2	Slow, deep, small & soft	02	35*	—	—
3	Superficial, rapid and equal	—	—	03	—
4	Sulb	—	—	—	—
	Total	25	36	03	—

*Damvi: 23 cases Statistically Highly Significant

** Balgami: 35 cases Statistically Highly significant

damavi mizaj patient as the Quwa and Afaal of digestive system was found average in 23 patients and week in 2 patients. Nervous system was found strong in 20 patient and weak in one patient. Reproductive system was found strong in 24 patients and week in one patient.

Out of 36 Balghami mizaj (Phlegmatic temperament) patients the digestive system was found strong in 10 patients and weak in 26 patients. Nervous system was found strong in 12 patients and weak in 24 patients. Respiratory system was found strong in 10 patients and weak in 26 patients. Reproductive system was found strong nil and found in average in 14 patients and weak in 22 patients.

Out of 3 safravi mizaj (choleretic temperament) digestive system was found strong in 2 patients and weak in one patient. Nervous system was found strong in 2 patients and weak in one patient. Respiratory system was found strong in 2 patients and weak in one patient. Reproductive system was found strong in 2 patients and weak in one patient (Table-5). It is also confirmed with the theory of Avicenna.

Table-5. Showing power (quwa) and functions (Af-aal) of different systems of angina cases in different temperament.

S.No.	Different Systems	No. of Cases (25)			No. of Cases (36)			No. of Cases (03)			No.o f Cases		
		Damavi			Balghami			Safravi			Saudavi		
		Strong	Average	Weak	Strong	Average	Weak	Strong	Average	Weak	Strong	Average	Weak
1.	Digestive System	23	—	02	10	—	26	02	—	01	—	—	—
2.	Nervous System	20	—	05	12	—	24	02	—	01	—	—	—
3.	Respiratory System	24	—	01	10	—	26	02	—	01	—	—	—
4.	Reproductive System	—	24	01	00	14	22	01	—	01	—	—	—

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Medicinal Importance of Narjeel Daryaee (*Lodoicea maldivica* (Poir.) Pers.) in Unani System of Medicine: A Review

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Abstract

Lodoicea maldivica is a plant of Arecaceae (Palmae) family and found growing in the Seychelles, but its fruits are obtainable on the Mumbai side. Fruit or nuts are of great size, frequently 40-50 1bs, in weight. The Kernel is used medicinally in the Unani system of Medicine as, general tonic, anti-diarrhoeal, antidote of opium, cardiac tonic. It's also used in brain diseases, paralysis, facial palsy and arthralgia since long time in Unani Medicine.

Key Words: Sea Coconut; Seychelles; Narjil-behri; Narjil-i-daryai

Introduction

Lodoicea maldivica is a plant of Arecaceae (Palmae) family and found growing in the Seychelles, but its fruits are obtainable on the Mumbai side. Fruit or nuts are of great size, frequently 40-50 1bs, in weight. They were formerly cast ashore on the west cost of the India and Ceylon from the Indian Ocean. They are now imported and used to some extent by the natives of the Northwestern India as food and medicine being regarded as preservatives and alexipharmic (Nadkarni, 1976).

Prior to the discovery of the Seychelles islands in 1743, the large and peculiar shaped nut of this palm, found floating in the Indian Ocean, was an object of curiosity which gave rise to many fabulous tales; it was called Sea Cocoanut and Coco-de-mer by Europeans Narjil-behri by the Arabs, Narjil-i-daryai by the Persians, and important medicinal virtues were attributed to it is not no longer valued by Europeans, but still in great repute among the Arabs and Indians as a tonic preservatives and alexipharmic (Dymock *et al*, 1890).

Peaces of Narjeel Daryaee *(Lodoicea maldivica)* (Poir.) Pers

Synonyms

English : Sea coconut.
Hindi : Darya-ka–naryal;
Ababic : Narjil bahri.
Persian : Narjil-i-daryai.
Urdu : Narjeel Daryaee;

Habitat and Distribution

Native of Seychelles Islands. It is grown on all type of soils. Thrives best in deep gorges with plant debris (Anonymous, 1992; Dymock *et al*., 1890; Kirtikar and Basu, 1987).

Botanical Descriptions

Thomas Moore, in the treasury of botany, says: "this magnificent palm, which is found only in two small islands, Praslin and Curieuse belonging to the Seyehelles group, requires a great length of time to arrive at maturity. The shortest period before it puts forth its flower buds are thirty years, and a hundred years elapse before it attains its full growth. From the age of 15 to 25 year it is in its greatest beauty, the leaves at this period being much longer than they are subsequently. The stem grows quite upright, straight as an iron pillars, and in the male trees frequently attain a hundred feet in height, the female being shorter at the age of thirty, it first puts forth its blossoms, the males forming enormous catkins about three feet in length and three inches in diameter, while the female are set upon a zigzag stalk, from which hang four or five, or some time as many as eleven nuts, averaging about 40 1bs. weight each. From the time of flowering to the maturation of the fruit, a period of nearly ten years passes, the full size, however, being attained in about four years at which time it is soft and full of a semi transparent jelly like substance.

The base of the stem as rounded and fits into a natural bowl or socket, which is pierced with hundreds of small holes about the size of a thimble, with hollow tubes corresponding on the out side through which the root penetrates the ground on all sides, however becoming attached to the bowl their partial elasticity affording an almost imperceptible but very necessary 'play' to the parent stem when struggling against the force of violent gales. This bowl is of the same substance as the shell of the nut, only much thicker; it rots very slowly, for it has been found quit perfect and entire in every respect sixty years after the tree has been cut down. (Dymock *et al,* 1890).

The fruits are covered externally with a thick fibrous husk and contain usually one, but sometime two or even three immense nuts with hard thick black shells, each being divided half way down into two lobes. The karnel is three quarter to one inch

thick and very hard and white having much the consistence of vegetable ivory; it has no odour or taste; when soaked in water it soften a little, and can be split into thin fibrous bundles (Dymock *et al*, 1890).

Trunk 18-30 m. high, straight, apparently destitute of bark, annulate, about 30-cm. diam., with scarcely any difference in size to the very top. Leaves 12-20, large, 2.4-3 m. long, 1.5-1.8m. Broad (sometimes up to 6 m. long and 3.6 m. broad), the youngest rising from the center, at first folded like a shut fan, and them clothed with a downy substances, later on broadly ovate with a central rib and regular folds diverging from it; margins more or less deeply cut, especially at the extremity; the colour bright yellow green; texture thin and dry. Spathes sheathing at the base of the spadices, small. Male and female flowers on different trees. Male spadix from the axils of the leaves, amentaceous, 60-120-cm. long 7.5-10 cm. diam. In the thickest part, cylindrical, tapering towards the apex closely covered on all sides with densely imbricated, semicircular, slightly convex scales. (Warrier *et al*., 1994).

The flowers in subreniform clusters in hollows of the axis imbricated in two rows. Sepals and petals oblong, yellowish–brown; the sepals rather larger and more angular than the inner. Filaments united at the base into one body; anthers linear, 2-celled, opening longitudinally, each cell terminating in two globular heads. Female spadix rising from the axils of the leaves, pendent, 60-120 cm. long, thick and woolly, tortuose, clothed with large sheathing, red-brown spath, which are singularly fimbriated, or more generally erose at the margin, and support several, more or less distantly placed, female flowers of different ages, at the same time, and of various size s. Sepals and petals almost semispherical and 2.5 cm. thick at the base; ovary almost concealed by the perianth, broadly ovate, narrow at the base above the insertion of the perianth. Fruit usually 1-seeded mostly 2- lobed (Warrier *et al*., 1994).

Macroscopic

The crude drug occurs in pieces of varying sizes 5-8 cm long, 2-2.5 cm broad and 2-5 cm thick, very hard and heavy. The outer surface of the kernel (endosperm) is moderately rough to smooth, dirty brown in colour, about 1mm in thickness, the inner side is rough, chocolate brown in colour, about 1mm in thickness, the inside is rough, dirty brown in colour with large number of tooth like projections. The cut end is shiny or glistening. The drug is odorless. Fracture vary hard, difficult to break but brittle, tasteless (Anonymous, 1992).

Microscopic

A transverse section of a portion of the kernel has shown an outer surface consisting of lignified cells, which are oval to polygonal in shape and 3-4 layers in thickness. These cells give positive test for oils and tannins. The walls of these lignified cells are thick and narrow. Simple to bordered pit canals connects these cells to each

other. Just bellow this layer there is another layer of 2-3 cells in thickness. These cells are thick–walled, somewhat oval to round in shape. The endosperm cells are spindle shaped having a central cavity with club shaped canal extending to the cells walls, where they are apposed to similar canal belonging to there neighbor cells. Occasionally oval to round simple starch grains are observed in the endosperm cells (Anonymous, 1992).

Phytochemistry

Chemical constituents

Organic: Glycosides, reducing sugars, steroids/triterpenes, and tannins. Inorganic: Sodium, potassium, calcium, iron, phosphate, and chloride (Anonymous, 1992).

Identity, Purity, Strength, and Assay: (Anonymous, 1992).

Foreign organic matter	Nil
Purity	100%
Identity	
Physico-chemical constant (%)	
Total ash	1.65
Acid insoluble ash	1.96 (w/w of ash)
Water soluble ash	79.27 (w/w of ash)
Loss of weight on drying at 110o C	1.70

Thin layer chromatography of Narjeel Daryaee

Extract	Solvent/ system	Spray/Treatment	No. of spot(s)	Rf. Value(s)
Petroleum ether	Petroleum ether	Orthophosphoric acid: water 1:1 heated to 115ºC for 15 minutes	5	0.05, 0.08, 0.15, 0.38, 0.78
Chloroform	Toluene: Ethyl format : formic acid 5:4:1	Observed under ultraviolet light exposed to iodine vapour	1 2	0.08 0.08, 0.22

(Anonymous, 1992)

Successive extractive values (%)

Petroleum ether (60-80)	6.58
Chloroform	0.44

Acetone	3.32
Ethanol	2.96
Distilled water	17.03
Quantitative estimation (%): Phenolics	0.22

(Anonymous, 1992).

Taste: Taste of the fresh fruit is sweetish and on ageing becomes bitter. (Anonymous, 1992).

Parts Used: The Kernel is used medicinally (Anonymous, 1992; Nadkarni, 1976).

Mizaj (Constitution)

Murakkab-ul-Quwa (compound constitution) (Ghani, 1913; Kabiruddin, 1937; Hussain, 1875). According to some Physicians, Hot^2 Wet^2 (Ghani, 1926; Khan, 1895). According to some, Hot Dry (Ghani, 1926; Kabiruddin, 1937).

Actions

General Tonic (Anonymous, 1992; Nadkarni, 1976; Dymock *et al*., 1890; Warrier *et al.,* 1994). It enhances hararate ghareezi (Innate heat or energy), antidote to poison (Kabiruddin, 1937; Khan, 1895). It protects body faculties, removes waste and toxic humours (Ghani, 1926; Hussain, 1875). It removes the effects of poisons from the deep tissues and also protects body faculties (Khan, 1895).

Therapeutic Uses

It is useful in cholera, hyperdipsia, odema, acute diarrhoea, colic and also as an antidote in opium and aconite poisoning. It is good cardio tonic (Kirtikar and Basu, 1987; Warrier *et al* 1994; Khan, 1895). It is useful in brain diseases, poisoning, paralysis, facial palsy, arthralgia (Ghani, 1926; Kabiruddin, 1937)

Side effects

It is harmful in hot ailments and also for the persons of hot constitution (Kabiruddin, 1937).

Muslehat (Correctives)

Arq gulab (Rose water), milk and black piper (Piper nigrum) (Kabiruddin, 1937).

Doses

750 - 1gm (Kabiruddin, 1937; Ghani, 1926), 0.5 - 1.0 gm. (Anonymous, 1992).

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Ethnomedicines and Vegetational Pattern of Atmakur Forest Division, Andhra Pradesh: Strategy for Conserving Biodiversity of Medicinal Plants*

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Abstract

Atmakur forest division is a rich source of medicinal plants diversity which is considerably declining in recent decades due to expansion of agriculture, rapid industrialization and urban development, over grazing, soil erosions, frequent incidence of forest fires and also due to export of medicinal plants. There is a heavy extraction of medicinal herbs from the dense forests which contributes for deterioration of vital habitats of medicinal plants. Hence most of the medicinal plants have become scarce and rare e.g. *Gloriosa superba* Linn. *Helicteres isora* Linn. *Bauhinia vahli* Wt. & Arn., *Smilax china* Linn. *Celastrus paniculatus* Willd. and *Holarrhena antidysenterica* (Buch-Ham) Wall ex G. Don. In the prevailing situation some measures are suggested for conserving biodiversity of medicinal plants.

The paper also presents some 16 contemporary folk recipes comprising 25 taxa of folk medicinal plants used by various tribal communities e.g *Chenchus, Erukas, Koyas* and *Telaga* agriculturists etc., for the treatment of various common ailments. Mode of administration is given for each recipe discussed. The study is likely to contribute material for discovery of new drugs of natural origin for many of the diseases and conditions, thus far incurable in modern medicine.

Key Words: Ethnopharmacological surveys, Tribal medicine, Atmakur forest division, Conservation & Biodiversity.

Introduction

Atmakur forest division is blessed with a rich biodiversity and a treasure of medicinal plants. The demand for the medicinal plants has been increasing year after year. A number of minor forest produce were not fully harvested for lack of funds in the past. At the same time it has been observed that many of the important plant species are fast disappearing due to various biotic factors such as fires, grazing illicit removal of medicinal plants and so on. The area has not been investigated exhaustively earlier in this direction except for some sporadic reports on medicinal uses of plants from the state. (Rao *et al.,* 1995; Chetty & Rao, 1989; Gupta *et al.,* 1997, 2005, 2007, 2008, Reddy *et al.,* 1989; Suryanarayana, 1996; Vedavathy & Rao, 1995; Kapoor & Kapoor, 1973; Khan, 1953; Pullaiah & Yasoda, 1989). Therefore, there is an urgent need of combined efforts to protect & conserve the rich biological diversity of the area.

Based on this rationale, an ethnopharmacological survey of Atmakur forest division of Kurnool district of Andhra Pradesh was undertaken in August 2008 and first-hand information on folk medicinal uses of plants for treatment of various diseases and conditions were recorded. The area from which data were derived is situated in

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North latitude between 15°30' to 16°15' and between East longitude of 78°15' and 79°. The area explored included 4 forest ranges namely (i) Atmakur (ii) Bairluti (iii) Srisalam (iv) Velugode. The important forest areas visited during field studies include Sivapuram, Nagluti, Bairluti, Rolapenta, Pasurutla, Velgode, GBM, Srisalam and Sunipenta.The study presents 16 folk medicinal species used by the tribals and other ethnic groups for various ailments from areas surveyed. Beides, vegetational pattern of Atmakur forest divison and development strategy for conservation and cultivation of high-demand priortized medicinal species has also been discussed.

Methodology

A systematic study for the identification, quantification, availability, conservation and cultivation of medicinal plants has been made. An ethno-botanical survey of Atmakur forests of Kurnool district was conducted during August 2008 with a view to study the medicinal herbs of the area and also to record the folk-wisdom of tribals known as Erukas, Chenchus, Koyas and Telaga agriculturists who have settled in the river side villages. The data on folk medicinal uses of plants were collected from the herbalists (medicine men) through their direct field interviews, who accompanied the survey team to the field or the tribals who have long been prescribing the folk-medicines for treatment of various diseases. Information about relative efficacy of the herbs was also recorded. Plant specimens of all folk drugs were collected, identified and voucher herbarium specimens have been deposited in the herbarium of Survey of Medicinal plants Unit, Central Research Institute of Unani Medicine, Hyderabad, for future reference and study.

Forests and Vegetation

The Andhra Pradesh Forests are next only to Madhya Pradesh, Orissa and Maharashtra. Bulks of the Andhra Pradesh forests are tropical dry deciduous with Teak as major species in Telangana region. There are pockets of moist deciduous forests in valleys in Vizag and Atmakur forest division. East and West Godavari districts covered by the north east monsoon winds. Mangrove forests occur at the mouth of the River Krishna and Godavari. Red Sandal occurs in the forests of Cuddapah and Chittoor districts and to a small extent in the district of Hyderabad and Tirupathi. Bamboo occurs in the forests of Atmakur, Khammam, Mehboobnagar, Kurnool, East and West Godavari, Vizag and Srikakulam districts.

The natural forest wealth is continuously depleting due to developmental programmes of irrigation projects, fuel wood consumption and increasing human and biotic interferences, so much so that some species of medicinal plants are at the verge of extinction. This has resulted not only in desertification but also loss of genetic diversity in biological resources. Seriousness of these problems has to be, therefore, considered in order to evolve proper strategy for the cultivation and availability of the regular supply of medicinal plants.

Survey team of this Institute initiated germplasm collection of 50 important medicinal plants from the different agro-climatic regions of Atmakur Forest division of Andhra Pradesh forest and the results of evaluation, multiplication and conservation of genetically important medicinal plants are encouraging.

Some of the medicinal plants used in Unani system of medicine need conservation and cultivation are; Adoosa (*Adhatoda vasica Nees)*; Gheekawar (*Aloe barbadensis* Mill*.);* Satawar (*Asparagus racemosus* Willd.); Sanna (*Cassia angustifolia* Vahl.); Guggul (*Commiphora mukul* (Hook. ex Stocks) Engl.); Amla (*Emblica officinalis* Gaertn.); Baobrang (*Embelia ribes* Burm. f.); Ushba-Hindi (*Hemidesmus indicus* R. Br.); Shitraj (*Plumbago zeylanica* Linn.) and Nirmali (*Strychnos potatorum* Linn. f.).

Enumeration of folk medicinal species

Adverting shortly to the scheme of text, the medicinal plants used as folk medicine in the study area are arranged in alphabetical order. Each entry gives the information in sequence; Plants scientific name with family (in parentheses), Field book no., local name(s); Unani name (wherever available); part(s) used, disease, condition and method of usage.

Achyranthes aspera Linn. (Amaranthaceae); CRI 8543; Uttareni; Chirchita; Seeds; Piles: five gm of fresh seeds of Uttareni are to be grinded with 100 ml of water and the paste is taken orally with rice water twice a day for a period of 2-3 weeks.

Acorus calamus Linn. (Araceae); CRI 8556; Vaja; Bach; Rhizome; Stomach ache: rhizome of Vaja and other two ingradients-voma and sonth are to be grinded to powder and 2 gm of the powder is taken orally 3 times a day for 2-3 days.

Albizzia lebbeck Benth. (Mimosaceae); CRI 8518; Dirisena; Siras; Stem bark; for longer healthy life: the bark of the plant is collected freshly and dried in shade and powdered, 5-6 gm of the powder is taken orally twice a day for 15-20 days.

Aloe barbadensis Mill. (Liliaceae); CRI 8599; Chinnakalabanda; Gheekawar; Leaves; Joints pain: the leaf is heated on gentle fire and the pulp is obtained by removing the epidermis. This pulp is to be tied as a plaster on the knee/affected parts.

Amaranthus spinosus Linn. (Amaranthaceae); CRI 8592; Mullatotakura; Chaulai khardaar; Leaves; Lactation problem; the leaves are cooked with pulse and eaten as vegetable.

Andrographis paniculata (Burm.f.) Wall. ex Nees (Acanthaceae); CRI 8550; Nelavemu; Kalmegh; whole plant; Snake bite: Nelavemu and bark of kuchla are grinded with sufficient quantity of water and 2 tea spoonsful of the juice is given orally to the victim alongwith 50 ml of rice water 2-3 times to cure the patient.

Bambusa arundinacea Retz. (Poaceae); CRI 8585; Bonguveduru; Bans; Tabasheer; Asthma: Tabasheer is used in the treatment of asthma.

Butea monosperma (Lam.) Taub. (Papilionaceae); CRI 8570; Moduga; Palas/ Tesu; Leaves; Eczema: leaves are to be shade dried and powdered and mixed with water to get a thick paste. Equal weight of lemon juice is added and applied as a plaster on the affected part of eczema.

Caesalpinia crista Linn. (Caesalpiniaceae); CRI 8576; Gachcha-kaya; Gajga; seeds; painful menstruation; 10 gm seeds grinded with 10 gm of sugar and made into pills (6 Nos.). One pill is to be taken orally twice a day for 3 days with water for painful menstruation.

Calotropis gigantea (L.) R.Br. (Asclepiadaceae); CRI 8538 ; Jilledu; Madar; Latex; Warts; latex is applied on affected part.

Chloroxylon swietenia DC. (Rutaceae); CRI 8530; Billydu; Root & Stem bark; Rheumatism: Root and stem bark powder with black pepper is useful for rheumatism.

Citrus aurantifolia (Christm.) Swingle.; (Rutaceae) CRI 8535; Imam; Nimbu; Fruit; Earache: lemon juice and juice of Aare wood in equel quantity is mixed and dropped in the ear.

Commiphora mukul (Hook ex stocks) Engl. (Burseraceae); CRI 8574; Guggul; Guqgul; Gum; leucorrhoea: 10 gm of the gum powder is boiled with water for 7 times and dried and added 20 gm of misri is and made into powder which is to be taken twice daily for leucorrhoea.

Crotalaria juncea Linn. (Papilionaceae); CRI 8532, Janumu; Sunn; Seeds; Burns; equal proportion of seeds are to be grinded with buffalo butter and applied to the affected part.

Curcuma domestica Valeton (Zingibraceae); CRI. 8507,; Pasupu; Haldi; Rhizome; Jaundice: Pasupu and lime, both the ingredients of equal weight are mixed and made into pills of pea size and given orally 5 times with an intervals of 12 hours.

Datura innoxia Mill. (Solanaceae); CRI 8502; Nellaummetta; Kaladhatura; Seeds; Chronic fever: seeds of Nallaummetta are soaked in lemon juice for 24 hours and dried. 10 gm of these seeds, 10 gm of black pepper and 5 gm of Raskapoor are powdered and made into pills of the size of ghungchi seeds. One pill is taken orally with water twice a day for 3-4 days.

Results and Discussion

Due to illicit cuttings, indiscriminate collections and number of other biotic interferences, the herbal wealth is diminishing at a fast rate even in Andhra Pradesh. About 25 plants which require conservation and cultivation to maintain the herbal treasure of Andhra Pradesh are listed in the present paper. In view of conservation and multiplication of selected medicinal plants, mass and clonal selections were adopted for the improvement of medicinal plant species at CRIUM, Hyderabad. The suitable location for cultivation for each plant has been given in Table-1.

Table-1. Important medicinal plants of Atmakur forest division suggested for conservation and cultivation.

S. No.	Botanical Name	Common Name	Habital
1.	*Plumeria alba* Linn.	Champa	Road sides and herbal gardens.
2.	*Semecarpus anacardium* Linn. f.	Bhilawa	In the deciduous forests of Narsapur
3.	*Solanum nigrum* Linn.	Mako	In beds of herbal garden and farms
4.	*Strychnos nux-vomica* Linn.	Kuchla	In the forest of Tirupati and Vikharabad.
5.	*Strychnos potatorum* Linn. f.	Nirmali	In the forests of Narsapur and Adilabad.
6.	*Terminalia arjuna* W. & A.	Arjuna	Sides of the roads and deciduous forests.
7.	*Terminalia belerica* Roxb.	Bahra	South A.P. Forests, gardens and road sides.
8.	*Withania somnifera* Dunal	Asgand desi	In herbal farms and Road sides.
9.	*Adhatoda vasica* Nees	Adosa	In waste lands
10.	*Aloe barbadensis* Mill.	Gheekawar	In waste lands
11.	*Aristolochia indica L.*	Tella ishvari	Shady places & herbal farms
12.	*Asparagus racemosus* Willd.	Satawar	In deciduous forests of A.P.
13.	*Bauhinia variegata* Linn.	Kachnar	Road sides of A.P.
14.	*Caesalpinia crista* L.	Gajaga	Hedges on the boundry of herbal farms
15.	*Cassia angustifolia* Vahl.	Sanna	Can be cultivated in herbal farms & Fields.
16.	*Celastrus paniculatus* Willd.	Malkangni	In all deciduous forests.
17.	*Centella asiatica* (L.) Urban	Brahmi	In farms and fields in moist places.
18.	*Clerodendrum serratum* (L.) Moon.	Bharangi	As a hedge in the herbal gardens.
19.	*Commiphora mukul* (Hook. ex Stocks) Engl.	Guggul	In deciduous forests of A.P.
20.	*Emblica officinalis* Gaertn.	Amla	Forests, gardens and road sides
21.	*Embelia ribes* Burm. f.	Baobarang	Forests of Narsapur and preferably in shady places.
22.	*Gloriosa superba* Linn.	Kalihari	In herbal gardens
23.	*Hemidesmus indicus* R.Br.	Ushba-desi	As a climber in herbal garden.
24.	*Operculina turpethum* (Linn.) Silva Manso	Dudh-Kalmi	Along hedges
25.	*Plumbago zeylanica* Linn.	Shitraj	In herbal farms.

The area of cultivation can be decided as per the market demand and availability of land.

In addition to above, a few other plants, which do not grow as wild in Andhra Pradesh forests but can be cultivated in herbal gardens, are *Rauwolfia serpentina* Benth. ex Kurz. (Asrol*); Tylophora indica* (Burm.f). Merill. (Anantamul); *Andrographis paniculata* Nees (Kalmegh); *Saraca indica* Linn. (Ashoka); *Piper longum* Linn (Piplamul) etc. Further certain forest species, which are not much used in medicines, but are rarely available may also be conserved and cultivated at least to maintain the association with naturally growing indigenous forest species. These are; *Buchanania lanzan* Spreng., *Aglaia elaeagnoidea* Benth., *Hardwickia piñnata* Roxb., *Albizzia amara* Boivin, *Chloroxylon swietenia* D.C, *Cassia auriculata* Linn., *Erythroxylum monogynum* Roxb., *Anogeissus latifolia* Wall., and *Peltophorum pterocarpum* Backer etc.

Joshi (1986) stressed the need for conservation and cultivation of medicinal plants in Gujarat. He mentioned that due to illicit cuttings, indiscriminate collections and number of other biotic interference, the herbal wealth is diminishing at a fast rate. Dave (1986) also expressed that there is a scope for cultivation of more species of medicinal plants in the forests on large scale namely, Khari Babul, Neem, Siras, Sheesham, Amla, Amaltas, Harda, Imli, Anar, Nariyal, Ashoka, Champa, Amrita etc.

Most of the medicinal species have been transplanted in the herbal garden of CRIUM, Hyderabad from their natural habitat of Atmakur forests. Efforts have been made to provide these species identical soil and climatic conditions including manure and fertilizer doses of NPK etc. A complete phenological data was also recorded for these species. In this direction the need for conservation and cultivation of medicinal species has already been stressed by previous workers (Anonymous, 1959, 1981; Chopra *et al*., 1956; Gamble, 1980; Gupta *et al*., 1997, 2005, 2007, 2008; Hooker, 1897; Khan, 1953; Kulkarni, *et al*., 1997; Sankara, 1997; Shailendra, 1986; Subba Rao & Aruna Kumari, 1997; Pullaiah & Yasoda, 1989; Hemadri, 1981, 1991, 1992).

For the success of this programme a combined approach of Ethnobotanists, Forest officers, Agriculturists and Pharmaceutical personnel is very essential.

In addition to systematic study for the identification and quantification of medicinal species, the step for their availability, conservation and cultivation have also been initiated at CRIUM, Hyderabad. Further, there is also a need to take up regeneration of important medicinal plants in the forests, wastelands etc. and a systematic coordinated approach by the State Government and private agencies in this direction is necessary with following consideration:

- Setting up of research-cum-production centers for the medicinal plants in forests, wastelands, farms and herbal gardens for conserving the rare medicinal plants.
- Training of personnel for proper identification and collection.

- Creation of infrastructure for storage, transport etc.
- Development of the market for such products or forest produces.

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We are grateful to the Director, Central Council for Research in Unani Medicine, New Delhi for encouragement and providing facilities for carrying out this study. Thanks are also due to Divisional Forest Officers of Atmakur forest division, Narsapur, Vikharbad, Adilabad and Hyderabad forest divisions respectively for their help rendered during the field-explorations.

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Pharmacognostic and Preliminary Phytochemical Studies on the Leaf of *Ruta graveolens* Linn.

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Abstract

The present communication attempts to investigate the pharmacognostical and preliminary phytochemical studies on leaves of *Ruta graveolens* Linn., Rutaceae. Different parts of this plant are being used in curing various diseases. Pharmacognostical, preliminary phytochemical and TLC studies of leaf have been described. Leaf extracts give positive test for the presence of steroids, alkaloids, coumarins, quinine, flavones, triterpenoids, amino acids, phenols, tannins and sugars. The studies carried out on pharmacognostic, preliminary phytochemical analysis and TLC reveals specific identities for the particular crude drug which will be useful in standardization of the raw drug.

Key Words: *Ruta graveolens* Linn., Pharmacognostical, Preliminary phytochemical, Fluorescence analysis, Physico-chemical, Thin Layer Chromatography.

Introduction

Sudab in Unani, Gucchapatra in Ayurveda is botanically equated to *Ruta graveolens* Linn., Syn. *Ruta angustifolia* Hook f., Rutaceae (Yoganarasimhan, 2000; Nadkarni, 1976) a strong-scented, erect, branched, glabrous, herb or sub-shrub, 30-90 cm in height. The root woody, branched and yellowish brown. Leaves alternate, pinnately compound; segments oblong to spathulate, strongly aromatic. Flower small greenish-yellow in corymbs. Fruits capsule covered with glands; seeds three edged, reinform, dark brown.

It is native to eastern part of Mediterranean region. In India some times it is cultivated in gardens (Anonymous, 1972, Rashmi, 2002). The chemical constituents of the leaves contain essential oil, rutarin glycoside, furanocoumarins, rabalinium, quaternary alkaloids, 1-methyl-2n-nonyl-4-quinolone, 2-nananone, 2-nonyl acetate, 2-undecyl acetate, â-sitosterol, campesterol and naphthaquinone, bergapten, rutamine, graveolinine, xanthotoxin, aliphatic ketone (Yoganarasimhan, 2000, Anonymous, 1972). All parts of the plant are medicinally useful. The plant is acrid, bitter, thermogenic, diuretic, laxative, aphrodisiac, febrifuge, abortifacient, digestive, carminative, emenagogue and tonic. Leaves and dried herb have been used in folk medicine to heal a number of diseases. It acts upon the periosteum and cartilage; it is used in ocular pain, eye strain, headache, painful affections of extremities, lameness, lassitude, weakness, despair, injured, brushed bones, catarrh, dyspepsia, paralysis, rheumatic pains, fits, convulsions, cardiac asthma, jaundice, infantile diarrhea (Kirtikar and Basu, 1998; Orient, 1996; Rashmi, 2002). The herb was hepatotoxic, contraindicated in kidney diseases and bleeding disorders (Khare, 2007).

Leaves and aerial parts of the *Ruta graveolens* Linn. are used in the preparation of important Unani formulation namely Jawarish-e-Kamooni Kabir, Jawarish-e-

Kamooni Mushil, Raughan-e-Seer and Zimad-e-Ushag Tehali (Anonymous, 2007).

Materials and Methods

Collection of drug

Leaves were collected from raw drug dealers, Chennai and it was identified with by the botanist and compared with the herbarium specimen of RRIUM, Chennai (Specimen No. 7665).

Analysis

The leaves were preserved in Formalo Acetyl Alcohol (FAA) in order to take free hand sections and epidermal peelings. Clearing and staining were done by the standard methods (Johansen, 1940).

Powder of the dried leaves of *Ruta graveolens* L. was used for chemical analysis. Physico-chemical studies like total ash, acid insoluble ash, water soluble ash, alcohol and water solubility and loss on drying at 105ºC methods were carried out as per the WHO guidelines (Anonymous, 1998). Preliminary phytochemical tests were done as per the standard methods (Harborne, 1973; Lala, 1993; Overton, 1963). The fluorescence behavior of the powdered drug in the day light and ultra violet light were carried out by moistening the powder in different solutions and viewing under the light of different wavelengths in a UV- chamber (Brain and Turner, 1975; Trease, and Evans, 1989).

Thin layer chromatography

Preparation of extract

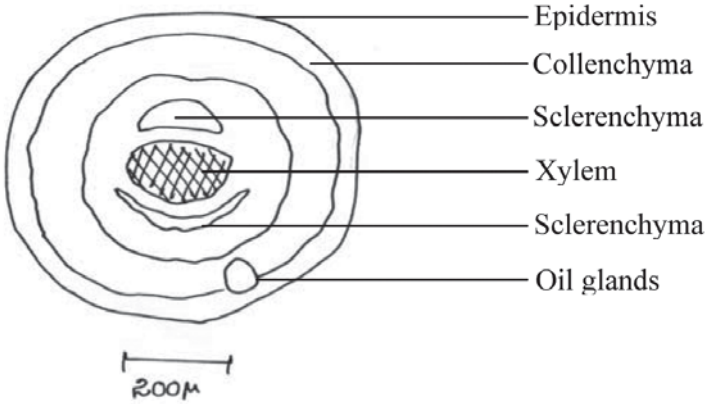
The powder of the drug (2g) was extracted using 30ml of chloroform, alcohol and the extracts were concentrated upto 10ml in a standard flask. These solutions were used for the TLC studies.

TLC analysis

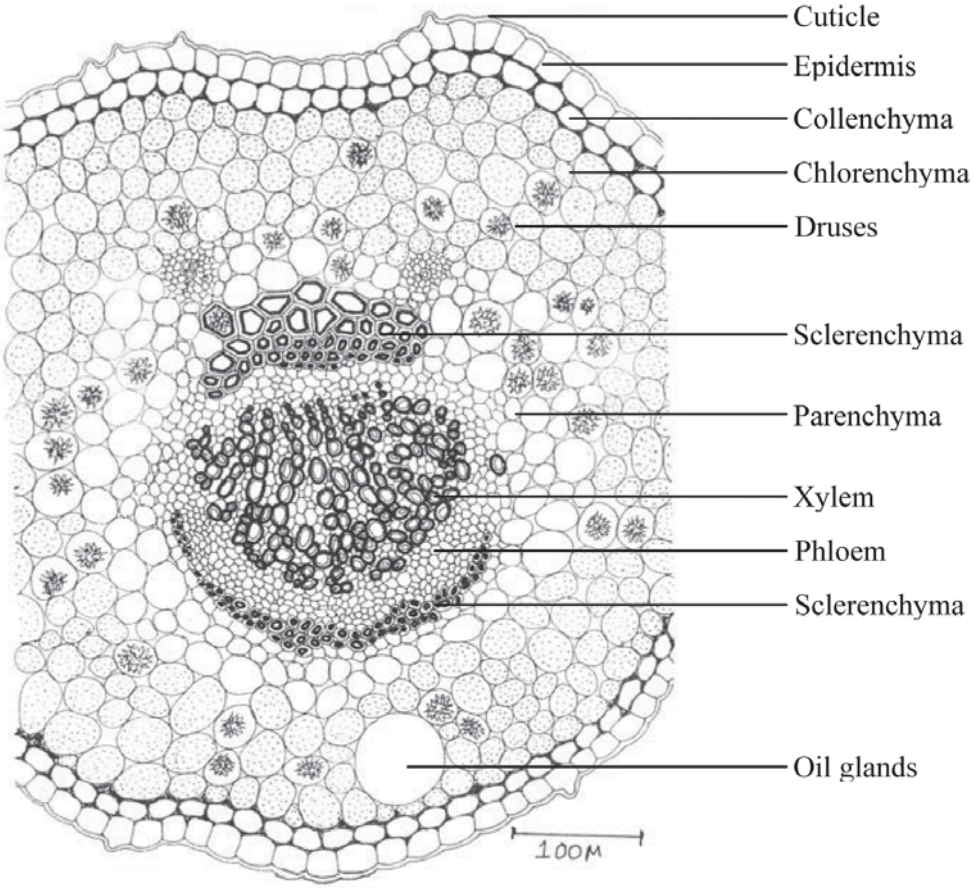
The TLC profile of chloroform and ethanol extracts were performed using precoated silica gel 60 F_{254} TLC plate (E. Merck) as adsorbent. TLC studies of these extracts were carried out using solvent systems like toluene : ethyl acetate, 9 : 1 and 1 : 1.3 respectively for the above extracts. After drying the plates were examined under UV -254, 366nm and observed the spots. Further the plates were dipped in vanillin-sulphuric acid reagent following by heating at 105ºC till the bright spots appeared (Wagner and Bladt, 1984).

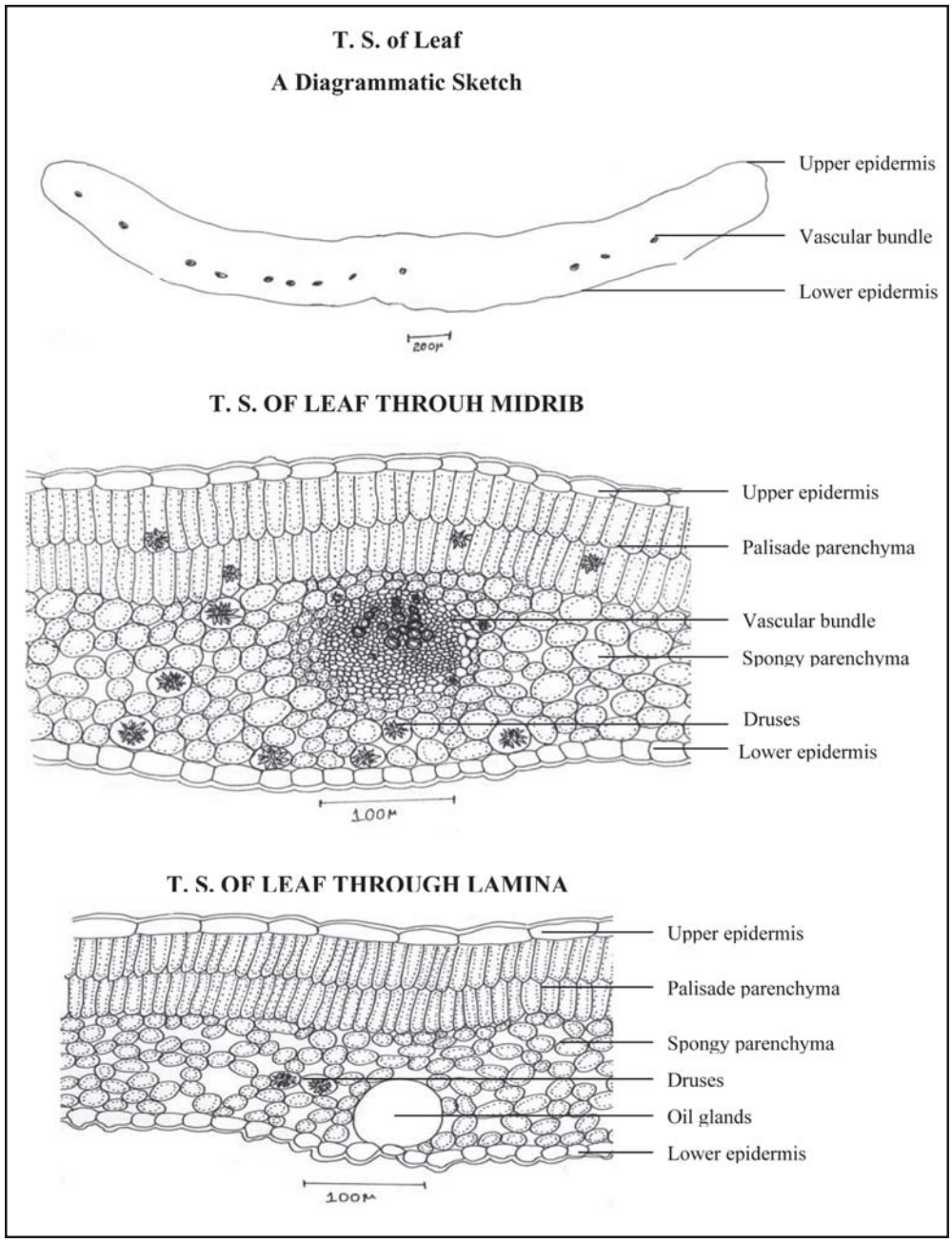
T. S. of Petiole

A Diagrammatic Sketch



T. S. OF PETIOLE

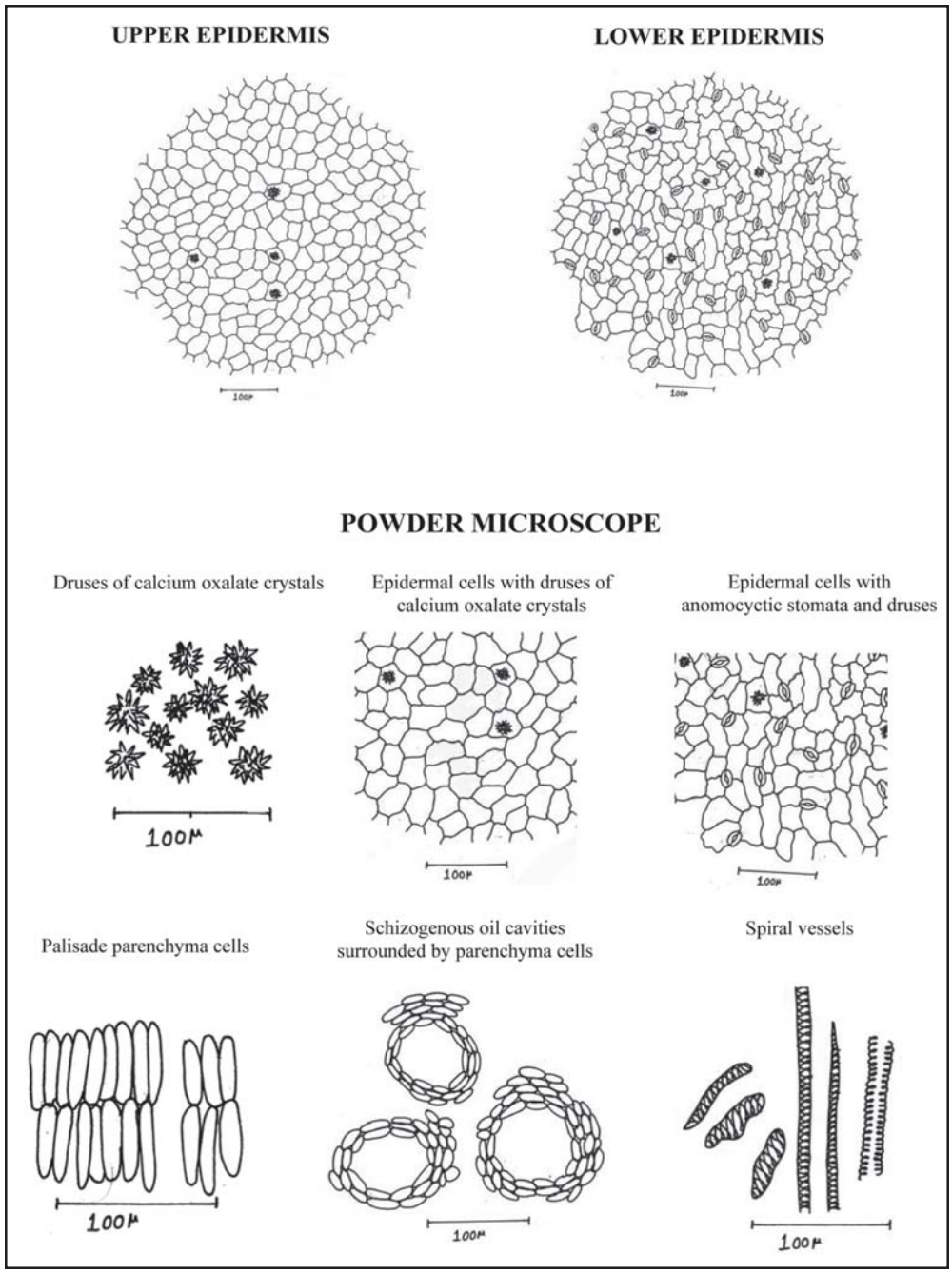




Results and Discussion

Macroscopical characters

Leaves alternate, tripinnate with a feathery appearance, very small with minute petiole, leaflets sessile, oblong, obtuse, dotted, glaucous, terminal leaflet obovate, cuneate, green to bluish green, 6 to 10mm long and 2 to 4mm wide, strongly aromatic odour and bitter in taste (Fig. 1).



Microscopic

Petiole: T.S. of petiole shows circular in outline; epidermis single layered covered with a thin cuticle; cortex consisting of outer region of 1 to 2 layers of collenchyma cells, middle region of 3 to 5 layers of chlorenchyma cells and inner region of 3 to 4 layers of parenchyma cells; pericycle consisting of strands of sclerenchyma cells present below and above the vascular bundle; vascular bundle arc shaped with xylem above and phloem below; druses of calcium oxalate crystals present in the cortex and schizogenous oil cavities present in the cortex.

Fig. 1. Leaf

Leaf

Midrib: T.S. of leaf shows epidermis single layered covered with thin cuticle; two layers of palisade parenchyma cells present below the upper epidermis and 2 to 3 layers of spongy parenchyma present above the lower epidermis and it is continuous in the midrib region also; vascular bundle present in the centre with xylem above and phloem below; druses of calcium oxalate crystals present in both the palisade and spongy parenchyma and in epidermal regions and schizogenesis oil cavities present.

Lamina: T.S. of leaf shows dorsiventral; epidermis single layered covered with thin cuticle; two layers of palisade parenchyma cells present below the upper epidermis and 2 to 3 layers of spongy parenchyma present above the lower epidermis, druses of calcium oxalate crystals present in palisade parenchyma, spongy parenchyma and in the epidermal cells; schizogenous oil cavities present, stomata present only in the lower epidermis, stomata number of the lower epidermis 55 to 62, stomata index of the lower epidermis 16 to 19, vein islet number 14 to 17, vein termination number 47 to 52 and palisade ration is 6 to 10.

Powder: Green; epidermal cells in surface view with anomocytic stomata, druses of calcium oxalate crystals upto 50µ; spiral vessels upto 20µ; palisade parenchyma cells and schizogenous oil cavities surrounded by parenchyma cells.

Physico-chemical data of the leaf of *Ruta graveolens* are summarised in Table-1. The loss on drying was obtained 14.73% and the content of total ash, acid insoluble ash were 10.41% and 1.62 % respectively present in the drug. The alcohol soluble extractive values 10.13% shows the extraction of polar constituents. The water soluble extractive 25.16 % indicates the inorganic contents present in the drug.

Phytochemical screening of *Ruta graveolens* leaf shows the presence of alkaloid, quinone, flavone, phenol, tannin, glycoside, steroids, coumarin, terpenoids and

Table-1. Physico-chemical parameters

Sl.No.	Parameters	Results (n=3) ± S.D
1.	% Foreign matter	1.07 ± 01
2.	% Loss on drying at 105ºC	14.73 ± 03
3.	% Ash	10.41 ± 0.09
4.	% Acid insoluble ash	1.62 ± 0.02
5.	% Solubility at room temp. a. Ethanol b. Water	 10.08 ± 0.02 25.16 ± 0.11

sugar in chloroform and alcohol extract. The results are given in Table-2. The observations of fluorescence analysis are shown in Table-3. The TLC profile of the chloroform and alcohol extracts showed in Fig. 2, 3 and the R_f values are recorded in Table-4 and 5.

The studies carried out will contribute to the existing knowledge in the standardization aspects of the raw drug *Ruta graveolens*.

Table-2. Preliminary phytochemical test

Sl.No.	Test	Chloroform extract	Alcohol extract
1.	Alkaloid	-	+ ve
2.	Quinone	+ ve	+ ve
3.	Coumarin	+ ve	+ ve
4.	Flavone	-	+ ve
5.	Steroid	+ ve	+ ve
6.	Phenol	-	+ ve
7.	Tannin	-	+ ve
8.	Glycoside/Sugar	-	+ ve
9.	Terpenoid	+ ve	+ ve
10.	Iridoid	-	-
11.	Amino acids	+ ve	+ ve

Table-3. Fluorescence analysis

Sl.No.	Powder	Day light	UV 254 nm	UV 366 nm
1.	Powder as such	Yellowish green	Green	Bluish green
2.	Powder + Water	Yellowish green	Green	Bluish green
3.	Powder + 1N NaOH	Brown	Violet	Violet
4.	Powder + 1N HCl	Brownish yellow	Bluish green	Blackish brown
5.	Powder + 50% H_2SO_4	Brown	Dark green	Light green
6.	Powder + petroleum ether	Yellowish green	Light green	Bluish green
7.	Powder + Chloroform	Green	Yellowish green	Violet
8.	Powder + Ethyl acetate	Yellowish green	Light green	Violet
9.	Powder + Ethanol	Yellowish green	Green	Blue

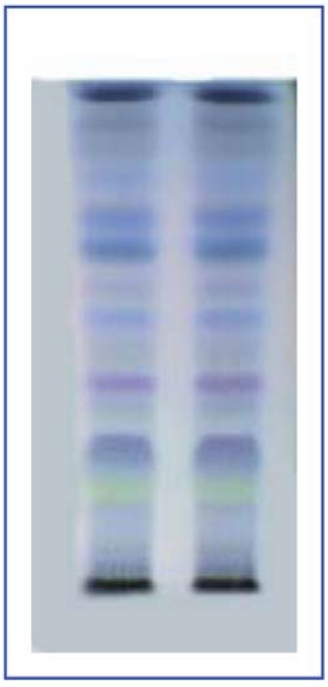


Fig. 2. TLC of Chloroform extract

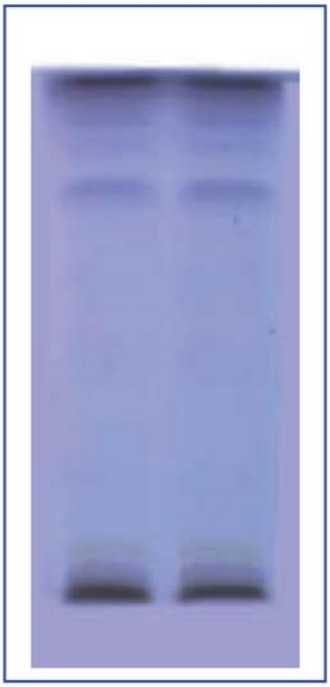


Fig. 3. TLC of Alcohol extract

Table-4. TLC data of the chloroform extract

Solvent system	R_f Values		
	UV 254 nm	UV 366 nm	V.S. Reagent
Toluene: Ethyl acetate (9 : 1)	0.91 (Light Pink)	0.79 (Light blue)	0.91 (Light blue)
	0.85 (Pink)	0.70 (Light blue)	0.85 (Light blue)
	0.65 (Green)	0.65 (Red)	0.80 (Light blue)
	0.57 (Yellowish green)	0.57 (Violet)	0.73 (Blue)
	0.53 (Pink)	0.53 (Light blue)	0.65 (Blue)
	0.44 (Yellowish green)	0.44 (Red)	0.52 (Blue)
	0.39 (Yellowish green)	0.33 (Red)	0.39 (Violet)
	0.20 (Pink)	0.20 (Fluorescent blue)	0.28 (Blue)
	0.11 (Pink)	0.10 (Blue)	0.19 (Yellowish green)

Table-5. TLC data of the alcohol extracts

Solvent system	R_f Values		
	UV 254 nm	UV 366 nm	V.S. Reagent
Toluene: Ethyl acetate (1 : 1.3)	0.85 (Pink)	0.94 (Blue)	0.92 (Grey)
	0.76 (Pink)	0.87 (Blue)	0.87 (Blue)
	0.70 (Yellowish green)	0.76 (Fluorescent blue)	0.79 (Violet)
	0.66 (Light pink)	0.66 (Blue)	
	0.61 (Light pink)	0.55 (Blue)	
	0.56 (Light pink)	0.24 (Blue)	
	0.24 (Pink)	0.16 (Red)	
	0.15 (Yellowish green)		

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Role of Chromatography in the Identification and Quality Control of Herbal Drugs 1 – HPTLC Finger Prints of Qurs-e-Istisqa

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Abstract

Standardization and quality control are the key factors in regulating the therapeutic efficacy of herbal drugs. Organoleptic parameters are not much reliable in establishing the standards of herbal drugs. Instrumental analysis of herbal drugs which gives a more concrete picture regarding the qualitative and quantitative aspects of bioactive molecules, which are responsible for therapeutic action, is widely accepted in the quality assessment of herbal drugs. However, such work is lacking or in infantile stage. In this study, a comparative account of the HPTLC finger prints of the ingredients and compound Unani formulation, Qurs-e-Istisqa are given and discussed in detail.

Key Words: Qurs-e-Istisqa, HPTLC, Quality Control.

Introduction

Qurs-e-Istisqa is a dark brown Unani tablet with odour like that of Sumbul-ut-teeb and Rewand chini and bitter taste and characteristic odour. It is made up of Aab-e-kasni (Hydro distillate of leaves of *Cichorium intybus L.),* Aab-e-Mako (Hydro distillate of leaves of *Solanum nigrum L.),* Gul-e-Surkh (Flowers of *Rosa damascena* Mill.), Luk Maghsool (Lac-Secretion of *Coccus lacca*), Maghz-e-Tukm-e-Khiyar (Cotyledons of *Cucumis sativus* L.), Mastagi (Gum-resin of *Pistacia lentiscus* L.), Rewandchini (Rhizome of *Rheum emodi* Wall.), Sumbul-ut-teeb (Roots of *Nardostachys jatamansi* DC), Tukm-e-Karafs (Seeds of *Apium graveolens* Linn.), Tukm-e-Kasoos (Seeds of *Cuscuta reflexa* Roxb.), Tukm-e-Khurfa (Seeds of *Portulacca oleracea* Linn.) and Zarishk (Seeds of *Berberis aristata* DC.). Therapeutic efficacy of compound herbal medicines is dependent up on the quality and the quantity of the constituent single drugs as they have specific bio-active marker chemical compounds with specific pharmacological actions. It is very difficult to identify the ingradients after the formulation is prepared and the Organoleptic parameters like taste, dour, colour etc. will not ascertain the standard quality of the medicine. There should be some analytical method to ensure the presence of all the ingradients in the formulation. Chromatographic finger printing of both the compound formulation and it's constitute single drugs will definitely ascertain the presence of the ingradients and the quality of the preparation. But such studies in Indian Systems of Medicines is lacking or scanty. Therefore, experiments have been designed to analyse the ingredient single drugs and the compound formulation, Qurs-e-Istisqa by HPTLC and the results are presented.

Materials and Methods

Preparation of the Formulation

It is prepared according to the composition of the formulation as given in National Formulary of Unani Medicine, Part-II, Vol.I, 2007.

Processing of Raw Material

Gul-e-Surkh, Luk Maghsool, Maghz-e-Tukm-eKhiyar, Mastagi, Rewandchini Sumbul-ut-teeb, Tukm-e-Karafs, Tukm-e-Kasoos, Tukm-e-Khurfa, Zarishk, the ingredients of the preparation, after identification and ascertaining the quality, were cleaned by the removal of foreign matter, if present, and by washing two to three times with sterile distil water, if needed.

The drugs were air dried in shade under aseptic conditions. Later, all the drugs except Mastagi were powdered separately by pulveriser and sieved through a mesh with a pore size of 150m.

Mastagi was powdered separately and gently in a metallic kharal.

Preparation of the Tablets

The tablets were prepared as per the procedure described by Mohammad Azam Khan. 1315 AD.

Required quantities of the powders were mixed thoroughly and moistened with Aab-e-kasni and Aab-e-Mako

Samagh-e- Arabi, ten percent of the total weight of the powders of the ingredients, was added to the powdered drugs to get a semisolid paste and subjected for granulation, using mechanical granulator.

The granules thus formed were dried in drier at low temperature or in the sun light.

The granules wee later subjected for making of tablets of desire weight using. Rotary tablet punching machine (Cadmach).

Determination of Physico-Chemical Standards

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

Preparation of Extract of the drug sample for HPTLC

Five grams powder of Qurs-e-istishqa was dissolved in 100 ml of methanol in a stoppered conical flask and was kept for 2 hours shaking in regular intervals. Later the contents were filtered through Whattmann No. 41 filter paper and evaporate the solution to 20 ml. The solution obtained was used as sample for the determination of components.

Development and determination of the solvent system

Sample Application	:	Sample drug solution of Bhel about 10µl.
Solvent system	:	Toluene : Ethyl acetate : Methanol (7 : 2 : 1)
Migration distance	:	98mm
Scanning wavelength	:	366nm

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

Development of HPTLC technique

After developing, TLC plates were dried completely and detected with the suitable detection system like UV Cabinet system for detection of spots at 366nm. Further it is scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366nm appearing a maximum number of components. A corresponding densitogram is then obtained as shown in the figure 2. in which peaks are appeared for the corresponding spots being detected in the densitometer while scanning and the peaks area under the curve corresponds to the concentration of the component in the sample for the concentration we applied on the TLC plate.

Results and Discussion

Analytical Profile

Organoleptic Characters

Dark brown Unani tablet with odour like that of sumbul-ut-teeb and rewand chini and bitter taste, Identification.

Identification

Microscopy

The preparation under high power of microscope shows parenchymatous cells containing rosettes of calcium oxalate crystals, polygonal cells filled with brown pigment and oval pollen grains (Gul-e-Surkh), wavy-walled sclereids and annular or

spiral xylem vessels in groups (Maghz-e-Tukm-eKhiyar); vessels with scalariform thickenings (Rewandchini) cork cells filled with oil globules (Sumbuluttib), elongated rectangular sclereids with sinuate walls and sphaero-raphides of calcium oxalate (Tukm-e-Karafs) small cuboid cells with starch grains and isodiametric lignified cells (Tukm-Kasoos), groups of oval to polygonal, thin-walled, parenchymatous cells, pitted and spiral vessels, fragments of cork cells rosette crystals of calcium oxalate and starch grains (Tukm-e-Khurfa).

Physico-Chemical Standards

Parameter		Sample		
		I	II	III
I.	Ash Values			
(i)	Total Ash	6.98-7.25	7.20-7.65	7.48-7.85
(ii)	Water soluble (% w/w)	2.01-2.25	2.00-2.20	2.30-2.75
(iii)	Acid insoluble (% w/w)	0.05-0.09	0.06-0.10	0.08-0.1.20
II.	Solubile Matter			
1.	Alcohol sol. matter (% w/w)	25.62-26.89	26.60-26.80	25.90-26.80
2.	Water sol. matter (% w/w)	40.02-42.35	40.02-42.35	40.02-42.35
III.	P^H Values			
A.	1% Aqueous Solution	4.50-4.70	4.54-4.80	4.52-4.76
B.	10% Aqueous Solution	4.54-4.66	4.56-4.86	4.57-4.96
IV.	Disintegration Time in Minute	10.00	10.02	10.10
V.	Total moisture content	7.00-7.20	7.25-7.50	7.40-7.6

(Loss of weight on drying at 105°C)

HPTLC Analysis: A comparative account of the Chromatograms of the methanolic extracts of Qurs-e-Istishqa and it's single drugs extracts. At UV 366nm wavelength and at 254 nm are given in figures 1 and 2 respectively. The Densitogram of formulation and it's ingredients at 366nm wave length is given in figure-3. The individual densitograms of compound formulation Qurs-e-Istisqa and it's ingradients Gul-e-Tesu, Luk Maghsool, Magh-e-Tukm-e-Khiyarain, Mastagi, Rewand Chini, Sumbul-ut-Teeb, Tukm-e-Karafs, Tukm-e-Kasoos, Tukm-e-Khurfa and Zarishk are given in figures from 4-13 respectively. A comparative account of the Rf Values of the formulation Istisqa and it's ingredients is shown in table-1. Where as the individual

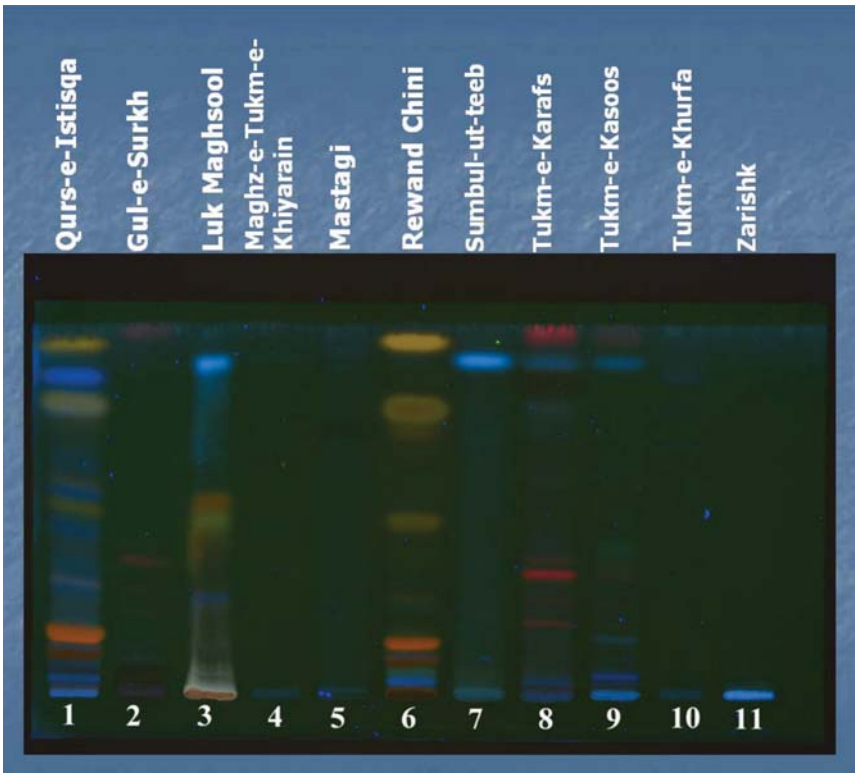

Fig. 1. Chromatograms of the methanolic extracts of Qurs-e-Istisqa and it's single drugs extracts At UV 366nm wavelength

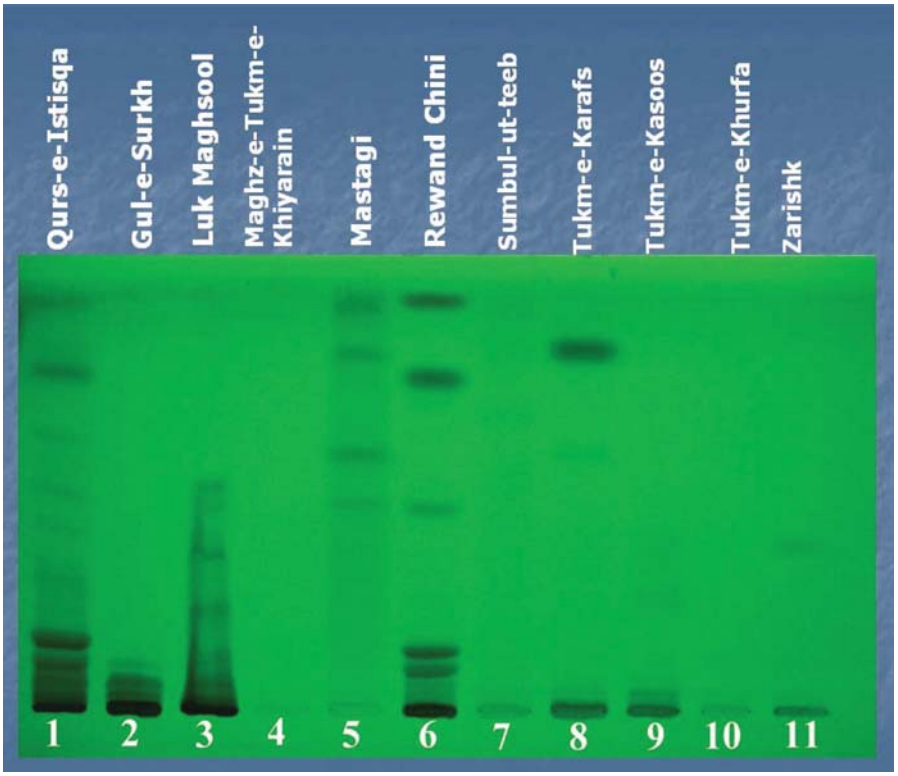

Fig. 2. Chromatograms of the methanolic extracts of Qurs-e-Istisqa and it's single drugs extracts At UV 254nm wavelength

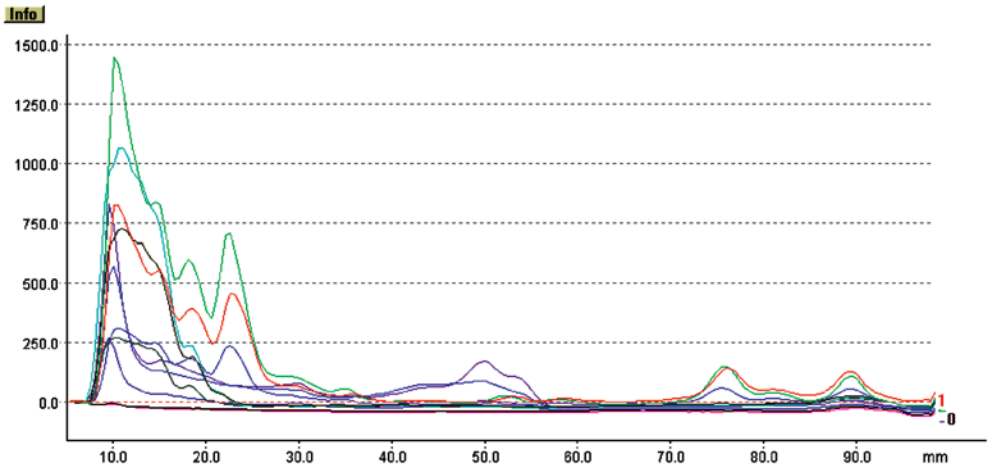

Fig. 3. Densitogram of formulation and ingredients at 366nm wavelength.

Table-1. Rf Values of the formulation, Qurs-e-Istisqa, corresponding to the specific Rf values of the ingradients.

Peaks	Q. Istisqa	G. Surkh	L. Maghsool	M.T. Khiyarain	Mastagi	Rewand Chini	Sumbul-ut-teeb	T. Karafs	T. Kasoos	T. Khurfa	Zarishk
1	0.03	0.03	-	-	-	-	0.03	-	-	0.03	0.03
2	0.09	-	-	-	-	-	-	0.09		-	-
3	0.13	-	-	-	0.13	-	-	-	-		-
4	0.17	0.17	0.17	-	-	-	-	-	-	-	-
5	0.32	-	-	-	-	-	-	-	-	-	-
6	0.39	-	-	-	-	-	-	-	-	-	-
7	0.51	-	-	-	-	-	-	-	0.52	-	-
8	0.57	0.57	-	-	-	-	-	-	-	-	
9	0.77	0.77	-	0.77	-	-	-	-	-	-	-
10	0.83		0.83	-	-	-	-	-	-	-	-
11	0.92	0.92	0.92	-	-	0.92	0.92	-	0.92	-	-

Rf values of Qurs-e-Istisqa and its ingradients Gul-e-Tesu, Luk Maghsool, Magh-e-Tukm-e-Khiyarain, Mastagi, Rewand Chini, Sumbul-ut-Teeb, Tukm-e-Karafs, Tukm-e-Kasoos, Tukm-e-Khurfa and Zarishk are given respectively in tables from 2-12. It is evident from figures 1 and 2 that the number of spots at UV 366nm wavelength

are more than that at 254 nm. It has been observed from table-2 that there are eleven spots in the chromatogram of Qurs-e-Istisqa at Rf values 0.03,0.09, 0.13,0.17, 0.32,0.39, 0.51, 0.57, 0.77, 0.83 and 0.92; from table-3 that there are eleven spots in the chromatogram of Gul-e-Surkh at Rf values 0.03,0.08, 0.12,0.17, 0.24, 0.31, 0.50, 0.57, 0.77, 0.83 and 0.92; from table-4 that there are twelve spots in the

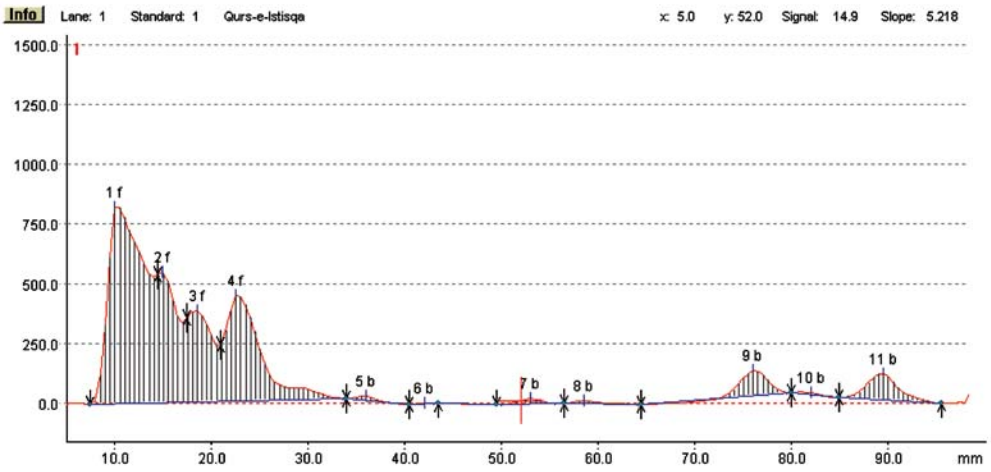

Fig. 4. Densitogram of the methanolic extract of Qurs-e-istisqa

Table-2. Rf values of various spots of methanolic extract of Qurs-e-Istisqa

Lane: 1 Type: Standard 1 Name: Qurs-e-Istisqa X-Position: 5.0 mm

Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.0	3545.12	42.7	809.70	f	0.03
2	:	15.0	1076.29	13.0	537.97	f	0.09
3	:	18.5	966.20	11.6	376.06	f	0.13
4	:	22.5	1798.45	21.7	437.56	f	0.17
5	:	36.0	39.58	0.5	19.90	b	0.32
6	:	42.0	3.73	0.0	2.28	b	0.39
7	:	53.0	59.23	0.7	20.68	b	0.51
8	:	58.5	37.92	0.5	11.74	b	0.57
9	:	76.0	346.36	4.2	102.29	b	0.77
10	:	82.0	19.35	0.2	8.36	b	0.83
11	:	89.5	414.17	5.0	108.89	b	0.92

chromatogram of Luk Mahgsool at Rf values 0.04,0.08, 0.12,0.17, 0.31, 0.038, 0.043, 0.56, 0.65, 0.76, 0.83 and 0.92; from table-5 that there are eight spots in the chromatogram of Mastagi at Rf values 0.04, 0.13, 0.23, 0.29, 0.37, 0.43, 0.58, 0.63 and 0.74; from table-6 that there are seven spots in the chromatogram of Mastagi at Rf values 0.04, 0.12, 0.29, 0.36, 0.59, 0.63 and 0.92; from table-7 that there are

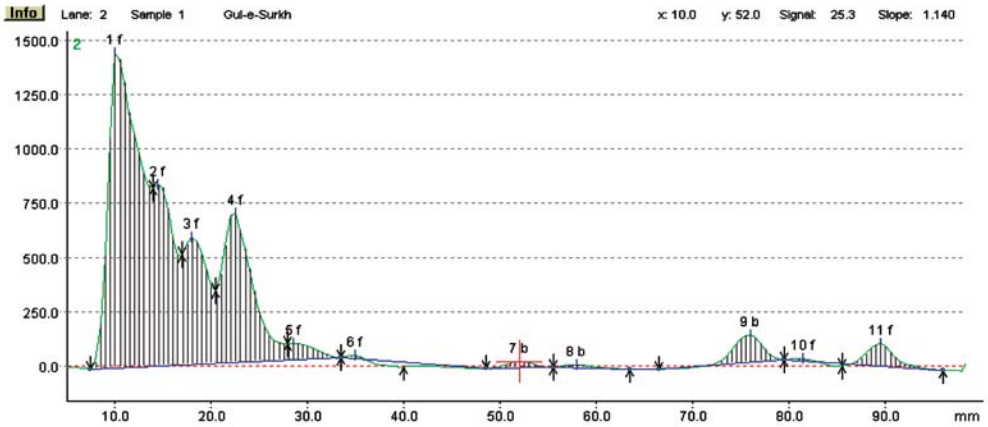

Fig. 5. Densitogram of the methanolic extract of Gul-e-Surkh

Table-3. Rf values of various spots of methanolic extract of Gul-e-Surkh

Lane: 2 Type: Sample 1		Name: Gul-e-Surkh		X-Position: 10.0 mm			
Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.0	5390.52	44.2	1429.41	f	0.03
2	:	14.5	1709.87	14.0	821.30	f	0.08
3	:	18.0	1487.09	12.2	578.37	f	0.12
4	:	22.5	2452.96	20.1	676.77	f	0.17
5	:	28.5	215.97	1.8	76.11	f	0.24
6	:	35.0	23.35	0.2	15.62	f	0.31
7	:	52.0	87.97	0.7	29.90	b	0.50
8	:	58.0	49.92	0.4	15.56	b	0.57
9	:	76.0	429.70	3.5	124.13	b	0.77
10	:	81.5	22.33	0.2	10.51	f	0.83
11	:	89.5	341.97	2.8	103.61	f	0.92

eight spots in the chromatogram of Rewand Chini at Rf values 0.04, 0.13, 0.23, 0.29, 0.37, 0.43, 0.58, 0.63 and 0.74 ; from table-8 that there are five spots in the chromatogram of Sumbul-ut-Teeb at Rf values 0.03, 0.43, 0.47, 0.79 and 0.92; from table-9 that there are six spots in the chromatogram of Tukm-e-Karafs at Rf values 0.03, 0.09, 0.26, 0.48, 0.62 and 0.79; from table-10 that there are eight spots in the

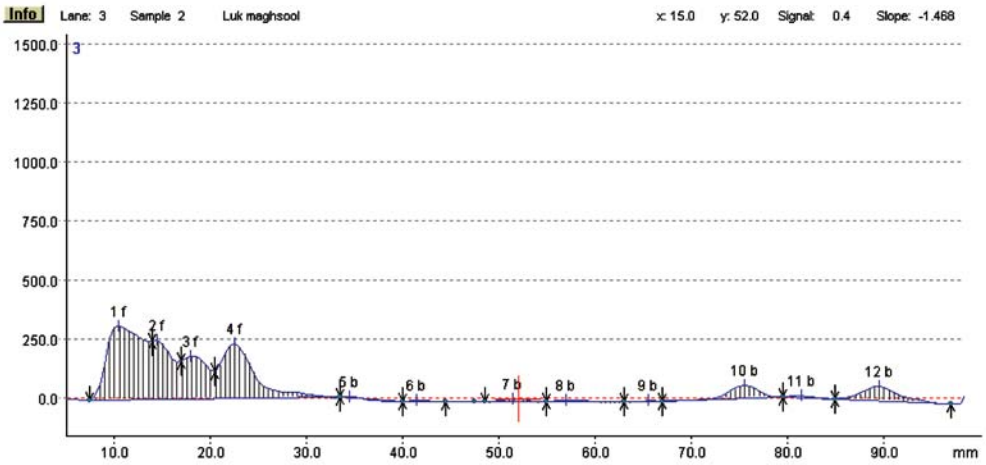

Fig. 6. Densitoogram of the methanolic extract of Luk-Maghsool

Table-4. Rf values of various spots of methanolic extract of Luk Maghsool

Lane: 3 Type: Sample 2 Name: Luk Maghsool X-Position: 15.0 mm

Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.5	1364.16	36.1	310.89	f	0.04
2	:	14.5	508.10	13.5	248.31	f	0.08
3	:	18.0	478.54	12.7	179.50	f	0.12
4	:	22.5	908.58	24.1	229.56	f	0.17
5	:	34.5	9.13	0.2	6.51	b	0.31
6	:	41.5	5.53	0.1	2.61	b	0.38
7	:	51.5	34.04	0.9	12.07	b	0.48
8	:	57.0	20.00	0.5	5.46	b	0.56
9	:	65.5	3.85	0.1	2.06	b	0.65
10	:	75.5	178.36	4.7	52.72	b	0.76
11	:	81.5	13.31	0.4	6.08	b	0.83
12	:	89.5	250.13	6.6	60.64	b	0.92

chromatogram of Mastagi at Rf values 0.04, 0.13, 0.23, 0.29, 0.37, 0.43, 0.58, 0.63 and 0.74; from table-10 that there are nine spots in the chromatogram of Tukm-e-kasoos at Rf values 0.03, 0.09, 0.26, 0.48, 0.52, 0.69 ,0.79, 0.92, and 0.97; from table-11 that there are nine spots in the chromatogram of Tukm-e-kasoos at Rf values 0.03, 0.09, 0.26, 0.48, 0.52, 0.69 ,0.79, 0.92, and 0.97; from table-12 that there are eight spots in the chromatogram of Tukm-e-Khurfa at Rf values 0.03, 0.13, 0.28, 0.42, 0.47, 0.67, 0.78, and 0.93; from table-13 that there are nine spots

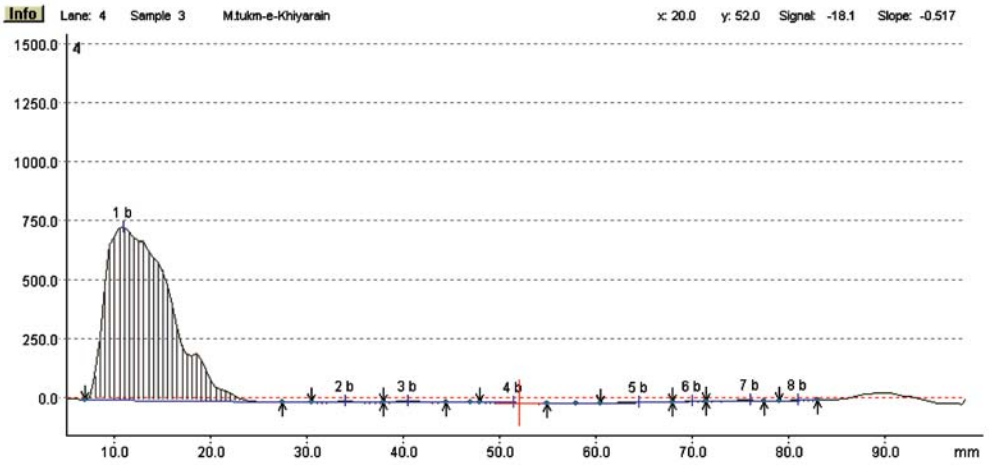

Fig. 7. Densitogram of the methanolic extract of Maghz-e-Tukm-e-Khiyarain

Table-5. Rf values of various spots of methanolic extract of Tukm-e-Khiyarain

Lane: 4 Type: Sample 3 Name: Tukm-e-Khiyarain X-Position: 20.0 mm

Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	11.0	5664.36	39.0	719.51	b	0.04
2	:	34.0	16.19	0.3	5.22	b	0.30
3	:	40.5	18.04	0.3	6.88	b	0.37
4	:	51.5	4.64	0.1	1.21	b	0.49
5	:	64.5	7.42	0.1	2.10	b	0.64
6	:	70.0	1.40	0.0	1.34	b	0.70
7	:	76.0	7.82	0.1	2.97	b	0.77
8	:	81.0	2.63	0.0	1.35	b	0.82

in the chromatogram of Zarishk at Rf values 0.03, 0.18, 0.42, 0.47, 0.67, 0.78 and 0.93.

It is of utmost significance to know whether all the required single drugs are mixed in the compound formulation because each and every single drug has it's own bioactive molecules responsible for a particular therapeutic activity. It is very difficult

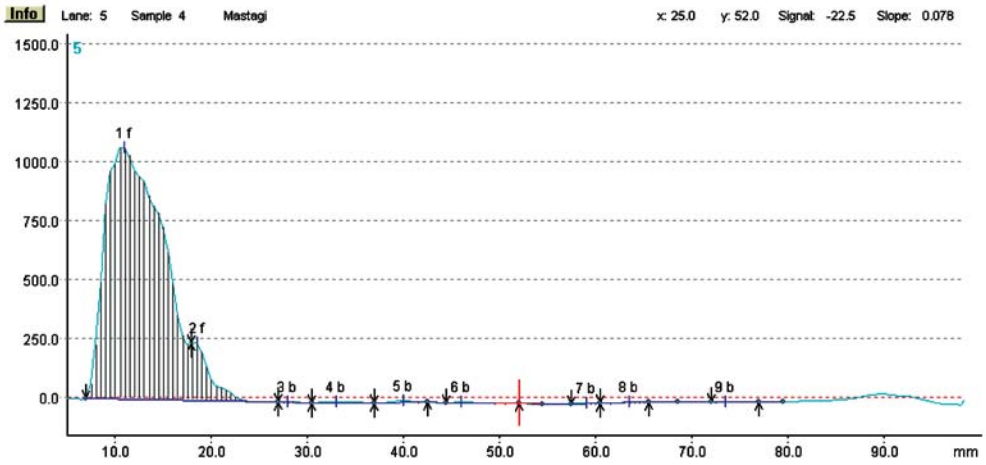

Fig. 8. Densitogram of the methanolic extract of Mastagi

Table-6. Rf values of various spots of methanolic extract of Mastagi

Lane: 5 Type: Sample 4 Name: Mastagi X-Position: 25.0 mm

Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	11.0	7288.94	33.5	1051.46	f	0.04
2	:	18.5	455.23	5.8	241.42	f	0.13
3	:	28.0	3.09	0.0	1.93	b	0.23
4	:	33.0	13.79	0.2	4.77	b	0.29
5	:	40.0	15.31	0.2	7.02	b	0.37
6	:	46.0	7.61	0.1	2.73	b	0.43
7	:	59.0	2.16	0.0	1.28	b	0.58
8	:	63.5	6.25	0.1	2.25	b	0.63
9	:	73.5	3.51	0.0	1.50	b	0.74

to identify the single drugs once they are powdered and mixed for preparing compound formulation. A comparative account of the finger print TLC of any compound formulation along with it's constituent ingradients will help in determining whether the genuine single drugs are mixed or not. Table-1 shows the Rf Values of the TLC spots of the formulation, Qurs-e-Istisqa, corresponding to the specific Rf values of the spots of the individual ingradients. It is evident from the table that there are eleven spots in the compound formulation with Rf values at 0.03, 0.09,

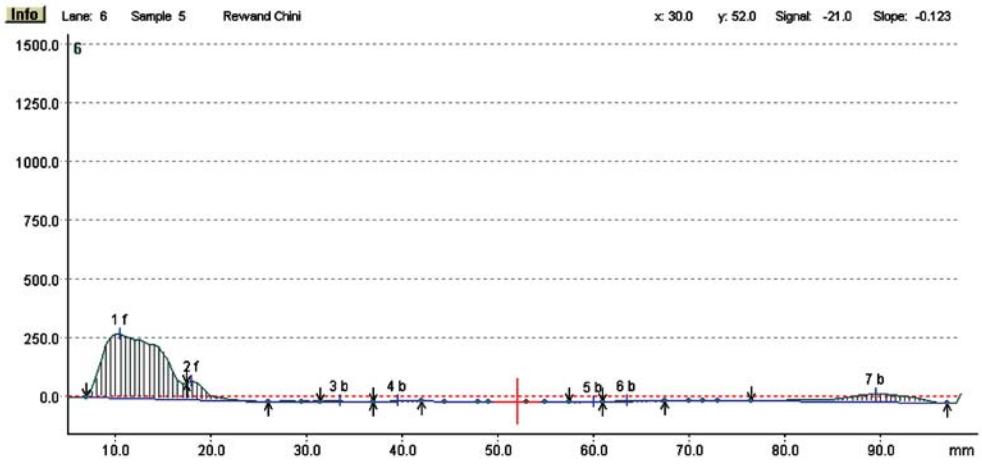

Fig. 9. Densitogram of the methanolic extract of Rewand Chini

Table-7. Rf values of various spots of methanolic extract of Rewand Chini

Lane: 6 Type: Sample 5 Name: Rewand Chini X-Position: 30.0 mm

Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.5	1904.74	81.2	268.21	f	0.04
2	:	18.0	150.56	6.4	75.65	f	0.12
3	:	33.5	4.98	0.2	1.74	b	0.29
4	:	39.5	5.42	0.2	2.41	b	0.36
5	:	60.0	2.75	0.1	1.43	b	0.59
6	:	63.5	7.09	0.3	1.62	b	0.63
7	:	89.5	270.32	11.5	33.87	b	0.92

0.13, 0.17, 0.32, 0.39, 0.51, 0.57, 0.77, 0.83 and 0.92 which correspond to the Rf values of various ingradients. Spot of compound formulation with Rf value at 0.03 corresponds to the spots specific to Sumbul-ut-Teeb, Tukm-e-Khurfa and Zarishk; Spot of compound formulation with Rf value at 0.09 corresponds to the spot specific to Tukm-e-Karafs where as spots of compound formulation with Rf values at 013. 0.51, 0.57, 0.77, 0.83 and 0.92 correspond to the spots specific respectively to mastagi, Tukm-e-kasoos, Gul-e-Surkh, maghz-e-Tukm-e-Khiyarain, Luk Maghsool and Rewand Chini indicating the presence of these ingradients in the compound formulation.

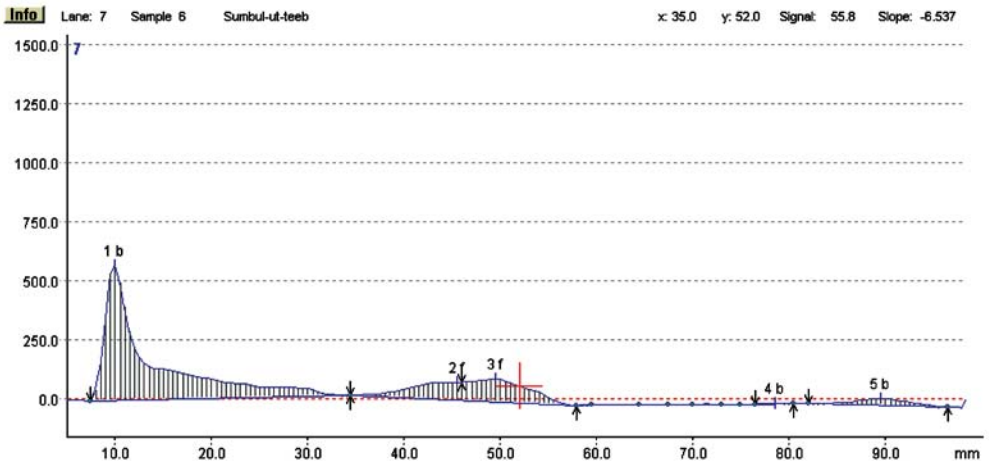

Fig. 10. Densitogram of the methanolic extract of Sumbul-ut-teeb

Table-8. Rf values of various spots of methanolic extract of Sumbul-ut-teeb

Lane: 7 Type: Sample 6 Name: Sumbul-ut-teeb X-Position: 35.0 mm							
Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.0	2364.88	69.6	560.67	b	0.03
2	:	45.5	432.28	10.1	75.15	f	0.43
3	:	49.5	664.34	15.6	95.16	f	0.47
4	:	78.5	5.16	0.1	2.87	b	0.79
5	:	89.5	194.29	4.6	29.67	b	0.92

Conclusion

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

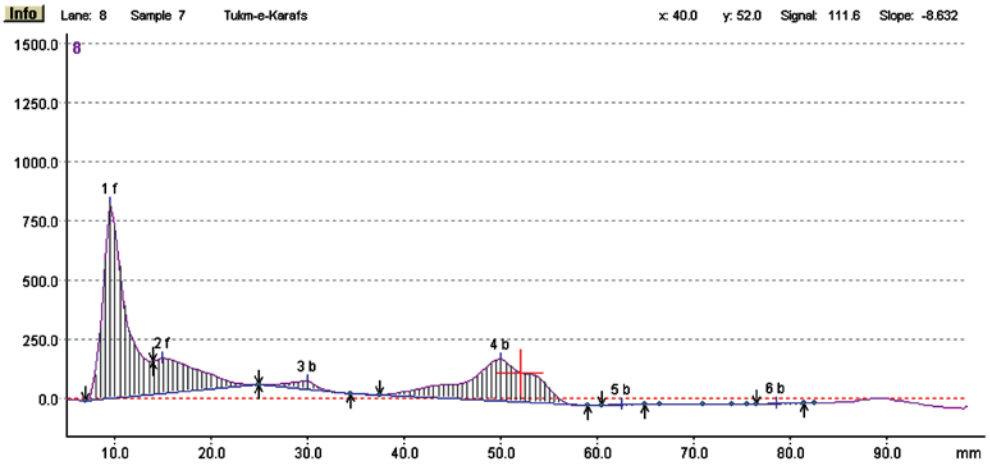

Fig. 11. Densitogram of the methanolic extract of Tukm-e-Karafs

Table-9. Rf values of various spots of methanolic extract of Tukm-e-Karafs

Lane: 8 Type: Sample 7 Name: Tukm-e-Karafs X-Position: 40.0 mm							
Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	9.5	2207.72	49.1	811.19	f	0.03
2	:	15.0	789.74	16.5	146.53	f	0.09
3	:	30.0	110.66	2.5	32.24	b	0.26
4	:	50.0	1425.13	31.7	172.25	b	0.48
5	:	62.5	3.13	0.1	1.46	b	0.62
6	:	78.5	5.55	0.1	2.65	b	0.79

Acknowledgements

The authors like to put on record their sincere gratitude to Dr. Mohammad Khalid Siddiqui, Director, CCRUM, New Delhi for his consistent encouragement and for providing financial help and all the necessary analytical equipment. The senior

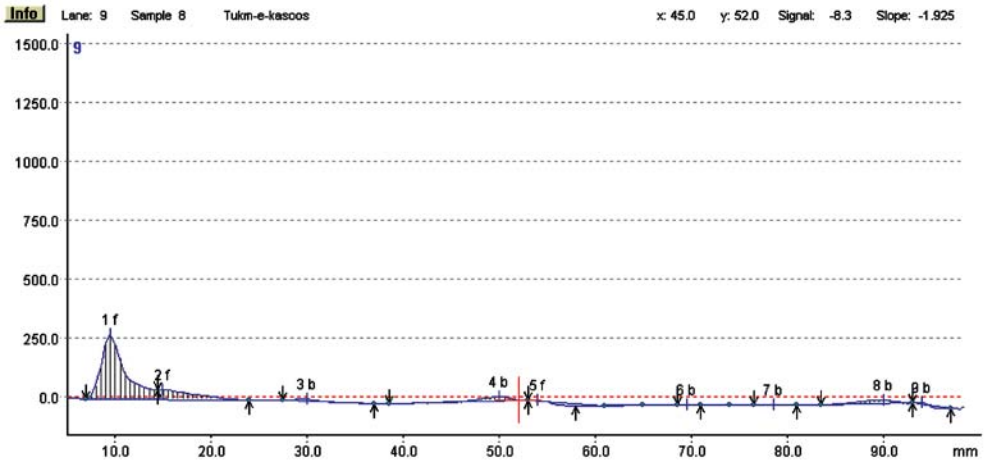

Fig. 12. Densitogram of the methanolic extract of Tukm-e-Kasoos

Table-10. Rf values of various spots of methanolic extract of Tukm-e-Kasoos

Lane: 9 Type: Sample 8 Name: Tukm-e-Kasoos X-Position: 45.0 mm							
Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	9.5	793.86	71.3	272.02	f	0.03
2	:	15.0	171.51	15.4	43.58	f	0.09
3	:	30.0	13.01	1.2	5.76	b	0.26
4	:	50.0	56.83	5.1	16.49	b	0.48
5	:	54.0	2.05	0.2	2.19	f	0.52
6	:	69.5	1.88	0.2	1.43	b	0.69
7	:	78.5	5.34	0.5	2.19	b	0.79
8	:	90.0	62.46	5.6	14.65	b	0.92
9	:	34.0	6.96	0.6	5.96	b	0.97

author also likes to thank the Dy. Director I/C of Central Research Institute of Unani Medicine, Hyderabad for providing the facilities.

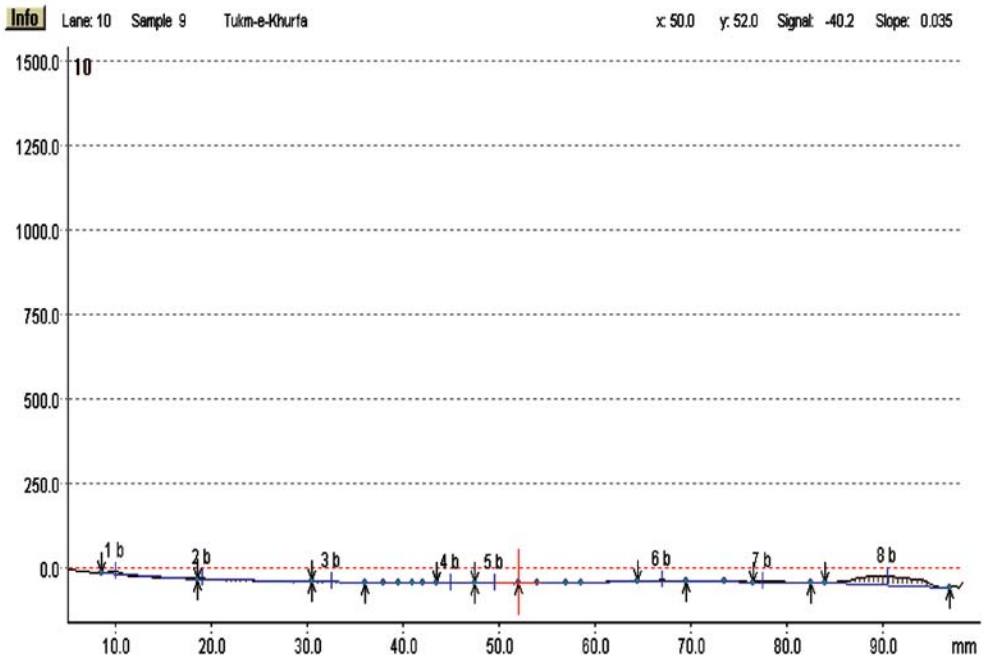

Fig. 13. Densitogram of the methanolic extract Tukm-e-Khurfa

Table-11. Rf values of various spots of methanolic extract of Tukm-e-Khurfa

Lane: 10 Type: Sample 9 Name: Tukm-e-Khurfa X-Position: 50.0 mm

Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.0	8.78	4.2	6.02	b	0.03
2	:	19.0	3.16	1.5	1.37	b	0.13
3	:	32.5	5.36	2.5	2.82	b	0.28
4	:	45.0	3.80	1.8	1.82	b	0.42
5	:	49.5	3.94	1.9	1.79	b	0.47
6	:	67.0	3.89	1.8	2.18	b	0.67
7	:	77.5	4.32	2.0	1.74	b	0.78
8	:	90.5	177.85	84.2	26.65	b	0.93

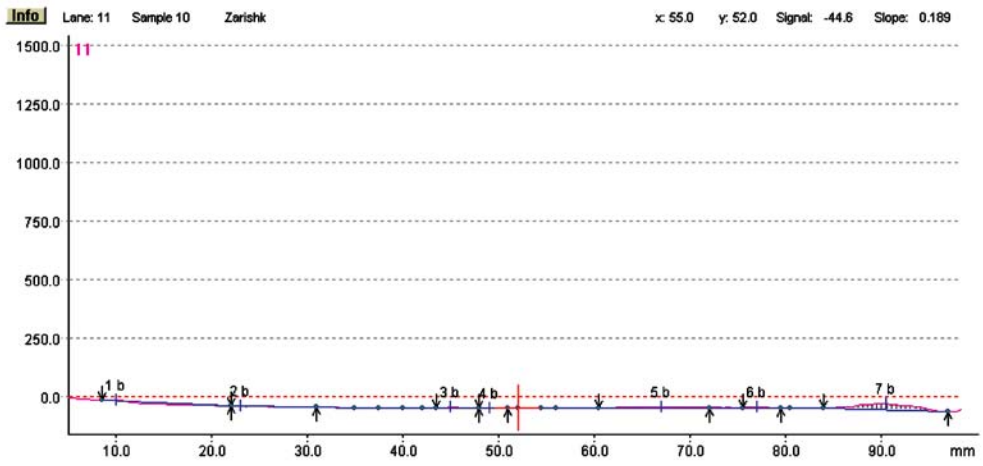

Fig. 14. Densitogram of the methanolic extract of Zarishk

Table-12. Rf values of various spots of methanolic extract of Zarishk

Lane: 11 Type: Sample 10			Name: Zarishk			X-Position: 55.0 mm	
Peak	Component Name	y-Pos (mm)	Area	Area (%)	Height	Type	Rf
1	:	10.0	7.27	3.5	5.08	b	0.03
2	:	23.0	1.19	0.6	1.23	b	0.18
3	:	45.0	5.17	2.5	2.60	b	0.42
4	:	49.0	2.32	1.1	1.51	b	0.47
5	:	67.0	27.71	13.2	5.02	b	0.67
6	:	77.0	5.18	2.5	2.52	b	0.78
7	:	90.5	160.32	76.7	24.89	b	0.93

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Chemical Examination of Majoon-e-Rewand – A Unani Formulation

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Abstract

The developing world is reliant on traditional medicine, and its acceptance in the developing world is increasing. In the past decades there has been a new interest in the usage of traditional medicines particularly in Unani System of Medicines for a wide range of diseases that affect urban population including arthritis, malaria, diabetes, filaria, jaundice, cardiac, hepatalgia, uteralagia and metralgia. Along side this growing interest there have been concern over the quality of traditional medicines. It seems evident that any evolving traditional system of medicine, that has served to society for several millennia such as those in India, China, Africa and Latin America could not survived without internal quality of standards. These traditional medicines remain undocumented because they are transmitted orally from generation to generation. This oral transmission, despite its remarkable efficacy, outreach and cost effectiveness is poorly understood. Since the drug Majoon-e-Rewand has many medicinal usages in the ailment of anti-inflammatory, analgesic, heptalagia, uteralagia and metralagia, this drug was taken for the development of standard method of preparation and to evaluate pharmacopoeial standards. In order to standardize and ascertain the quality of this popular Unani formulation the SOP's, pharmacognostical, physico-chemical, thin layer chromatography, microbial load, aflotoxin, heavy metal, pesticide residue studies were carried out.

Key Words: Microscopy, Physico-chemical analysis, TLC studies, Heavy metals, Microbial load, Aflatoxins, Pesticide residues.

Introduction

In the past few decades there is a sense of awareness among the developing world population, about the importance of traditional system of medicines such as Unani, Ayurveda and Siddha in maintaining health without the side effects. Due to this scientific awareness a scenario has created to undertake the research activities like quality standardization of traditional medicines and development of scientific methods for the manufacture of quality medicines. Majoon-e-Rewand is one of the important Unani medicine categorized under the Majooniath categories, listed in the National Formulary of Unani Medicine, part-II. The drug is being used in ailment of anti-inflammatory, analgesic, heptalagia, uteralagia and metralagia (Anonymous, 2007). Since the drug has many medicinal properties, it was undertaken for the development of standard method of preparation as well as evaluates its pharmacopoeial standards. In order to lay down SOP's and pharmacopoeial standards the drug was prepared in three different batches at laboratory scale. The present investigation has been designed to studies like microscopical, physico-chemical, and thin layer chromatography. Further it was subjected to analyze the parameters such as microbial contamination, aflotoxin level, pesticide residue and heavy metal studies to ascertain its quality.

Material and Methods

Preparation of Powders

In order to develop a scientific method for the preparation of this formulation, the raw drug ingredients were procured from local raw drug dealers Chennai. All the ingredients were procured with the knowledge of Unani physician and identified by botanist using pharmacognostical methods. All the ingredients were powdered separately and passed through sieve number 80. The Majoon-e-Rewand was prepared as per the formulation composition given in NFUM part-II (Anonymous, 2007).

Formulation Composition

S. No.	Unani name	Botanical/ English name	Part used	Quantity taken for SOP	Quantity as per NFUM part-II
1.	Rewand Chini (UPI–II)	*Rheum emodi* Wall.	Rt.	40 g	10g
2.	Waj (UPI–V)	*Acorus calamus* Linn.	Rz.	40 g	10g
3.	Bekh-e-Karafs (API–VI)	*Apium graveolens* Linn.	Rt.	40 g	10g
4.	Badiyan (UPI–I)	*Foeniculum vulgare* Linn.	Fr.	40 g	10g
5.	Anisoon (UPI–II)	*Pimpinella anisum* Linn.	Fr.	40 g	10g
6.	Nankhwah (API–I)	*Trachyspermum ammi* (L.) Sprague.	Fr.	40 g	10g
7.	Zard Chob (API–I)	*Curcuma longa* Linn.	Rz.	40 g	10g
8.	Luk Magsool (Wallis, 1997)	Coccus lacca (Animal origin)	–	40 g	10g
9.	Qand Safaid	Sugar	–	960 g	200g

Preparation of Majoon-e-Rewand

All the ingredients were taken of pharmacopoeial quality. Cleaned, dried and powdered the ingredients number 1 to 8 of the formulation composition separately. Dissolved the specified quantity of sugar, as per the formulation composition in 900

ml of water on slow heat. Added 0.1 % of citric acid, mixed thoroughly and filtered through muslin cloth. Then the filtrate was boiled on slow heat and prepared the quiwam of 78 % consistency. Removed the vessel from the fire. While hot condition added the mixed powders of ingredient number 1 to 8 along with 0.1 % sodium benzoate and mixed thoroughly to prepare the homogenous product. Allowed it to cool to room temperature. Packed it in tightly closed container to protect from light and moisture. The formulation was prepared in three batches separately by same method.

Powder Microscopy

5g of the sample was weighed and mixed with 50ml of water in a beaker with gentle warming, till the sample completely dispersed in water. The mixture was centrifuged and decanted the supernatant. The sediment washed several times with distilled water, centrifuged again and decanted the supernatant. A few mg of the sediment was taken and mounted in glycerine and observed for the following characters. Camera lucida drawings were done for the salient features of the drug (Johansen, 1940).

Chemical Analysis

The prepared three batch samples were subjected for chemical analysis. Physico-chemical studies like total ash, acid insoluble ash, water soluble ash, alcohol and water solubility, loss on drying at 105º, microbial load and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). Aflotoxin, pesticide residues were carried out by standard methods (Anonymous. 2000). The bulk density, sugar estimation and *pH* values for 1% and 10% aqueous solution were also carried out (Anonymous, 1987).

Thin layer chromatography

Preparation of extracts for TLC

2g of drug samples were soaked in chloroform and alcohol separately for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made upto 5ml in a standard flask separately.

Method of developing for TLC

The chloroform and alcohol extracts were applied on precoated silica gel 60 F_{254} TLC plate (E.merck) as absorbent and developed the plate using solvent systems, toluene : ethyl acetate 9:1 and 1: 1 respectively. After developing, the plates were

dried and observed the colour spots at UV-254, UV-366 nm and vanillin-sulphuric acid spraying reagent (Wagner *et. al*., 1984).

Results and Discussion

Majoon-e-Rewand is blackish brown, semi solid with agreeable odour and sweetish bitter in taste. The drug was spreaded in a petridish and observed, it did not show any filth, fungus or objectionable extraneous matter.

Microscopical Observation

Rewand Chini - Rosette of calcium oxalate crystals upto 100m; **Waj** - Groups of parenchyma cells filled with spheroid starch grains, each starch grains single rarely compound 2 or 3 unite, 2 to 10m interrupted by arenchymatous space; Bekh-e-Karafs - Cortical parenchyma cells in surface view with the presence of large predominant secretary cells, many times larger than the cortical cells; **Badiyan** - Reticulate lignified parenchyma cells, very narrow thin walled cells arranged parallel to one another in groups of 5 to 7, these groups of cells at an angle of adjacent groups (paraquetry arrangement); **Anisoon** - Epidermal cells in surface view with occasional anomocytic stomata and numerous conical, mostly unicellular occasionally bicellular thick walled warty trichomes; **Nankhwah** - Papillose epidermal cells in surface view with small club shaped, thick walled trichomes and broken trichome bases; **Zard Chob** – Parenchyma cells with amorphous masses of gelatinized starch grains. (Microscopic diagram of the drug was shown in Figure-1).

Chemical analysis

Moisture content of this drug shows 25.35%. The alcohol soluble extractive (55.48%) might be due to the extraction of polar chemicals constituents and the water soluble extractives 51.52% indicate the presence of inorganic constituents. The physico-chemical data of the drug are shown in table-3. The total bacterial count was found with in the permissible limit (Table-4). The studies of other parameters like aflotoxin such as B_1, B_2, G_1 and G_2 were not found in the drug. The pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion were also not detected in the drug samples. The study carried out on heavy metals such as lead is present with in the permissible limit, and other elements cadmium, arsenic and mercury were found below the detection limit (Table-4).

Thin Layer Chromatography analysis

Thin layer chromatography studies of chloroform and alcohol extract of all the three batch samples showed identical spots in various detector ranges. The R_f values of

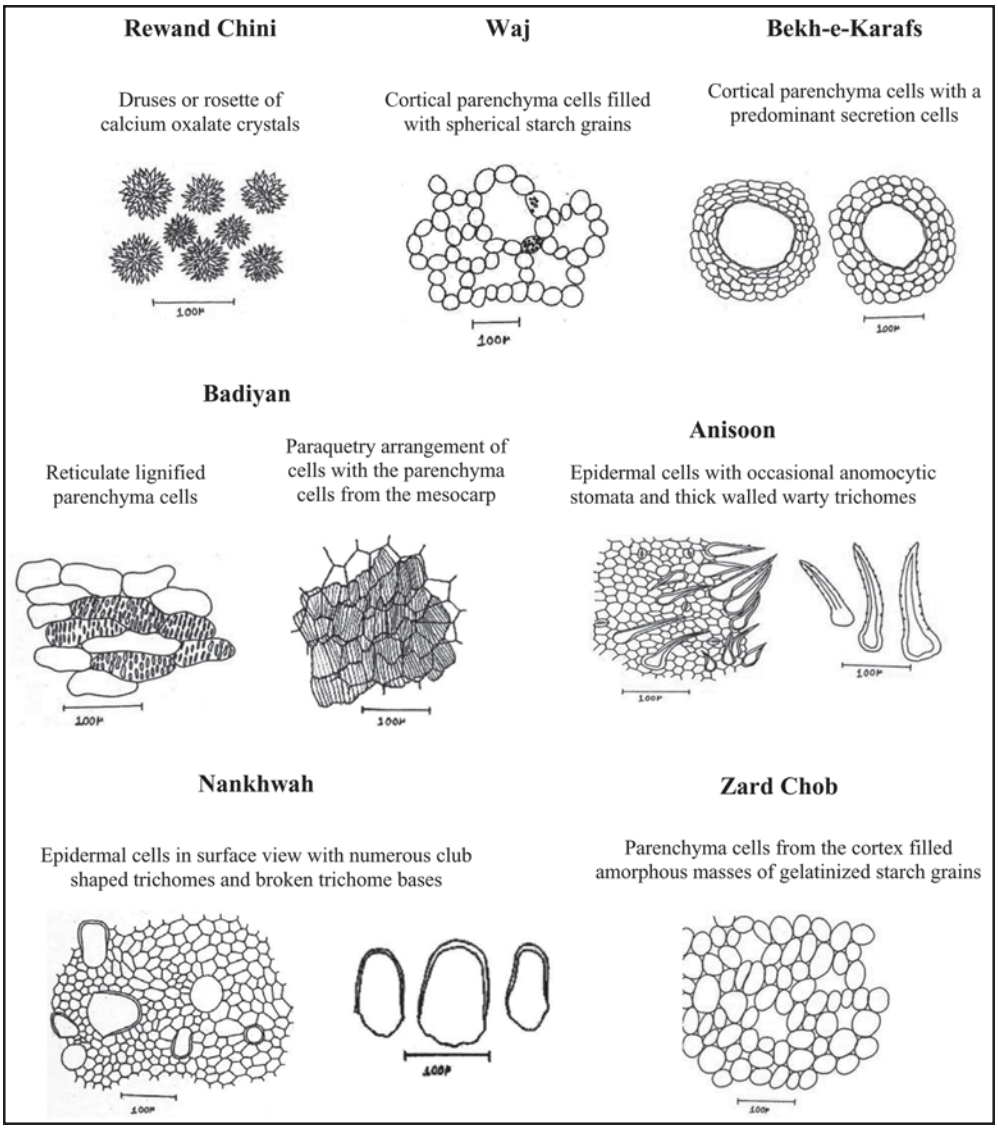

Fig. 1. Powder microscopy of Majoon-e-Rewand

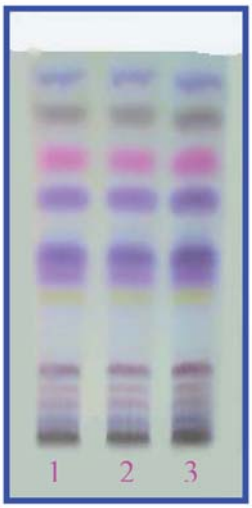

Fig. 2. Chloroform Extract

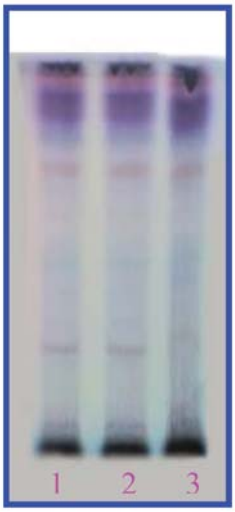

Fig. 3. Alcohol Extract

Table-1. R_f Values of chloroform extract

Solvent system	R_f Values		
	UV 254nm	UV 366nm	V. S. Reagent
Toluene: Ethyl acetate (9 : 1)	0.83 Yellow	0.83 Yellowish green	0.92 Violet
	0.61 Dark Pink	0.58 Blue	0.81 Brownish green
	0.54 Dark Pink	0.49 Sky blue	0.68 Pink
	0.49 Dark Pink	0.36 Dark brown	0.59 Violet
	0.15 Dark Pink	0.25 Sky blue	0.46 Violet
	0.36 Yellowish green	0.18 Yellow	0.41 Violet
	0.31 Pink	0.12 Yellow	0.36 Yellow
	0.18 Yellow		0.18 Brown
	0.12 Yellow		0.12 Pale brown

Table-2. R_f Values of alcohol extract

Solvent system	R_f Values		
	UV 254nm	UV 366nm	V. S. Reagent
Toluene: Ethyl acetate (1 : 1)	0.96 Greenish yellow	0.96 Yellowish orange	0.94 Orange
	0.89 Pink	0.89 Sky blue	0.91 Violet
	0.86 Greenish yellow	0.86 Brown	0.84 Violet
	0.71 Yellowish green	0.78 Sky blue	0.79 Light blue
		0.71 Yellowish green	0.72 Brown
		0.51 Sky blue	0.48 Bluish green
		0.24 Violet	0.26 Violet
		0.21 Blue	

Table-3. Physico-chemical parameters

Parameters	Batch Number					
	I	Mean value	II	Mean value	III	Mean value
Alcohol soluble matter (% W/W)	55.16 55.28 55.52	55.32	55.29 55.48 55.64	55.47	55.36 55.64 55.96	55.65
Water soluble matter (% W/W)	51.20 51.36 51.68	51.41	51.16 51.52 52.04	51.57	51.28 51.60 51.92	51.60
Total ash (% W/W)	1.47 1.50 1.67	1.54	1.46 1.48 1.51	1.48	1.34 1.55 1.61	1.50
Acid insoluble ash (% W/W)	0.43 0.48 0.50	0.47	0.42 0.46 0.52	0.46	0.41 0.42 0.49	0.44
pH values 1% Aqueous solution	6.00 6.20 6.40	6.20	6.10 6.30 6.40	6.26	6.10 6.20 6.40	6.23
pH values 10% Aqueous solution	5.10 5.30 5.40	5.26	5.20 5.30 5.50	5.33	5.10 5.30 5.40	5.26
Sugar estimation Reducing sugar (% W/W)	37.31 37.38 37.91	37.53	37.26 37.53 37.77	37.52	37.32 37.41 37.57	37.43
Non reducing sugar (% W/W)	3.67 3.71 3.93	3.77	3.11 3.57 3.70	3.46	3.39 3.58 3.70	3.55
Moisture (% W/W)	25.12 25.42 25.49	25.34	25.20 25.31 25.62	25.37	25.07 25.45 25.52	25.34
Bulk Density	1.3987 1.4021 1.4276	1.4094	1.3958 1.4065 1.4311	1.4111	1.3895 1.3978 1.4057	1.3976

Table-4. Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	26,000 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	Nil/gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	Staphylococcus aureus	Absent / gm	Nil

Table-5. Heavy metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Below detection limit	10 ppm
2	Cadmium	Below detection limit	0.30 ppm
3	Lead	0.85 ppm	10 ppm
4	Mercury	Below detection limit	1.0 ppm

the chloroform and alcohol extracts were shown in table-1 and 2. The plates were developed using vannilin-sulphuric acid and heated at 105º till appears colored spots (Figure 4 & 5).

Acknowledgement

The authors are extremely thankful to the Director, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities.

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Effect of Vermicomposting on Kalmegh (*Andrographis paniculata* Wall. ex Nees)

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Abstract

Investigations were carried to find out the influence of vermicompost (VC), farmyard manure (FYM) and chemical fertilizers as growth promoter on Kalmegh (*Andrographis paniculata* Wall. ex Nees). Various types of growth promoter were applied in the experimental plots. Vermicompost and FYM (@ 8 t ha^{-1}) was found best for the total yield. While, poorest yield were recorded in the combination of vermicompost + FYM + chemical fertilizers.

Key Words: *Andrographis paniculata* Wall. ex Nees, Vermicompost, FYM.

Introduction

Tropical forests are an abode of medicinal plants. They are in great demand due to the increased acceptance of ayurveda and traditional medicines, because of their property of less or no side effects. In fact, large scale collection of medicinal plants from forests has accelerated the depletion of tropical forests, thus making them endangered and threatened group of plant species. In order to maintain a sustained supply of raw materials to the drug industry, these plants are encouraged to be cultivated outside the forest ecosystem in recent years. Kalmegh (*Andrographis paniculata* Nees.) is one such medicinal plant with numerous medicinal properties. It is an erect branched annual herb of height 0.3 to 0.9 m, with branches sharply quadrangular, leaves lanceolate, flowers small, white, solitary with yellowish brown seeds.

The fresh and dried leaves of Kalmegh and juice extracted from the herb is an official drug of Indian pharmacopoeia. It is a source of several diterpinoids of which andrographolide is informant. The drug is used for general debility, certain forms of dyspepsia, chronic malaria, jaundice, and dysentery. Some scientists have observed that andrographolide has the potential to be included in the cocktail vaccine against AIDS by virtue of its antagonistic property with HIV II virus (Wibo, 1995). It is already being used in treating cancer as it promotes cell differentiation in tumour cells (Matsude *et al*., 1994). Though leaves contain maximum andrographolide the entire plant is sued for extracting the active ingredient.

The current day emphasis is an sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus, use of biofertilizers play an important role in sustainable agriculture. Vermicompost are known to improve the nutritional status of soil growth and development of plants. Hence, in the present investigation, it was envisaged to screen and select an appropriate doses of vermicompost for optimum Kalmegh cultivation,.

Materials and Methods

The experiments on agrotechnical practices using vermicomposting and fertilizers for crude drug production on *Andrographis paniculata* were carried out during 2006-

07 at the Vermiculture research station, D.S. College, Aligarh, U.P., India. The experimental site lies between 27º54'50"N latitude and 78º4'26"E longitude, represents almost a dry climate, the cold weather lasts longer than in the eastern districts and it extends from middle of October after close of the rains to the end of the March. The mean annual rainfall is 16.41 mm, the mean number of rainy days was 48 per annum. The mean maximum and minimum temperature were 30.75ºC and 18.06ºC respectively. The relative humidity often as 66.93 percent. The study was conducted during the period from 15 July 2006 to 15 December 2007. The weather condition prevailed during the experimental period are presented in Table 1, Fig. 1.

Table-1. Monthly mean weather data for the period from July 2006 to Dec. 2007.

Month	Max. Temp. (ºC)	Min. Temp. (ºC)	Relative humidity	Rainfall
		2006		
July	38.17	27.52	71.62	1.15
August	33.64	25.00	90.12	8.12
September	33.76	24.21	82.60	2.80
October	33.52	17.26	76.87	-
November	25.87	13.48	84.12	9.00
December	17.73	9.31	84.75	1.10
		2007		
January	21.71	8.96	88.87	-
February	22.22	7.80	79.62	-
March	29.71	14.77	68.12	-
April	38.22	20.07	45.87	-
May	38.08	23.68	52.62	-
June	41.75	26.61	55.62	2.80
July	32.52	25.06	78.97	87.90
August	31.98	24.47	87.12	82.50
September	33.51	22.81	79.00	17.20
October	26.22	18.36	73.85	0.40
November	23.70	12.03	80.20	6.20
December	24.44	9.27	24.44	-

Cropping history of experimental site

Field where no experiments were conducted during the three previous seasons was selected to conduct field trials (Table 2).

The planting material was collected from Rajasthan Agromedicinal Corporation Ltd. Sonamukhi Nagar, District Jodhpur (Rajasthan). Farmyard manure and chemical fertilizers was purchased locally and vermicompost was prepared from cattle manure and agriculture wastes. The chemical composition of the experiment materials used in study are described in Table 3.

Experiment details

The design and layout of the experiments (RBD) are given below along with a brief outline of the technical programme followed (Table 4). The plot size was uniform for the field experiments, 9.45 m^2 (6.3 m x 1.5 m) grossplot and 7.2 m^2 (6 m x 1.2 m) netplot.

The experimental area was ploughed twice, harrowed, leveled and brought to good tilth. The field was laid out as per the design of the individual experiment in raised beds with channels (40 cm) separating the plots.

Table-2. Physical and chemical characteristics of the soil prior to the experiment

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

Table-3. Chemical composition of the experimental materials used in the study.

Nutrient	Planting material	FYM	Vermicompost
N (%)	0.30	0.89	1.50
P (%)	0.12	0.47	1.07
K (%)	0.51	0.37	0.046
Ca (%)	1.09	0.72	1.25
Mg (%)	0.24	0.39	0.15
S (%)	0.27	0.18	0.23
Fe (ppm)	3044.00	1727.00	1520.00
Mn (ppm)	188.00	252.90	176.60
Zn (ppm)	72.60	65.90	61.50
Cu (ppm)	22.00	85.10	85.60

Table-4. Layout of field experiment

M: Mainplot S : Subplot

T3:M1S3	T2:M1S2	T4:M1S4
T1:M1S1	T4:M1S4	T3:M1S3
T2:M1S2	T1:M1S1	T2:M1S2
T4:M1S4	T3:M1S3	T1:M1S1
T5:M2S2	T8:M2S4	T7:M2S3
T5:M2S1	T7:M2S3	T5:M2S1
T8:M2S4	T5:M2S1	T6:M2S2
T7:M2S3	T6:M2S2	T8:M2S4
T13:M4S1	T13:M4S1	T15:M4S3
T15:M4S3	T14:M4S2	T14:M4S2
T16:M4S4	T14:M4S3	T16:M4S4
T14:M4S2	T16:M4S4	T13:M4S1
T12:M3S4	T10:M3S2	T12:M3S4
T9:M3S1	T12:M3S4	T10:M3S2
T10M3S2	T9:M3S1	T11:M3S3
T11:M3S3	T11:M3S3	T9:M3S1
R1	R2	R3

Experiment 1

F: vermicompost level P: vermicompost: Fert. Proportion

T11:F2P5	F10:F2P4	F16:F3P5
T9:F2P3	T2:F1P1	T11:F2P5
T12:F3P1	T4:F1P3	T15:F3P4
T8:F2P2	T15:F3P4	T5:F1P4
T4:F1P3	T5:F1P4	T12:F3P1
T10:F2P4	F16:F3P5	T4:F1P3
T16:F3P5	T12:F3P1	T6:F1P5
T15:F3P4	T6:F1P5	T10:F2P4
T5:F1P4	T8:F2P2	T2:F1P1
T3:F1P2	T13:F3P2	T7:F2P1
T6:F1P5	T7:F2P1	T14:F3P3
T2:F1P1	T9:F2P3	T14:F3P3
T14:F3P3	T11:F2P5	T9:F2P3
T1:Control	T14:F3P4	T3:F1P2
T7:F2P1	T1:Control	T13:F3P2
T13:F3P2	T3:F1P2	T8:F2P2
R1	R2	R3

Experiment 2

There was heavy weed infestation since the crop was slow growing with limited ground coverage. Hand weeding was carried out at 2 and 4 months after planting. Earthing up was carried out simultaneously with weeding and top dressing of fertilizers. In general, plant protection was taken up as and when necessary. Biometric (plant height, no. of leaves, length of roots, canopy spread, dry roots production) observations on five plants were taken and the arithmetic mean was recorded.

Observations and Results

Appropriate technological practices were recorded to ensure adequate availability of quality crude drug and fetch height returns for the farmers.

In the present investigation the plant height was found maximum (66.51 ± 1.06) in the R6 (8 t ha^{-1} VC + 3t ha^{-1} FYM + 0.02 gm DAP). While the minimum was recorded (47.24 ± 1.58) in the 8t ha^{-1} VC + 8t ha^{-1} FYM. The maximum branches of plant was found (17.20 ± 1.52) in the 8t ha^{-1} VC + 0.04 gm urea + 0.02 DAP after 6 MAP (month after planting) and minimum branches was recorded (12.40 ± 1.52) in the treatment of 8t ha^{-1} VC + 8t ha^{-1} FYM. The no. of leaves (maximum) was recorded (176.50 ± 1.40) in the treatment of 6 t ha^{-1} VC + 0.04 gm urea and minimum was recorded (112.58 ± 2.55) in the treatment of 7t ha^{-1} VC at 6 MAP stage. The capsules per plant was maximum in the (133.66 ± 3.65) treatment of 8t ha^{-1} VC + 8t ha^{-1} FYM and minimum recorded (80.75 ± 2.98) in the treatment of

Table-5 Kalmegh

Treatments	Plant height	No. of branches	No. of leaves	Capsule	Flowers	Total yields kg/ha	
						Green	Dry
R_0	62.89 ±3.75	14.0 ±2.99	112.58 ±2.55	121.25 ±0.84	67.83 ±2.16	641.20 ±0.81	166.60 ±1.09
R_1	63.64 ±3.12	16.40 ±2.03	176.50 ±1.40	116.50 ±4.53	76.61 ±2.40	455.80 ±1.08	148.20 ±0.52
R_2	57.80 ±0.93	13.50 ±3.63	121.91 ±1.14	111.33 ±3.28	45.75 ±3.96	432.20 ±1.30	130.20 ±2.80
R_3	57.99 ±0.89	13.80 ±1.08	122.66 ±1.92	125.76 ±2.01	32.03 ±5.37	426.20 ±2.75	142.80 ±1.0
R_4	55.10 ±3.81	17.20 ±1.56	126.58 ±1.83	100.66 ±6.24	66.50 ±2.95	42.960 ±1.0	144.90 ±3.59
R_5	47.24 ±1.58	12.40 ±1.52	125.41 ±1.14	133.66 ±3.65	83.08 ±6.60	446.70 ±1.86	134.90 ±1.13
R_6	66.51 ±1.06	13.60 ±1.0	121.63 ±4.81	80.75 ±2.98	41.41 ±2.83	541.30 ±1.22	158.60 ±1.04

8t ha^{-1} VC + 3t ha^{-1} FYM + 0.02 gm DAP at 6 MAP stage. The maximum flower was recorded (83.08 ± 6.60) in the 8t ha^{-1} VC + 8t ha^{-1} FYM and minimum was recorded (41.41 ± 2.83) at 8t ha^{-1} VC + 3t ha^{-1} FYM and 0.02 gm DAP at 6 MAP stage. Maximum yield/plant was recorded (641.20 ± 0.81) in the 8t ha^{-1} VC and minimum (426.20 ± 2.75) was recorded in 6t ha^{-1} VC + 0.04 gm urea + 0.02 gm DAP 6 MAP stage. The maximum dry yield was recorded (166.60 ± 1.09) in the 8t ha^{-1} VC and minimum was found (130.20 ± 2.80) in the treatment of 8t ha^{-1} VC + 0.04 gm urea + 0.02 gm DAP at 6 MAP stage.

Discussion

A crop is cultivated not only for the quality of yield but also for the quality of produce. Usually there exists an inverse relation between quantity and quality as the quality components are formed from quantity components. Hence, the time of harvest needs to strike an ideal balance between the two on the one hand and be economically viable on the other hand.

Singh *et al*. (2001b) domesticated and cultivated three wild types of *Andrographis paniculata* and reported that the population exhibited wide variation among themselves with respect to growth, behaviour, maturity period dry biomass and leaf yield and percent andrographolides content and their yield.

Nemade *et al*. (2001) reported that growth and yield attributes of *Andrographis paniculata* were not influenced by date of harvest but date of planting. Srivastava *et al*. (2000) noted that *Mentha arvensis* aged 3-4 months gave higher yields of high quality oil. Bahl *et al*. (2000) reported that the full flowering stage of *Ocimum basilicum* offered the most profitable time of harvest. Menon and Potty (1998, 1999) reported that FYM application led to more balanced development of yield components. Riba (2000) observed better growth of ginsing due to the addition of cow dung. Kasera and Saharan (2001) reported that application FYM at 8t ha^{-1} was suitable for obtaining maximum plant growth and biomass in *Evolvulus alsinoides*.

Singh *et al*. (2000b) conducted that higher yields of *Plantago ovata* could be achieved by sowing in ridges with the application of organic fertilizer Ulrich-23. Shiva and Mantolia (1998) further recorded that quality of medicinal plants was influenced by seasonal effects and hence they suggested that generally,d rugs should be collected in autumn season. According to the Pusalkar and Aruna (1990) major cultivation of medicinal plants starts only after 1980 and the last decade witnessed their large scale cultivation. Pereira *et al*. (1998) noted that organic fertilization increased caumarin concentration in *Mikenia glomerata*.

Acknowledgement

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Protein Analysis of *Cassia sophera* Linn. and *Cassia sophera* Var. *purpurea* Linn. for Correct Botanical Differentiation

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Abstract

Kasondi is an important Unani Medicine used in many diseases e.g. bronchitis, gonorrhoea, in snake bite as an antidote, as a cathartic and diuretic. There are two varieties *Cassia sophera* Linn., and *Cassia sophera* Var. *purpurea* Linn., with minor morphological differences and almost same chemical components, hence creating problems to identify them when dry. In this paper an attempt has been made to established a marker particularly proteins which can be helpful for differentiating these two phenotype similar varieties. The bioactive compound may change or reduce in quantity while processing the plants for medical uses but it is likely that protein may retain its nature if not burnt. The SDS-PAGE method was employed to get the protein bands and the molecular weights were calculated. The differences in the proteins and their molecular weights are used here as tool for identification of the two varieties. The nitrogen and protein percentage were also estimated.

Key Words: *Cassia sophera* Linn., *Cassia sophera* Var. *purpurea* Linn., Kasondi, SDS-PAGE, Gel electrophoresis, Protein.

Introduction

Protein is the most abundant macromolecules of living organisms. It is the most important final product of information pathway and considered as molecular instrument through which genetic information is expressed. Among the protein products, enzymes are the most varied, specialized and virtually the catalyst of all cellular reaction (Lehninger, 2000). Some pharmacologically active proteins that are found in plants are Robin isolated from locust bark, Abrin from *Abrus precatorius* (Ghomchi) seed (Evans 2002) and Ricin from castor beans (Lenninger, 2000; Evans 2002) and legheamoglobin from leguminous plants. Rubisco the most abundant protein varies from 50%-60% of total soluble protein in green tissue (Lehninger 2000). It is known fact that different part of plant contain different protein complement moreover it is also true that the different tissue with in a plant at different developmental stage contain unique complement of protein. The molecular weight (MW) of protein are generally 10- 50 Kilo Dalton (Kda), although protein as small as 50 Dalton and greater than 1000 Kda and multi-chain protein complex of greater than 200 Kda are frequently encountered.

No attempt has been made, so far, to identify the closely related species on the basis of various proteins present in the green tissue, that protein might be expressing their differential characters. With regard to the herbal drugs it is noted that the market samples are mostly the broken part of the plants and some time it becomes difficult to identify on basis of macroscopical and microscopical character particularly when two species are very closely related with minute differential characters. In this paper an attempt has been made to study the protein profile of two closely related

species rather varieties for making any conclusion. The varieties are *Cassia sophera* Linn. and *Cassia sophera* var*. purpurea* Linn. The only difference in these two varieties is the presence of the purple color twig and leaf stalk in the variety *purpurea* (Hooker, 1982), and this difference is in-differentiable when the plants are dry. The chemical compositions are also almost same Both are known as Kasondi and used for the same purposes e.g. bronchitis, gonorrhoea, in snake bite as an antidote, as a cathartic and diuretic.

Material and Methods

Plant material

The leaves and young stem of *C. sophera* L. and *C. sophera* var*. purpurea* were collected from the plants cultivated in the herb garden of department of Ilmul Advia AMU, Aligarh, Figure 1 & 2. The plants were growing in the same agro-climatic conditions and that of same age. After collection, the material was stored at 4°C. The herbarium specimens are deposited in the museum of department of Ilmul Advia (Voch. No. 273, 274). The identification was made with the help of published literature (Kiritkar and Basu, 1986; Hooker, 1982). Further confirmation was made with the help of Prof. Wazahat Husain, Department of Botany, AMU, Aligarh.

Determination of Nitrogen

The total percentage of nitrogen was estimated in the air dried powder of the leaves using the method of Lindner & Harley (1942) after digesting it in the concentrated sulphuric acid to convert all the organic nitrogen into inorganic nitrogen.

Fig. 1. Cultivated plant of *Cassia sophera* Linn.

Fig. 2. Cultivated plant of *Cassia sophera* var*. purpurea* Linn.

Determination of Amino acid

The amino acids were extracted by the method documented by Afaq *& Siddiqui* (1983) and Afaq & Latif (1988). The descending paper chromatography on Whatman N0.1 filter paper was made by the standard method using the BAW (n-butanol: acetic acid and water (4:1:5) as a mobile phase). The percentage composition of the different amino acids, with out correction, was determined and the graph was plotted by the Toshniwal densitometer. The percentage composition was estimated by comparing the area of the peaks so obtained.

Preparation of protein sample

The material (5 g) was grounded in a mortar pestle with 1ml of 10µg/ml Phenyl methyl sulfonyl fluoride (PMSF) dissolve in acetone. Further 2 ml of 5 mM of Tris HCl buffer was added in the content and macerated. The content was centrifuge at 4000 rpm for 10 minutes and the supernatant was taken out.

Determination of Proteins

The supernatant (0.5 ml) was taken and 0.5 ml of distilled water was added to make the volume 1 ml. The total protein was estimated using the systronic spectroscopy by the method of Lowry *et al* (1951)

Gel electrophoresis

25µl of supernatants (protein sample) was used with 25µl of sample buffer for Sodium Dodisyl Sulphate-Polyacrylamide Gel Electrophoresis. SDS-PAGE was done with 12% polyacrylamide concentrations separating gel and 15 mA current was supplied continuously for six hours using gel electrophoresis equipment (Laemmlli, 1970). For the visualization of protein gel was stained with silver stain (Morrissey, 1981) and commasi brilliant blue R-250 according to standard method.

Molecular weight (MW) standard curve

Protein marker (PMW-M) was obtained from Genei, Banglore, and SDS-PAGE runs to obtain the relative mobility (Rm values). Simultaneously the protein sample was also loaded. Logarithmic scale was set and a typical plot of molecular weight of standard proteins and their relative mobility were made. The standard proteins were Phosphorylase b (97.4 Kda), Bovine serum albumin (66 Kda), Ova albumin (43 Kda), Carbonic anhydrase (29 Kda), Soyabean trypsin inhibitor (20.1 Kda), and lysozyme (14.3 Kda). The various proteins separated from the sample were identified using the standard curve with relation to Rm values; further the molecular weights were also calculated.

Result and Discussion

The results of present study show marked differences in protein profile of two closely related varieties of *Cassia sophera.* Figure 3 shows nine different and clearly visible protein bands on silver staining in *Cassia sophera* var. *purpurea, w*hile *Cassia sophera* shows eleven clearly visible protein bands (Figure 4). Figure 5 shows standard protein marker to calculate molecular weights of observed proteins. The difference in MWs of proteins of two plants is clearer and suggestive (Table 1). The protein with higher molecular wt. of *C. sophera* var*. purpurea* is 110 Kda while that of *C. sophera* it is 81 Kda. The lowest molecular wt. protein of *C. sophera* var*. purpurea* was found to be 16.2 Kda that of *C. sophera it* was 19.5 Kda. The MW of some of proteins however was found to be almost the same or is very close to each other such as protein of 61.1 Kda in var. purpurea and 67.1 Kda in *C.sophera* similarly 38.95 Kda in variety *purpurea* nearly same or sub unit of 37.2 Kda protein of *C .sophera*. The results of present study demonstrated that the two closely related medicinal plants shearing common morphological characters and chemical properties possess different protein profile. The Nitrogen percentage of *C .sophera* and *Cassia sophera* var. *purpurea* were noted to be 1.5 mg/100mg and 1.25 mg/ 100mg respectively. The total protein in *C .sophera* and *Cassia sophera* var. *purpurea* were noted to be 378 mg/10 gm and 140 mg/10 gm respectively. The comparative high percentage of nitrogen and total protein in *Cassia sophera* is in accordance to the above results as the greater number of protein is present in this variety. The amino acids in both the samples are same but the percentage composition is different (Table 2). The observed differences in the protein profile may prove to be a great help in identification and standardization of these two drugs. The strategy could be of great help in authentication of several other medicinal plants, which on account of physical and chemical resemblance are difficult to be identified and standardized.

Figure 3

Figure 4

Figures 5

Fig. 3. Proteins bands of *Cassia sophera* var*. purpurea* Linn. after silver staining.
Fig. 4. Proteins bands of *Cassia sophera* Linn. after silver staining.
Fig. 5. Standard protein Molecular Weight marker.

Table 1. Molecular Weights of two closely related varieties of herbal drug, *Cassia sophera* var. *purpurea* and *Cassia sophera*.

S.No.	M. Ws. of *Cassia sophera* var. *purpurea* proteins	M. Ws. of Cassia *sophera* proteins
1	110.00	81.00
2.	105.00	67.60
3.	73.20	63.00
4.	67.10	53.40
5.	44.70	50.10
6.	38.95	37.20
7.	36.50	34.10
8.	24.20	30.20
9.	16.20	29.50
10.	-	21.30
11.	-	19.50

Table 2. The percentage composition of amino acids in *Cassia sophera* and. *Cassia sophera* var. *purpurea*

S.No.	Amino acids	% composition in *Cassia sophera* var. *purpurea*	% composition in *Cassia sophera*
1	Histadine	4.75	4.90
2.	Cystine	8.10	10.85
3.	Serine	15.62	10.06
4.	Glutamic acid	8.80	14.69
5.	DL- alanine	21.06	5.97
6.	Dl-2-amino-n-butyric acid	13.11	11.79
7.	Proline	11.05	4.31
8.	Tryptophane	9.55	8.70
9.	Lucine	22.4	14.93
10.	Nor-lucine	21.00	13.99

Conclusion

Finally it can be concluded that large number of drugs which are identical, albeit vary in pharmacological affect may be correctly identified when present as a single drug or even incorporated in compound formulation. One or more specific proteins from the medicinal plant can be chosen standard marker to identify specific variety or species of any herbal drug. The difference in the pharmacological properties in very closely relative species, with common chemical components may also be due to the proteins of different molecular weight present in the plant.

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Traditional Phytoremedies from Chandouli District of Eastern Uttar Pradesh, India

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Abstract

Based on an ethnobotanical survey, the medicinal uses of local flora from Chandouli district of eastern Uttar Pradesh are reported in this paper. A total of 35 taxa belonging to 29 families were collected through interaction with traditional healers and other knowledgeable village elders during the fieldwork. For each plant species are given the scientific and vernacular names, the part used, claimed medicinal use(s), mode of preparation and administration. A comparison of data obtained in this study against the available ethnobotanical literature has revealed some interesting and new phytotherapeutic uses.

Key Words: Ethnobotanical survey, herbal remedies, Chandouli, Eastern Uttar Pradesh.

Introduction

Eastern Uttar Pradesh is extremely rich in cultural heritage and relatively varied flora. This geographical region of northern India has an old tradition of herbal healing. Therefore, explorations undertaken by investigators interested in the cultural attributes of indigenous and local communities have yielded valuable medicinal uses of the plants. Consequently, a wealth of knowledge on traditional and folk medicines of different rural areas and tribal pockets across the region has been accumulated in the literature (Ahmad, 1996; Bajpai et al., 1995; Gupta, 1979; Khanna et al., 1996; Maheshwari and Singh, 1988; Pandey, 2003; Maheshwari et al., 1986; Prakash and Singh, 2000-2001; Singh, 1994, 1999; Singh et al., 1987, 1994, 2002. Singh and Maheshwari, 1983, 1992; Singh and Singh 1985; Singh and Prakash, 1996; Srivastava, 1985; Tewari and Tewari, 1986). A review of this literature reveals that no separate report is available on medicinal plants which are in therapeutic use among the indigenous societies of Chandouli district. The present paper, therefore, enumerates information on certain traditional phytoremedies collected during an extensive ethnobotanical survey of this area of the country.

Chandouli district forms a part of Upper Gangetic Plain and lying between 24°42'00" – 25°03'55" N latitude and 83°03'24" – 83°22'55" E longitude in the eastern Uttar Pradesh (Fig. 1). There are nine forest ranges (namely: Chakia, Chahania, Chandouli, Chandrapirbha, Jaimohni, Majhgaeen, Mughalsarai, Naugarh and Rajpath) which fall in Kashi Wildlife Forest Division Ramnagar, Varanasi. Only Chakia, Chandrapirbha and Naugarh forest ranges are rich in natural forests and wildlife. The area is predominantly inhabited by various tribal communities such as Chero, Gour, Gond, Kharwar, Kol and Musahar. These people have their own diverse religious, cultural and social customs as well as a rich tradition of phytotherapy.

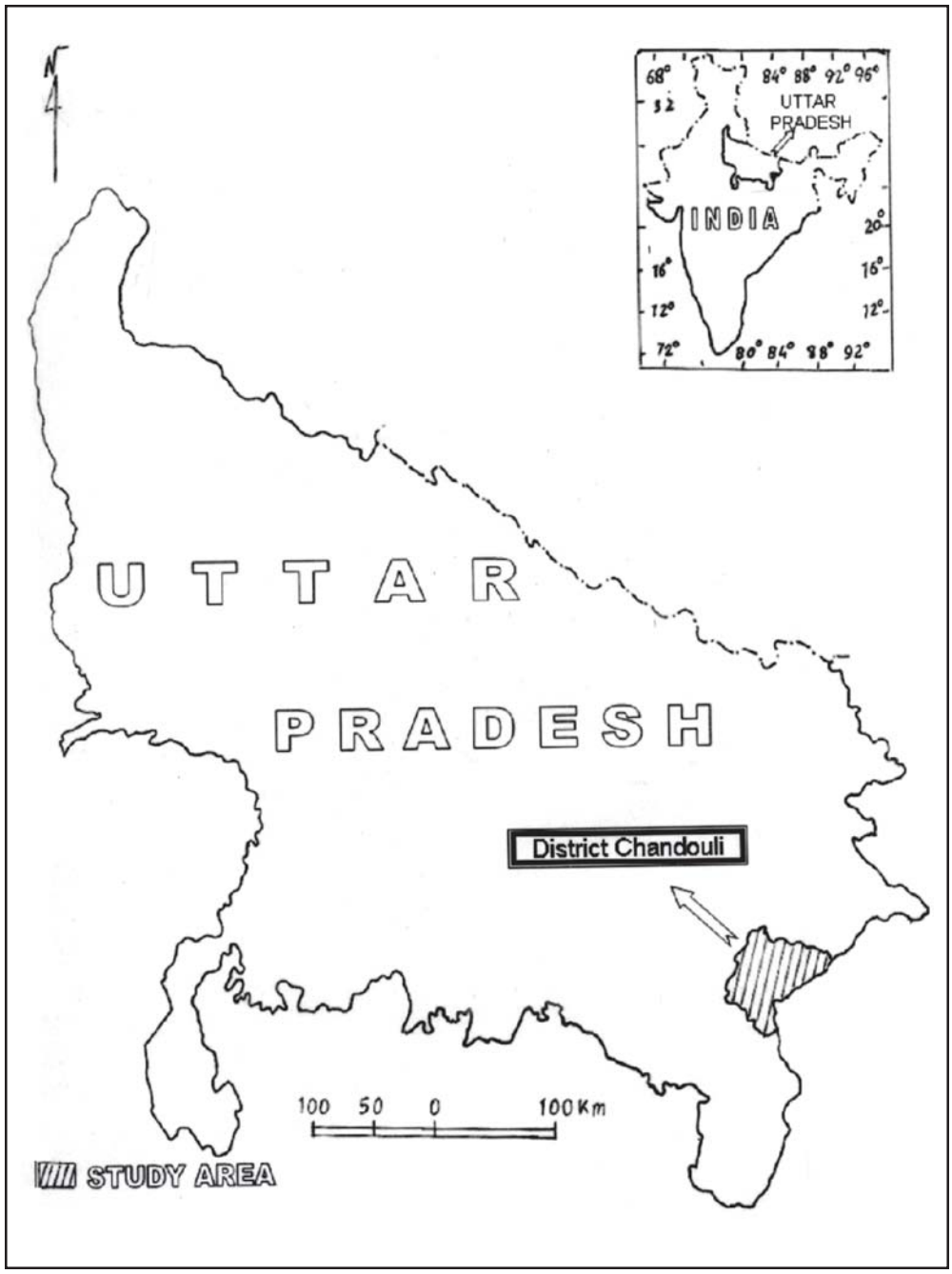

Fig. 1. Study area: Chandouli District, Uttar Pradesh, India.

Methodology

Fieldwork was carried out in November and December 2007. Information on medicinal uses of plants was obtained through interaction with traditional healers and other knowledgeable village elders. Data on common name of the plant or crude drug, medicinal use(s), the part used, other ingredients added (if any), method of drug preparation, mode of administration, dosage and duration of treatment were recorded for each claim. All botanical specimens along with relevant field information were

collected. Plant species were later identified by the authors with the help of related floras (Hooker, 1872-1897; Duthie, 1903-1922; Kanjilal, 1993). Voucher specimens were prepared and deposited in the herbarium of Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India.

Observations

In the following enumeration plants are arranged in an alphabetic order by their scientific names. Each entry gives botanical name, family in parenthesis, local name, locality from which a particular use was recorded, voucher specimen number and use(s).

Acacia leucophloea (Roxb.) Willd. (Mimosaceae), 'Reuan', Chakia (*SMPUA 7975*). Children suffering from marasmus are advised to give water bath under the tree in the morning and left their used clothes.

Achyranthes aspera L. (Amaranthaceae), 'Chirchiri', Latif Shah (*SMPUA 7841*). Long pieces of the stems of 'chirchiri' and 'bareira' (*Sida rhombifolia* L.) are tied together around the forearm of the patient for allaying fever.

Anogeissus latifolia (Roxb. ex DC.) Wall. ex Guill. & Perr. (Combretaceae), 'Dho', Rajdari (*SMPUA 7820*). About 50 g powdered stem bark is boiled in water; the resulting decoction is given to treat cough.

Blumea membranacea DC. (Asteraceae), 'Kukrounda', Chakia (*SMPUA 7848*). Leaf paste is applied locally on boils. It is also used to check the bleeding in fresh cut and wounds.

Butea monosperma (Lam.) Taub. (Fabaceae), 'Dhak', Chakia (*SMPUA 7893*). To prevent scars in chicken pox, lukewarm decoction of the flowers is used to take bath.

Carica papaya L. (Caricaceae), 'Papita', Sapahi (*SMPUA 8022*). Fresh latex of this cultivated plant is applied over the skin in fungal infection.

Cissampelos pareira L. (Menispermaceae), 'Bhikma', Chakia (*SMPUA 7902*). The paste of 10 g root, obtained by crushing, is given orally 2-3 times a day for three days to treat common fever.

Cissus quadrangula L. (Vitaceae), 'Hadjor', Mughalsarai (*SMPUA 7943*). A paste of the plant is mixed with little powder of alum and plastered around the fractured limb after setting the bones right.

Clerodendrum viscosum Vent (Verbenaceae), 'Bhot', Sapahi (*SMPUA 7920*). Tender twig is made into toothbrush and used daily to prevent tooth decay and pyorrhoea.

Cuscuta reflexa Roxb. (Cuscutaceae), 'Akashbanwar', Chahania (*SMPUA 7871*). The paste of the plant is applied on scrotum sac for hydrocele.

Dalbergia sissoo Roxb. (Fabaceae), 'Shisham', Chakia (SMPUA 7960). Leaf paste is applid locally for fungal infection of groin.

Diospyros melanoxylon Roxb. (Ebenaceae), 'Tendu', Rajdari (*SMPUA 7822*). Ripe fruits are taken at bedtime for treating constipation.

Echinops echinatus Roxb. (Asteraceae), 'Untkanta', Mubarakpur (*SMPUA 7985*). Fresh leaf juice is instilled for removing redness of eyes.

Euphorbia nivulla Buch.-Ham. (Euphorbiaceae), 'Sehund', Chandrapirbha (*SMPUA 7828*). Two to three spoonful of aqueous decoction of dried leaves are given orally to children twice a day for 3-5 days to treat cough and common cold.

Euphorbia tirucalli L. (Euphorbiaceae), 'Nerya', Chahania (*SMPUA 7872*). Aerial parts are cut into small pieces, boiled in mustard oil, then filtered and allowed to cool: this oil is applied on painful joints twice a day till the cure is obtained.

Ficus rumphii Blume (Moraceae), 'Girra', Chakia (*SMPUA 7815*). Fresh latex, obtained from the leaf, is applied to an aching tooth.

Gardenia turgida Roxb. (Rubiaceae), 'Kharhar', Chandrapirbha (*SMPUA 7929*). To prevent from epidemic diseases, cattle are tied with the trunk of the plant or with a peg prepared from the long stem.

Grewia hirsuta Vahl (Tiliaceae), 'Ghursakri', Chandrapirbha (*SMPUA 7819*). Leaf paste in the dose of 15 g is given orally two times a day for one month to treat spermatorrhoea.

Hemidesmus indicus (L.) R. Br. (Asclepiadaceae), 'Kapuri', Chandrapirbha (*SMPUA 7899*). About 20 g of the root paste are given with water twice a day for 5 days to treat burning micturition.

Holarrhena pubescens (Buch.-Ham.) Wall. ex. G. Don (Apocynaceae), 'Kuraiyya' and 'Dudhkhira', Chakia (*SMPUA 7806*). Fresh stem bark paste (15 g) is given with water three times a day for one week to treat sunstroke.

Ipomoea carnea Jacq. ssp. *fistulosa* (Mart. ex Choisy) D. Austin (Convolvulaceae), 'Behaya', Jaimohini (*SMPUA 7898*). Leaf paste is applied as poultice for traumatic pain.

Lannea coromandelica (Houtt.) Merrill (Anacardiaceae), 'Jigna', Naugarh (*SMPUA 7939*). Decoction of about 50 g powdered stem bark is given orally for cough and common cold.

Leucas cephalotes (Koen. ex Roth) Spreng. (Lamiaceae), 'Goom', Rajdari (*SMPUA 7825*). Leaf juice is used as an ear drop for earache.

Litsea glutinosa (Lour.) C.B. Robinson (Lauraceae), 'Medh', Chandrapirbha (*SMPUA 7927*). Fresh inner stem bark is ground and used as plaster to treat bone fracture and sprain.

Manilkara hexandra (Roxb.) Dub. (Sapotaceae), 'Khirna', Chakia (*SMPUA 7897*). Ripe fruits are taken for constipation.

Murraya paniculata (L.) Jack (Rutaceae), 'Ainthil', Chandrapirbha (*SMPUA 7827*). Ghee of the sheep is applied on a long stick prepared from the stem and used as walking stick by the patient suffering from fungal infection in palm.

Nyctanthus arbor-tristis L. (Oleaceae), 'Paraajata', Chakia (*SMPUA 7835*). Equal quantities of leaves and flowers are boiled in water and cooled. The resulting decoction is given for cough with fever.

Opuntia dillenii (Ker-Gawler) Haworth (Cactaceae), 'Naghphan', Mughalsarai (*SMPUA 7947*). Two to three spoonful of juice, obtained by heating the phylloclade, are given orally with honey for cough and cold.

Pongamia pinnata (L.) Pierre (Fabaceae), 'Kanji', Sakaldiha (*SMPUA 7864*). Tender twig of stem is made into toothbrush and used twice daily for foul odour of the mouth.

Rauvolfia tetraphylla L. (Apocynaceae), 'Dholbarua', Chakia (*SMPUA 7836*). Root paste (10 g) mixed with little crystalline sugar and given three times a day for 15 days for treating jaundice.

Selaginella bryopteris Baker (Selaginellaceae), 'Patharchati', Chakia (*SMPUA 7809*). About 12-15 g of the leaf paste are given orally twice daily for one month to treat spermatorrhoea.

Sida cordifolia L. (Malvaceae), 'Faridbuti', Naugarh (*SMPUA 7938*). About 10 g of the leaf paste are given early in the morning for 15 days to treat menorrhagia.

Ventilago denticulata Willd. (Rhamnaceae), 'Keoti', Rajdari (*SMPUA 7830*). Seed oil is applied on the body to treat sunstroke. It is also used against skin disorders.

Vitex leucoxylon L.f. (Verbenaceae), 'Meyor', Latif Shah (*SMPUA 8005*). A paste is prepared by grinding of the leaves of 'meyor' and 'har' (fruits of *Terminallia chebula* Retz.) and applied on wounds of cattle.

Zingiber squarrosum Roxb. (Zingiberaceae), 'Banhaldi', Chandrapirbha (*SMPUA 8021*). Root paste as poultice is applied on painful joints.

Discussion and Conclusions

The present study on Chandouli district forests of eastern Uttar Pradesh has led to the documentation of 35 taxa belonging to 29 families that are commonly used by the natives as folk drugs for treatment of common illnesses and injuries like boils, bone fracture, burning micturition, constipation, cough, cold, cut and wounds, earache, fungal infection, jaundice, joint pain, spermatorrhoea, toothache, etc. The data are authentic and based on direct field interviews of reliable informants. These herbal

remedies are in use since time immemorial and have wide local acceptability. Furthermore, on comparison with the pertinent literature (Anonymous, 1948-1976, 2001; Chopra et al., 1956; Jain, 1991; Kirtikar and Basu, 1935; Nadkarni, 1954; etc.), it was found that some of these uses are new and interesting and enrich our existing traditional and contemporary ethnobotanical knowledge. These reported claims need scientific testing as information given by the informants is based on traditional wisdom.

Such observations have special utility and significance from drug discovery point of view. As these may provide a useful lead in the development and discovery of new plant-based pharmaceuticals. Therefore, urgent scientific field surveys should be conducted among the traditional societies of ethnobotanically unexplored or under-explored areas of the country in order to document the wealth of knowledge on folk medicine before it will be lost by the erosive effect of modernization.

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The Effect of Unani Aphrodisiac Agent Khulanjan (*Alpinia galanga* Root) on Potency in Normal Male Rats.

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Abstract

One of the advantages of Unani Medicine over Western Medicine is that it claims to possess many effective and safe Sexual Function Improving (Aphrodisiac) drugs. However, most of them have not been studied scientifically. One such drug, Khulanjan (*Alpinia galanga*) was shown by us to improve sexual function in normal male rats, with some indication that it increases Potency. Therefore, in the present it was subjected to a specific test (Penile Reflexes Test) for Potency in normal male rats. The drug was shown to significantly increase the penile reflexes. Thus, the drug was shown to possess Potency-improving effect in normal rats. The study therefore, scientifically corroborated the Unani claim about Sexual Function Improving Effect of Khulanjan and also indicates that the drug is likely to be effective also in sexual dysfunction due to organic causes.

Key Words: *Alpinia galanga*, Aphrodisiac, Impotence.

Introduction

One of the distinctive features of Tibb-e-Unani is that it possesses a large number of Sexual Function Improving (Aphrodisiac) drugs. On the other hand Western Medicine lacks such agents. So it would be expected that this group of drugs would be studied on a preferential basis like other classes of drugs of traditional medicines that are not available in Western Medicines such as Hepato-protective agents, Adaptogens etc. But quite surprisingly the Unani drugs claimed to improve the Sexual Function have been completely ignored during the process of scientific evaluation of the drugs of Traditional Medicines.

The first Unani preparation to be thus studied was a combination of three Unani drugs reported to possess Moqawwi-e-Bah (Sexual Function Improving Action) (Ibn Baytar, 1197-1248 CE), namely, Salab-Misri (*Orchis latifolia*, root), Tukhm-e-Konch (*Mucuna pruriens*, seed) and Khulanjan (*Alpinia galanga,* root).The study demonstrated highly remarkable action in the formulation (Ambekar et al, 1991). In a subsequent study, one of the three drugs, namely, Khulanjan (*Alpinia galanga,* root), was individually studied for effect on mating behaviour and androgenic effect in sexually normal male rats (Khan et al, 1993). The drug was shown to produce a significant increase in all parameters of mating behaviour.

Despite being an over-all entity the sexual function can be usefully conceived to be comprised of certain elements, chiefly Potency and Libido.

Although, the general mating behaviour also provides some indication about Potency and Libido as individual elements, Intromission Frequency and Mounting Frequency being the markers for them, respectively, (Davidson, 1982) but it would be better to study them by independent tests.

The study of the effect of Khulanjan on general mating behaviour of rats indicated it to improve both Potency and Libido as it significantly increased both Intromission Frequency and Mounting Frequency (Khan et al, 1993). However, in order to confirm its effect specifically on Potency, in the present study it was tested by a specific test for Potency, namely, the Penile Reflexes Test according to the method of Hart and Haugen (1968) and Hart (1979) modified by us (Ambekar et al, 1991).

Material and Methods

Preparation of Extract

The test drug, i.e., Khulanjan (*Alpinia galanga*, root) was procured from Dawakhana Tibbia College, A.M.U, Aligarh.The identity of the drug was confirmed pharmacognostically.The test drug was powdered and stored in air tight glass container and was administered in the form and by the route used in Tibb-e- Unani, i.e., the whole crude herb by oral route. The test drug was dissolved in distilled water immediately before the administration .The dose of the drug was also also comparable with its Unani clinical dose. The human dose described in Unani texts was multiplied by appropriate conversion factors (Dhawan, 1982) to obtain animal dose expected to be equivalent to the former. The dose determined was 100 mg/ 100 gm body weight/ day, for albino rats.

Penile Reflexes Test

The test was carried out according to the methods of Hart and Haugen (1968) and Hart (1979). Twenty male albino rats were used. They were divided into 2 groups of 5 animals each. The animals in Gp.II were administered with Khulanjan (*A. galanga*) in the dose of 100 mg/100 gm body weight, orally once a days for 7 days. The control group (Gp. I) was administered with equal volume of distilled water. On the 8 th day the test for Penile Reflexes was carried out by placing the rat on its back in a glass cylinder for partial restraint. The preputial sheath was pushed behind the glans of penis by means of thumb and index finger and held in this manner for a period of 15 minutes. Such stimulation elicites a cluster of genital reflexes. The following components of cluster of reflexes were recorded.

Erections (E) Quick Flips (QF) and Long Flips (LF). The frequency of these parameters observed in control and test group were statistically analysed and compared by the Student's 't' Test. The three reflexes were also aggregated to obtain the total penile reflexes, which were also analysed in the same manner.

Observations and Results

Penile Reflexes Test

The effect of the 3 test drugs on Penile Reflexes was evaluated by the methods of Hart and Haugen (1968) and Hart (1979) modified by Amin etal (1991) .Twenty albino rats were divided into 2 groups of 5 animals each.

The mean Erection (E.) in the control group were 10.00 ± 1.59 and in the animals treated with Khulanjan (*A. galanga*) the mean Erection (E.) were 14.60± 0.67 ($p<0.05$). Thus it can be seen that all the test drug produces a significant increase in Erections. The mean score of Quick flips (Q.F) was found to be 5.80 ± 0.92 in the control animals and 7.20 ± 0.58 in the animals treated with Khulanjan. The mean frequency of Long Flips (L.F) was found to be 2.8 ± 0.16 in the control group animals and 5.40 ± 0.92 in the test animals. Thus, although Khulanjan was seen to increase Quick Flips and Long Flips but this increase was not significant statistically. The mean aggregate penile reflexes were found to be 19.4 ± 6.74 in the control animals while they were increased to 27.2 ± 1.72 ($p<0.001$). Thus, the test drug was shown to significantly increase the aggregate penile reflexes. The results are presented in Table-1.

Discussion

One of the important principles of modern research in Tibb-e-Unani and other systems of traditional medicine is to give priority to the study of the drugs and actions whose counterparts are lacking in main-stream medicine (Nitya Anand, 1989).Hepato-protective action, adaptogenic action and anti-arthritic action etc. have been identified as the top priority areas of research in the traditional medicine in the light of this principle. One such field is that of Sexual Function Improving Action. In one study of healthy, married, middle aged men 40% were

Table-1. Effect Of Khulanjan (*Alpinia Galanga* Root) On Penile Reflexes (Potency) In Male Rats

S.No.	Groups	Mean Frequency ± S.E.			
		Erections (E.)	Quick Flips (Q.F.)	Long Flips (L.F.)	Total
1.	Control	10.00 ± 1.59	5.00 ± 0.92	2.0 ±0.16	19.4 6.74
2.	*A.galanga*	14.60 ± 0.67*	7.20 ± 0.58	5.40 ± 0.92	27.2 ± 1.72**

* = $p<0.05$

** = $p<0.001$

found to have sexual dysfunction, primarily impotence and premature ejaculation (Reich, 1987). So it can be seen that male Sexual dysfunction is a problem of gigantic proportion.

Therefore, we initially subjected a combination of the three Unani drugs namely, Salab-Misri (*Orchis latifolia*, root), Tukhm-e-Konch (*Mucuna pruriens*, seed) and Khulanjan (*Alpinia galanja,* root).The study demonstrated highly remarkable action in the formulation (Ambekar et al, 1991). In a subsequent study, one of the three drugs, namely, Khulanjan (*Alpinia galanga,* root), was individually studied for effect on mating behaviour (Khan et al, 1993). The drug was shown to produce a significant increase in all parameters of mating behaviour. Although, the male sexual function is a holistic phenomenon but it can be analysed to comprise of certain distinct elements, two of them being Potency and Libido, which have somewhat different physiological and molecular bases. The therapeutic interventions in their dysfunction too, though overlapping, have some differences. So, it is useful to assess the effect of a therapy specifically on Potency etc. The general mating behaviour provides some indication about Potency. The Intromission Frequency is considered to be a marker of Potency (Davidso0n, 1982). Thus, Khulanjan, which significantly increased Intromission Frequency, was indicated to augment Potency of male rats (Khan et al, 1993). However, the specific effect on Potency should preferably be confirmed by specific tests for this aspect of sexual function. We utilized two tests for Potency carried out in the area of animal physiology to develop a specific test for studying the effect of drugs on Potency and used it to study Khulanjan for Potency-increasing effect.

Our study showed Khulanjan to significantly increase the aggregate of all types of penile reflexes in the rat. As mentioned above, an earlier study (Khan et al, 1993) also provided some indication about the Potency-increasing effect of Khulanjan. So, it can be rather conclusively surmised that Khulanjan possesses Potency-improving effect. The proportion of Erections and Long Flips was greater than Quick Flips in the aggregate of Penile Reflexes. Thus, the study provides some indication that the test drug increases Potency in a manner that would clearly improve the actual sexual performance.

Since, the drug is specifically shown to improve the component of Potency in the Sexual Function; it is likely to be effective also in cases where the sexual function is compromised due to organic causes or non-psychogenic Impotence. Secondly, as the test drug was shown to augment Potency in normal male rats, it is likely that it will produce a striking increase in hypo-sexual subjects.

The study, therefore, provides scientific validation for the Unani claim that Khulanjan (*Alpinia galanga* root) possesses Muqawwi-e-Bah (Sexual Function Improving) Effect. It also shows the drug to specifically improve the component of Potency in Sexual Function.

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Pharmacognostic Studies on the Roots of *Cissampelos pareira* Linn.

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Abstract

Pharmacognostic studies on the roots of *Cissampelos pareira* Linn. has been carried out to lay down standards for the genuine drug. The diagnostic characters of dried roots in microscopy reveal anomalous secondary growth, sclerenchymatous cells forming more or less discontinuous ring outside the patches of secondary phloem, secondary vascular strands in the form of radiating strips alternating with medullary rays, simple starch grains and prismatic calcium oxalate crystals. Other parameters studied include physico-chemical constants, fluorescence behavior, U.V. Spectrophotometry, Chromatography etc.

Key Words: *Cissampelos pareira* Linn., Pharmacognosy, Drug Standardisation, Patha.

Introduction

Cissampelos pareira Linn. (Family-Menispermaceae) in trade is known as 'Patha'. The drug consists of dried roots of this twining perennial shrub. The roots are also commercially exploited as 'false pareira brava'. However, the true 'pareira brava' is reported to be derived from *Chondodendron tomentosum* Ruiz et Par. (Family- Menis-permaceae), which is a tropical African species. *Cissampelos* Linn. genus (*Kissos*-ivy and *ampelos*-a vine) have the characters of ivy in its resembling branches that of the vine from the fruits being in recemes. *C. pareira* Linn. is native of south America. The leaves of the plants are applied to abscess. *C. pareira* Linn. is anthelmintic, antidote to poison, antilithic, astringent, cardiac, carminative, diuretic, expectorant, febrifuge, sedative, supportive and toxic in action. It is medicinally used for asthma, cold and cough, colic, diarrhoea and dysentery, fever, indigestion, inflammatory affections of the bladder and kidney (chronic cystitis), nephritic disorders, piles and ulcers. Some authors equate 'Laghupatha' with *C. pareira* Linn. and 'Patha' with *Stephania hernandifolia* Walp. In South India *C. pareira* Linn. is accepted as medicinal plant, but not as 'Patha'. *Cyclea peltala* Diels and other allied species belonging to family Manispermaceae are used as patha (Herman, 1868, Kirtikar & Basu, 1933; Anonymous, 1950, Aiyer and Kolamal, 1953-66; Chopra *et al*. 1956; Day, 1980).

Methodology

Drug samples were collected from different places with a view to find out any significant difference present within the same species. Hand sections were stained and mounted in Canada balsam for anatomical studies. Lignification on smoothed cross-surfaces was studied with phloroglucinol-HCl. For studying powder, Jackson

and Snowdon (1968) was followed. To determine physico-chemical constants, Indian Pharmacopoeia (Anonymous, 1966) was consulted and for fluorescence study schedules mentioned by Trease and Evans (1972) were followed. Colours were named by consulting Rayner (1970). Standard prescribed procedures for Histochemical studies (Johanson, 1940; Youngken, 1951; Cromwell, 1955, Trease and Evans, 1978), Organic group detection (Robinson, 1963), Elemental quantitation (Khan, *et. al*., 1985), U.V. Spectrophotometry (Willard, *et. al*., 1965) and Chromatography (Shellard, 1968, Stahl, 1969, Smith and Feinberg, 1972) were adopted.

Systematics

Family: Menispermaceae. Endl. Gen.825, Lindl. Veg. Kingd. 307, Gen. Pl. I: 30.

The family is represented by about 65 genera and 350 species of warmer regions of the world. In India, 17 genera and 42 species are distributed chiefly in tropical and sub-tropical parts.

Genus: *Cissampelos* Linn. Gen. n.1138, Gen. Pl. I: 37, FBI 1:103.

There are thirty species of this genus which are distributed in tropics. In India, this genus is represented by only one species. This species is distributed throughout topical and sub-tropical India.

C. pareira Linn. Sp. P1.1031, 1753, FBI 1:103, FUGP 1: 30, FFSC 18; Diels, Pfrieich 46: 286, 1910; Forman, Kew Bull. 22: 356, 1968, Parker, For. Fl. 11; FD 52.

Synonyms: *C. convolvulacea* Willd., *C. cumingiana* Turcz., *C. discolor* DC., *C. discolor* DC. var. *cardiophylla* Gray, *C. mucronata* Rich.

A slender, twining and perennial shrub, Leaves triangularly broad cordate, obtuse to retuse, usually peltate, mature ones glabrous above, glacous beneath. Flowers pale green, minute unisexual, the male and female occurring on different plants. Male flowers in axillary, facicled, pilose cymes, bracts linear. Sepals subequal, obovate spatulate. Petals connate into a cup shaped structure, hairy without glabrous within staminal colum. Female flowers usually solitary and subsessile or shortly stalked sepals long. Carpels 1, style short, 3 fid or toothed. Fruits drupe, ovoid-subglobose, red when ripen, pilose with sub basal persistant stylar scars (Plate-I).

Flowering and Fruiting: April-August,

Fruiting: September-November.

Distribution: Common throughout the tropical and subtropical parts of India. It is cosmopolitan in warm countries. (Anonymous, 1976).

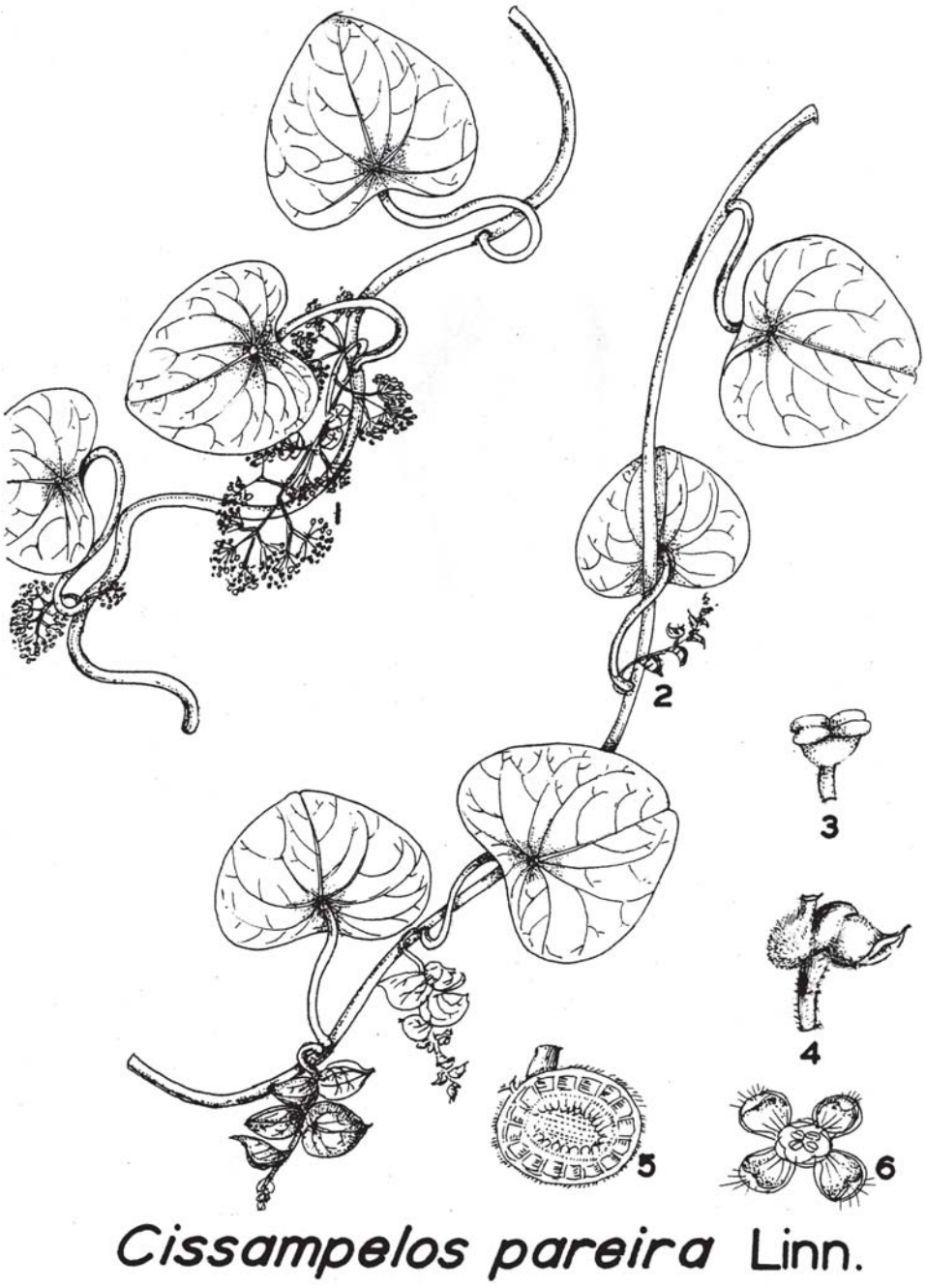

Plate-I. Habit and taxonomic details of drug plant (*Cissampelos pareira* Linn.)

1. Flowering twig of male plant, 2. Flowering twig of female plant, 3. Stamen, 4. Female flower, 5. Fruit and 6. Male flower.

Observations

I. Organoleptic Characteristics

A. The drug occurs in the from of dried, cylindrical pieces of perennial and seldom branched matured tap roots. The drug varies in size and measures 15.0-24.0 cm in length and 1.0-2.5 cm in diameter. The pieces of roots obtained from the closer portion of shoot system are woody in comparison to other portions obtained from deeper parts of the root. The other portions are generally more fleshy and tuberous. The dried roots are brownish to grey in colour, corky in texture, compressed, entire or splitted longitudinally. The external minute pits and wavy. It also shows vertically branched cracks or fissures. The older pieces of drug exhibits longitudinally ridged surface with transverse cracks (Plate-II). The fracture of the root is short and splintery. There is faint aromatic odour. The taste is at first sweetish and then bitter.

B. Powdered Drug: The powdered drug is brown in colour with faint aromatic odour. It has bitter taste which is at first sweetish on chewing.

II. Micro-Morphological Characteristics

A. Transverse section of matured roots is wavy in outline (Plate-III). Phellem consists of six to ten layers of tangentially elongated, radially arranged thin walled cells. Outer few layers of phellem are crushed. Phellogen consists of one or two layers of narrow, elongated, and thin walled cells. The phellogen is followed by phelloderm which is a narrow zone consisting of three to six rows of oval to tangentially

Plate-II. Macrosopical feature of drug (Dried roots of *Cissampelos pareira* Linn.)

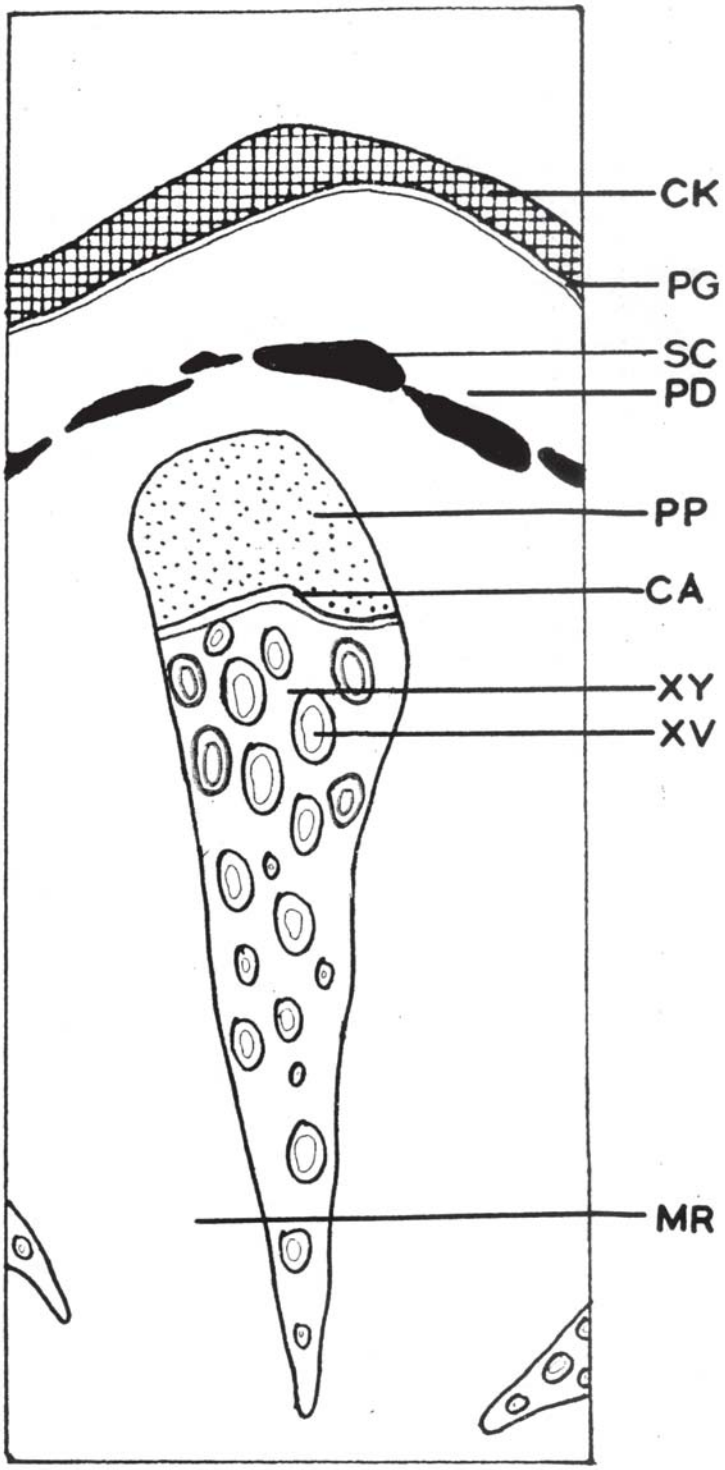

Plate-III. Diagramatic representation of transection of drug (Dried roots *Cissampelos pareira* Linn.), 25 X

Abbreviations: CA-Cambium, CK-Phellem, MR- Medullary ray, PG-Phellogen, PD-Phelloderm, PP-Secondary phloem, SC-Stone cells, XV-Xylem vessels and XY- Secondary xylem.

elongated cells (Plate-IV A). These cells are compactly arranged and some of the cells contain starch grains and prismatic crystals of calcium oxalate. The starch grains are simple and compound with three to five components. The simple starch grains are round to oval in shape and some of them are cup shaped with small break. Towards the inner side of phelloderm a few cells are lignified and are grouped in two to three layers adjoining the pericycle and appear as a discontinuous ring of sclerenchymatous cells (Plate-IV B). In the more matured roots these groups of sclerenchymatous cells are bridged together and appear to be a complete ring. Some times a few phloem fibres are also associated with these cells. These selerenchymatous cells vary in shape and size being cubical rounded to oval and tangentialy elongated with thick lignified walls having simple pits. The stele is broad in old roots and occupies the entire area below the groups of selerechymatous cells. It consists of three to twelve radiating strips of vascular strands with masses of madullary rays (Plate-IV B, C, D). Strips of vascular strands comprising phloem and xylem vary in radial length. Longer vascular strands encroach upto the center of the root, while smaller strands which are newly formed are restricted at the periphery or approaching towards center. The phloem which is just below the selerenchymatous ring consists of small strands of phloem parenchyma, sieve tubes, companion cells and few phloem fibres. (Plate IV B). In the phloem region parenchymatous cells are thin walled, circular to polyhedral with patches of sieve tubes accompanied by companion cells in somewhat collapsed from. Most of the phloem parenchyma cells contain starch grains and calcium oxalate crystals similar to those found in phelloderm region.The xylem is composed of a number of converging wedges separated by broad medullary rays and consists of vessels, trachieds, fibres and xylem parenchyma (Plate IV E). The vessels are usually drum shaped, wide and have straight or oblique articulations with simple pits on their walls. Trachieds have tapering ends with simple and bordered pits. Xylem fibres are elongated having pointed ends and show simple pits on their walls. Lignified xylem parenchyma is thick walled, pitted and rectangular in shape. Most of these cells also contain starch grains and calcium oxalate crystals identical to that of phelloderm region. The cambium in vascular strands generally appears to be compressed or collapsed. Medullary rays are uniseriate to triseriate and are much wider than strands of xylem and phloem. The medullary ray cells are mostly thin walled, non-lignified and a few of ray cells are lignified and thick walled. The ray cells are rectangular and radially elongated in xylem part and tangentially extended in the phloem region. Some of the ray cells also show recticulate thickening. Starch grains are also present in ray cells and are identical in nature to that of phelloderm region.

Microscopical measurements of individual cells of different tissues and cells contents in microns are give below (Table 1).

b. Powdered Drug: The histological elements of powdered drug includes fairly common fragments of phellem which occur in both surface and transactional view, thin walled phelloderm cells containing starch grains and occasional crystals of calcium oxalate, small groups of selerenchymatous cells which are not abundant

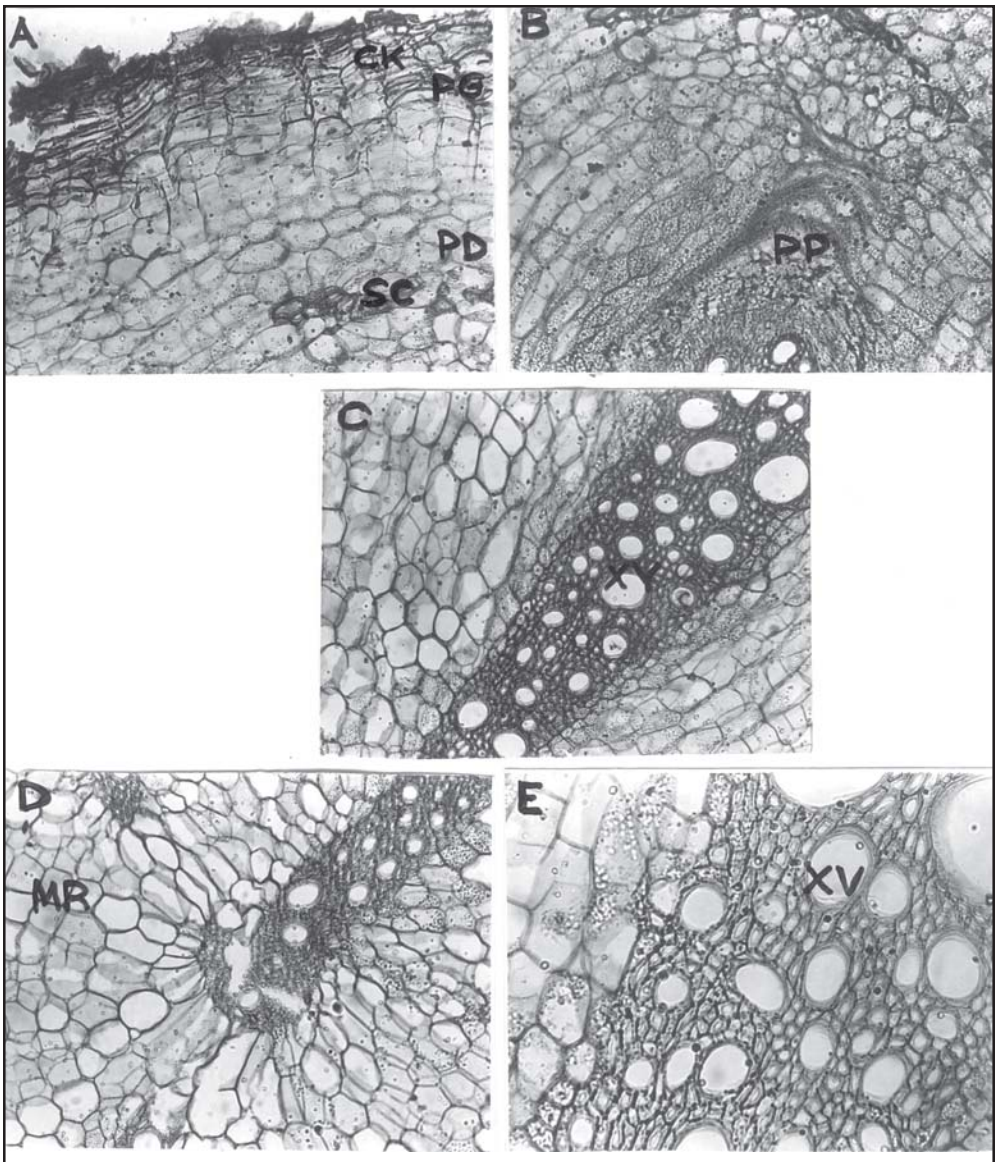

Plate-IV. Photomicrographs of transection of drug (Dried roots of *Cissampelos pareira* Linn.)

Transection of drug showing a portion of –

A. Periderm (phellem, phellogen and phelloderm), 600 X.

B. Discontinuous ring of sclernchymatous cells and part of radiating strip of vascular strand comprising of secondary phloem, compressed cambium and secondary xylem, 600 X.

C & D. Part of radiating strip of vascular strand comprising secondary xylem and medullary ray, 600 X.

E. Enlarged portion of secondary xylem and medullary ray cells, 1200 X.

Abbreviations: CK-Phellem, MR- Medullary ray, PD-Phelloderm, PG-Phellogen, PP-Secondary phloem, SC-Sclerenchymatous cells, XV-Xylem vessels and XY- Secondary phloem.

Table 1. Dimensional data of Cellular elements in transactions and cell contents.

Sl.No.	Cellular Elements/Cell Contents	Measurements in microns
1.	Phellem cells	20.0-60.2 x 12.1-33.0
2.	Phellogen cells	33.2-70.5 x 9.0-14.8
3.	Phelloderm cells	40.0-100.8 x 30.0-48.0
4.	Sclerenchymatous cells	28.2-72.0 x 12.0 x 36.2
5.	Phloem parenchyma cells	9.0-36.2 x 5.4-20.2
6.	Cambium cells	16.0-32.2 x 8.0-17.0
7.	Xylem vessels	16.2-106.5 x 12.0-84.8
8.	Medullary ray cells	42.0-72.6 x 36.0-62.0
9.	Starch grains	5.0-7.2 (D)
10.	Calcium oxalate crystals	3.0-10.0x2.5-5.5

Abbreviation: 'D' – Diameter

and fairly common rectangular, thin walled medullary ray cells, containing starch grains. The vessels and trachieds are found singly or in groups but usually fragmented. Vessels have articulations with simple pits on wall. starch grains are mostly simple and some of them are compound with three to five components. Individual starch grains are round to oval some of them are cup shaped. The occasional calcium oxalate crystals are found scattered or enclosed in cells and are usually in the form of single prisms.

III. Histochemistry

A. Micro-Chemical Tests and Behaviour of specific reagents towards Plant/Drug Tissues: Observations and results pertaining to micro-chemical tests and behaviour of specific reagent towards plant tissues are presented in Table-2.

B. Organic Groups of Chemical Constituents – The extracts of the drug were tested for presence of different organic groups and results are presented in Table-3.

IV. Identity, Purity & Strength

A. Physico-Chemical Constants: The analytical values in respect of physico-chemical constant of drug were established and results are reported in Table-4.

B. Medicinal Inorganic Elements: The quantitative data in respect of medicinal inorganic elements detected in drug are presented in Table-5.

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

Sl. No.	Reagent	Test for	Infe-rence	Histological zone/cell contents responded.
1.	Dragendorff's reagent	Alkaloids	+	Most of the cells of phelloderm, phloem, xylem and few medullary ray cells.
2.	Marme's reagent	Alkaloids	+	Same as above.
3.	Wagner's reagent	Alkaloids	+	Same as above.
4.	Potassium hydroxide solution (5% w/v)	Anthocynin	-	Not Responded
5.	Sulphuric acid (66% v/v)	Anthocynin	-	Not Responded
6.	Acetic acid	Calcium oxalate	+	Prismatic calcium oxalate crystals.
7.	Potassium hydroxide solution (5% v/v) + Hydrochloric acid	Calcium oxalate	+	Same as above.
8.	Sulphuric acid	Calcium oxalate	+	Same as above.
9.	Kedde reagent	Cardiac glycoside	-	Not Responded
10.	Iodine Solution followed by Sulphuric acid	Cellulose	+	Cells of phelloderm, certain cells of phloem xylem and few medullary ray cells.
11.	Sudan III	Fixed oil and fats	-	Not Responded
12.	Chlor-zinc-Iodine Solution	Latex	-	Not Responded
13.	Aniline sulphate Solution followed by Sulphuric acid	Lignin	+	Most of the Cells of phellem, sclerenchymatous cells, phloem, few cells of xylem, vessels and medullary ray cells.
14.	Phloroglucinol HCl	Lignin	+	Same as above
15.	Lugol's solution	Protein	+	Most of the cells of phelloderm, phloem and xylem.
16.	Millon's reagent	Protein	+	Same as above
17.	Picric acid	Protein	+	Same as above
18.	Heating with KOH (5% w/v) + H_zSO_4	Suberin	+	A few of cells of phellem
19.	Sudan III	Suberin	+	Same as above
20.	Weak Iodine solution	Starch	+	Starch grains
21.	Potassium hydroxide solution (5% w/v)	Starch	+	Same as above
22.	Sulphuric acid	Starch	+	Same as above

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

Table-3. Major Group of Organic Chemical Constituents of Drug.

Sl. No.	Organic Groups of Chemical Constituents	Reagents/Tests	Inference
1.	Alkaloid	Dragendorff's and Mayer's reagents	+
2.	Anthraquinone	Borntrager reaction	+
3.	Coumarin	Alcoholic potassium hydroxide	-
4.	Flavonoid	Shinoda reaction	+
5.	Glycoside	Mollisch's test	+
6.	Protein	Xanthoprotein test	+
7.	Resin	Ferric chloride regent	+
8.	Saponin	Libermann-Burchard reaction	+
9.	Steroid	Salkowski reaction	+
10.	Tannin	Gelation test	+

Table-4. Analytical Values of Physico-chemical Constants

Sl.No.	Physico-Chemical Constants	Analytical values
1.	Moisture content, % w/w	6.5
2.	pH	6.4
3.	Crude fibre, % w/w	15.5
4.	Total Ash, % w/w	5.0
5.	Acid insoluble ash, % w/w	1.6
6.	Alcohol soluble extractive % w/w	5.2
7.	Water soluble extractive % w/w	16.0
8.	Total Alkaloids, % v/w	3.2

V. Fluorescence & Spectroscopy

A. Fluorescence Characteristic of Powdered drug under Ultra-Violet Light: Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight and under ultra-violet light were noticed and are presented in Table-6.

Table-5. Quantitative estimation of Medicinal Inorganic Elements.

Sl.No.	Physico-Chemical Constants	Analytical values Mg/g of ash
1.	Cadmium	0.0011
2.	Calcium	0.2620
3.	Copper	0.2240
4.	Iron	1.1482
5.	Magnesium	0.4000
6.	Manganese	0.0173
7.	Nickle	0.0650
8.	Potassium	10.0700
9.	Sodium	20.9321
10.	Zinc	0.0093

Table-6. Fluorescence Characteristic of Powdered Drug under Ultra-Violet Light.

Sl. No.	Treatments	Colour in day light	Nature of colour in fluorescence
1.	Powder as such	Brown	White
2.	Powder with		
	Carbon tetra chloride	Colourless	Colourless
	Ethyl acetate	Colourless	Colourless
	Hydrochloric acid	Brownish	Yellowish brown
	Nitric acid + water	Deep Orange	Greenish yellow
	Sodium hydroxide + methanol	Yellowish brown	Greenish yellow
	Sodium hydroxide + water	Yellowish brown	Yellowish brown
	Sulphuric acid + water	Brownish tinge	Yellowish tinge
	Buffer- pH 5	Brownish	Yellowish green
	Buffer- pH 7	Yellowish tinge	Yellowish green
	Buffer- pH 9	Brownish yellow	Bluish green

B. Ultra-Violet Spectroscopy: The data related to Ultra-Violet Spectrophotometric characteristics as computed in Table-7.

VI. Chromatography

A. Paper Chromatography: The amino acids and free sugars were resoluted and detected by paper chromatographic techniques. The comparison of Rf. Values of reference standards of different amino acids and free sugars confirms the presence of –

(i) Amino Acids – DL-2-Amino-n-butyric acid, L-Histidine monohydrochloride,

DL- Methionine, DL- Serine and DL- Threonine.

(ii) Free Sugars –Sucrose.

B. Thin-Layer Chromatography: Best separation for TLC fingerprinting were obtained by using different layers and solvent systems. Inferences are shown in Table-8.

Discussion

The previous investigations on the micro-morphology of the *Cissampelos pereira* Linn., have made some erroneous observations, which have been remarked by

Table-7. Ultra-Violet Spectrophotometer characteristic of drugs.

Sl.No.	Specifications	Data
1.	Tincture dilution ml/ml	0.02
2.	Maximum absorption peak	1.984, 2.065 & 0.852
3.	l Maxima at, nm	206 & 234 & 281

Table-8. TLC fingerprinting data

Sl.No.	Technical details	I	II
1.	Layer	Silica gel GF,	Silica gel GF, Buffered layer, NaOH, 0.1N
2.	Solvent system	n-butanol-acetic acid-water (3:1.5:5.5, v/v)	Methanol
3.	No. of spots	07	07
4.	h Rf. Values of visualised spots	12.7, 22.7, 30.9, 35.5, 41.8, 50.0 and 81.8	5.5, 11.0, 21.2, 59.2, 85.8, 88.1 and 95.2

Prasad *et al.* (1962). Datta and Mukerjee (1950) reported the scelerenchymatous ring in the secondary phloem and have shown a continuous ring of phloem in the diagram with the sclerenchymatous ring outside the secondary phloem, while present studies elucidate that the phloem occurs as strands not a continuous ring being separated by medullary rays. The authors also mentioned that the cells of phelloderm are like the cortical cells while no more cortex exists after the secondary growth. Besides, they also mentioned that xylem is composed of broad medulary rays, but rays are of two types - narrow uni-to triseriate medullary rays and broad multiseriate medullary rays composed from ray cells which are distinct from xylem tissues. However, medullary rays also include part of delignified xylem parenchyma often. Mukerjee (1953) has also made same erroneous statements. Aiyer and Kolamal (1953-66) have show the absence of starch grains and calcium oxalate crystals in phelloderm but reported in stone cells. The authors also mentioned that no elements of mechanical tissues are associated with phloem proper and that ridges in outline correspond the number to the wedge like masses of xylem elements. These observations are contrary to the findings of present studies. The findings of Prasad *et al* (1962) are in agreement with the observations of present studies and confirm the present findings.

Acknowledgement

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A Comparative Pharmacognostic Study of *Amaranthus spinosus* Linn. growing in non-polluted & polluted areas

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Abstract

Comparative pharmacognistic studies of *Amaranthus spinosus* Linn., growing in non-polluted & polluted areas are described. It has considerable food value and used in Ayurveda for gonorrhoea, menorrhoea, metorarrhagiae, stomach troubles, curing piles, leprosy and to control vomiting. Attempt is made to study the differences in pharmacognostic characters of plants, grown in polluted area. (Ester India Chemicals, Ghaziabad) and non polluted / cultivated lands. Total ash, insoluble ash, sulphated ash & alcoholic extractive are found more in plants which were grown in polluted areas. The preliminary colour reaction tests showed presence of alkaloids, tannin, carbohydrate, saponin, proteins, glucosides and absence of oils and flavanoids. The study also includes some other characters like stomatal index, palisade ratio, vein islet number, vein termination number etc. TLC and fluorescence behaviours.

Key Words: Pharmacognostic, *Amaranthus spinosus* L., Pollution.

Introduction

Amaranthus spinosus Linn. (Amaranthaceae) is an erect, 30 – 60 cm. high, much branched, spinosus herb varying in colour from green to red or purple and commonly known as **jangali chaulai**. The Plant has considerable food value and contains, moisture-85%; protein-3%; fat-0.3%; carbohydrates - 8.1% and mineral matter - 3.6%; Ca-0.8%; P-0.5%; Fe-22.9 mg/100gm (Wealth of India, 1950). It contains hentriacontane, a-spinosterol, a saponin mixture composed of oleanolic acid, D-glucose and D-glucuronic acid, b-sito-sterol stigmasterol, campsterol, cholesterol and steric, oleic and linoleic b-D glucopyranosyl, 1–3 – oleanolic acid (Kirtikar and Basu, 1935; Chopra *et al.,* 1956). It is an important medicinal plant of Ayurveda and used in many diseases like gonorrhoea, menorrohoea, uterine pains, stomach troubles and to stop bleeding & control vomiting. The pollens are used in allergic asthma (Kirtikar and Basu, 1935; Chopra *et al.,* 1956).

The detailed information on pharmacognostic study of this drug plant seems to be not available, particularly on the samples collected from polluted and non polluted areas. Hence, the present studies. This is with a view to check substandard quality of drug and adulteration in raw material in order to lay down correct botanical identification for its value as a crude drug.

Vernacular Names

Amaranthus spinosus Linn. have several names in different languages - Hindi - Jangli Chaulai; Sanskrit – Tanduliya; Bengali – Kanta halya; Marathi – kante math

Gujarati – Kantanudant; Tamil – Mulluk kirai; Kannad – Mullu Harive Soppu; Malyalam – Kattu – Mullen Kerru (Wealth of India, 1950).

Materials and Methods

The fresh material was collected from both sites non-polluted (ALTT Centre,Ghaziabad,India.) and polluted (Ester India Chemicals, Ghaziabad, India) area. For microscopical studies, material was fixed in F.A.A.

After conventional methods of dehydration, the material was embedded in paraffin wax in usual manner (Johansen, 1940). Serial microtome sections were cut and safararine & fast green combination was used in making DPX mount. For anatomical studies (Metecalfe, 1947); for powder studies (Jackson & Snowdon 1968) and for chemical analysis (Johansen, 1940; Youngken, 1951; Cromwell, 1955 & Trease and Evans, 1983) were followed. TLC was performed with the extract of plants.

The plant powder (5 gm.) was extracted with 25 ml. ethanol for 30 minutes on a water bath under reflux. The filtered extract concentrated to 10 ml. The extract was spotted on precoated silica gel G, plates and developed with chloroform: methanol, 9:1 solvent mixture. After development the spot were observed in day light and UV-light (254 and 365 nm.) and Rf values were calculated.

Results and Discussion

Macromorphology

It is an erect, 30-60 cm. high, much branched, spinosus herb. Stem is erect and cylindrical Leaves are ovate or deltoid ovate, obtuse, glabrous, usually notched at the apex and base truncate. Details are shown in table-1 (Plate-1).

Microscopical Study

The non-polluted stem shows single layer of epidermis covered with thin cuticle and both types of trichomes (glandular and non glandular), collenchyma 6-7 layered, Chlorenchyma 2-3 layered, Parenchyma 3-4 layered, Pericycle in patches, 3-4 layers of cambium, vascular bundles are arranged in continuous ring and contain phloem towards periphery and xylem towards centre; vessels with spiral & scalariform thicking. Medullary vascular bundles are conjoint, collateral, open and endarch. But in case of polluted plants stem contains, thick cuticle, collenchyma 3-4 layered, parenchyma 3-5 layered, chlorenchyma, endodermis and pericycle absent. Secondary phloem and cambium are in

Table-1. Macromorphological differences between *Amaranthus spinosus* Linn. growing in non- polluted and polluted areas.

Parameter	Non-Polluted	Polluted
Leaf colour	Dark green	Light green
Length of shoot (cm)	39.850 ± 12.910 cv = 32.396	26.140 ± 10.870 cv = 41.583
Length of root (cm)	13.000 ± 4.210 cv = 32.384	10.900 ± 3.680 cv = 33.761
No. of leaves/Plant	106.000 ± 26.250 cv = 24.764	81.600 ± 23.620 cv = 28.946
Leaf area (cm^2)	18.687 ± 12.884 cv = 69.086	11.062 ± 4.893 cv = 44.239
Spikes/plant	50.460 ± 8.921 cv = 17.677	41.500 ± 9.810 cv = 23.640
Petiole size (cm)	4.011 ± 1.366 cv = 33.915	2.500 ± 1.250 cv = 50.000
Lamina size (cm)	1.5 – 2.6	0.5 – 1.6

a b

PLATE-1

Morphological differences of *Amaranthus spinosus* Linn. Growing in non-polluted and polluted areas.

Fig. a. Plant growing in non-polluted areas.

Fig. b. Plant growing in polluted areas.

patches. Medullary vascular bundles are conjoint, collateral and close. Micro, rosette and acicular crystals of calcium oxalate are present. (Plate-2, fig. a & b)

Non-polluted leaf characterized by single layer of epidermis bearing glandular and non-glandular trichomes. Glandular trichomes contain 3-9 celled stalk and 1-2 celled head. Nonglandular trichomes are unicellular with warts, bicellular and multicellular. Stomata anomocytic and more frequent on lower surface. 1-2 layered collenchyma above the lower epidermis, 4 vascular bundles in midrib. Mesophyll differentiated into 1-2 layered palisade and 2-3 layers of spongy parenchyma. Micro, rosette and prismatic crystal of calcium oxalate present throughout the mesophyll. But the polluted leaf shows 3-4 layered palisade, single vascular bundle in mid rib and absence of bicellular trichomes, collenchyma & prismatic crystals. The vascular bundles of lamina contain bundle sheath in both the cases. The main differences are shown in table-2a & 2b (Plate-3, fig. 3a, b, c & d.)

Preliminary Chemical Studies

shows presence of alkaloids, saponin, tannin, lignin, protein, carbohydrate, suberin, glucosides and absence of flavanoids and oils. Differences in colour reaction tests are given in table-3 which shows easy and quick differentiation between plants grown in polluted and non polluted area.

Physical Evaluation

(a) Fluorescence behavior of the plant drug variously tested are presented in table-4.

(b) Ash values and Extractive values are present in table-5 & 6 respectively.

TLC

It was found that the number of chemical compounds were higher in non-polluted plant samples than the polluted plant samples. It is due to the more number of spots, 3 to 9 in non-polluted plants samples while 3 to 4 spots were found in polluted plants, presented in Table 7.

The above observations indicate that the percentage, number of the chemical constituents and biomass / cell size decreased in the plants which were grown in industrial areas. The above result matched with (Dhar et al 2003) and (Ghouse et al 1985).

We are accordingly inclined to conclude that the plants from non polluted areas should be taken for quality production, since all the parameters reflect declining values in plants taken from polluted area.

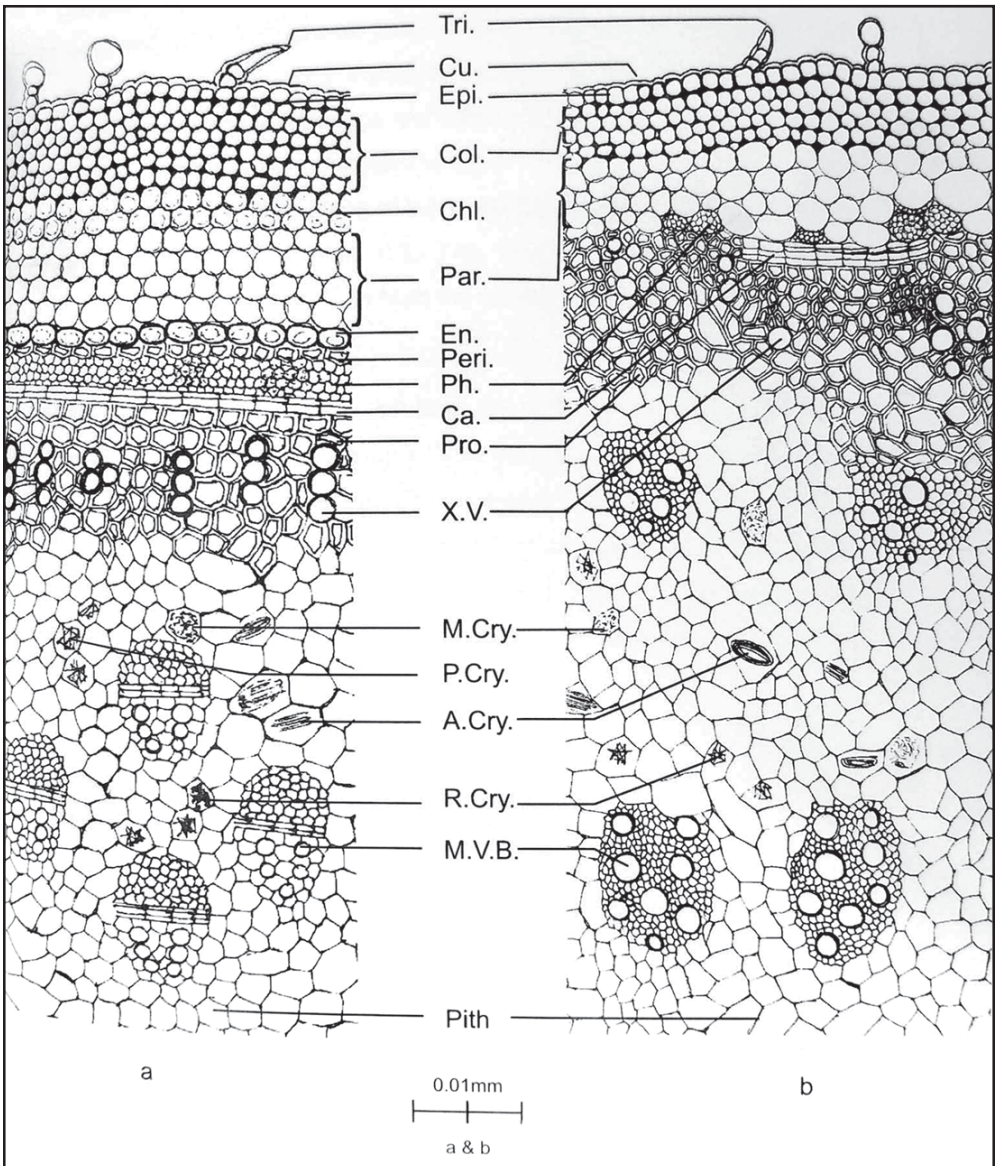

PLATE-2

Anatomical differences in the stem of *Amaranthus spinosus* Linn. Growing in non-polluted and polluted areas.

Fig.a- T. S. of stem of plant growing in non- polluted areas.

Fig.b- T. S. of stem of plant growing in polluted areas.

Abbreviations: A. Cry-acicularcrystals; B.S.-bundlesheath; Ca-cambium; Chl-chlorenchyma; Col-collenchyma; Cu-cuticle; En-endodermis; Epi-epidermis; M.Cry-microcrystals; M.V.B.-medullary vascular bundle; Par-parenchyma; P.Cry-prismatic crystals; Peri-pericycle; Ph-phloem; P.L.-palisadelayer; Pro-prosenchyma; R.Cry-rosettecrystals; S.Par-spongy parenchyma; Tri- trichomes; V.B.-vascular bundle; X.V.-xylem vessel.

Table-2 a. Anatomical difference of *Amaranthus spinosus* Linn. (Stem) growing in non-polluted and polluted areas.

Characters	Non-polluted	Polluted
(1) Trichomes (stem)	Present	Absent
(2) Collenchyma*	L = 0.045 ± 0.003 cv = 6.666 W = 0.030 ± 0.001 cv = 3.333	L = 0.041 ± 0.006 cv = 14.634 W = 0.030 ± 0.001 cv = 3.333
(3) Parenchyma*	L = 0.068 ± 0.005 cv = 10.294 W = 0.033 ± 0.006 cv = 18.181	L = 0.093 ± 0.019 cv = 20.430 W = 0.056 ± 0.012 cv = 21.428
(4) Endodermis	Single Layer	Absent
(5) Pericycle*	Group of patches L = 0.037 ± 0.005 cv = 13.514 W = 0.022 ± 0.007 cv = 31.818	absent
(6) Cambium	Continuous	Discontinious
(7) Phloem	Compressed	In group
(8) Prosenchyma*	L= 0.067 ± 0.002 cv = 2.985 W = 0.035 ± 0.006 cv = 17.142	L= 0.048 ± 0.001 cv = 2.083 W = 0.032 ± 0.004 cv = 12.500
(9) Xylem vessels*	L = 0.049 ± 0.001 cv = 2.040 W = 0.034 ± 0.001 cv = 2.941	L = 0.048 ± 0.012 cv = 25.000 W = 0.033 ± 0.012 cv = 36.363
(10) Pith cells*	L = 0.097 ± 0.006 cv = 6.185 W = 0.078 ± 0.008 cv = 10.256	L = 0.078 ± 0.026 cv = 33.333 W = 0.075 ± 0.023 cv = 30.666
(11) Acicular crystal	Absent	L = 0.165 ± 0.012 cv = 7.272 W = 0.049 ± 0.002 cv = 7.081
(12) Microcrystal	Absent	Present
(13) Rosett Crystal	Absent	Present

Table-2b. Anatomical difference of *Amaranthus spinosus* Linn. (Leaf) growing in non- polluted and polluted areas.

Characters	Non – Polluted	Polluted
1. Cuticle*	Thin cuticle W = 0.0029 ± 0.001 cv = 3.448	Thick cuticle W = 0.032 ± 0.004 cv = 1.246
2. Epidermis*	Single layer. F = 221/mm^2 L = 0.048 ± 0.004 cv = 8.333 W = 0.033 ± 0.003 cv = 9.090	Single layer. F = 320/mm^2 L = 0.035 ± 0.004 cv = 11.428 W = 0.030 ± 0.001 cv = 3.333
3. Trichomes	Two types trichomes present	Same
Glandular*	glandular hair with uni & bi cellular head. F = 6–8 / unit area. L = 0.069 ± 0.040 cv = 57.971 W = 0.034 ± 0.012 cv = 35.294	Glandular hair with uni & bi cellular head. F = 13–16 / unit area. L = 0.053 ± 0.041 cv = 77.358 W = 0.030 ± 0.011 cv = 33.333
Non-glandular		
Unicellular & Warty*	F = 3–9 / unit area L = 0.101 ± 0.012 cv = 11.881 W = 0.030 ± 0.002 cv = 6.666	F = 5–11 / unit area L = 0.081 ± 0.013 cv = 16.049 W = 0.020 ± 0.002 cv = 10.000
Bicellular*	F = 4–6/unit area L = 0.062 ± 0.003 cv = 4.838 W = 0.026 ± 0.001 cv = 6.666	Absent
Multicelluar*	3–4 celled. F = 3–7/unit area L = 0.109 ± 0.004 cv = 3.669 W = .028 ± .001 cv = 3.571	3–5 celled. F=10–14/unit area L = .729 ± .004 cv = 0.548 W = .025 ± .002 cv = 8.000
4. Collenchyma*	2 layer (only on lower surface) L = 0.040 ± 0.004 cv = 10.000 W = 0.030 ± 0.003 cv = 9.230	absent
5. Palisade layer*	1-2 layer of Palisade L = 0. 460 ± 0.006 cv = 1.304 W = 0.033 ± .003 cv = 9.090	3-4 layer of Palisade L = 0.088 ± 0.009 cv = 10.629 W = 0.031 ± .001 cv = 3.333

Characters	Non – Polluted	Polluted
6. Palisade ratio	R = 6.500 – 6.750 SD = 6.937 ± 0. 325 cv = 4.685	R = 9.250 – 11.750 SD = 10.825 ± 0.944 cv = 8.720
7. Spongy Parenchyma*	2-3 layer L = 0.042 ± 0.009 cv = 21.176 W = 0.032 ± 0.003 cv = 9.231	2-layer L = 0.078 ± 0.015 cv = 19.885 W = 0.060 ± 0.006 cv = 10.000
8. Crystals		
Micro crystal	4 in unit area.	6 in unit area
Rosette crystal*	2-3 unit area L = 0.093 ± 0.021 cv = 22.629 W = 0.048 ± 0.012 cv = 24.844	5-10 unit area L = 0.113 ± 0.012 cv = 10.619 W = 0.062 ± 0.013 cv = 20.967
Prismatic Crystal*	1-2 unit area L = 0.169 ± 0.021 cv = 12.426 W = 0.089 ± 0.012 cv = 13.483	absent
9. Parenchyma/ Ground tissue*	L = .1073 ± 0.013 cv = 12.115 W = 0.070 ± 0.014 cv = 20.000	L = 0.080 ± 0.037 cv = 46.770 W = 0.078 ± 0.037 cv = 47.742
10. Stomatal index		
Upper surface	R = 21.428 – 23.437 SD = 22.342 ± 0.773 cv = 3.459	R = 20.262 – 22.727 SD = 21.281 ± 1.006 cv = 4.727
Lower surface	R = 23.148 – 25.000 SD = 24.14 ± 0.772 cv = 3.198	R = 20.389 – 23.489 SD = 22.163 ± 1.196 cv = 5.396
Guard cell-size*	L = 0.150 ± 0.012 cv = 8.000 W = 0.450 ± 0.033 cv = 7.333	L = 0.105 ± 0.012 cv = 11.619 W = 0.035 ± 0.004 cv = 10.000
Pore Size*	L = 0.104 ± 0.008 cv = 7.692 W = 0.042 ± 0.003 cv = 7.058	L = 0.075 ± 0.012 cv = 16.266 W = 0.032 ± 0.004 cv = 12.500
11. Vein islets numbers	R = 12 – 32 SD = 21.333 ± 8.219 cv = 38.570	R = 32.56 SD = 44.00 ± 9.797 cv = 22.265
12. Vein termination number	R = 4 – 12 SD = 6.660 ± 3.771 cv = 56.606	R = 12 – 32 SD = 16.000 ± 8.000 cv = 50.00

* The values are in mm.

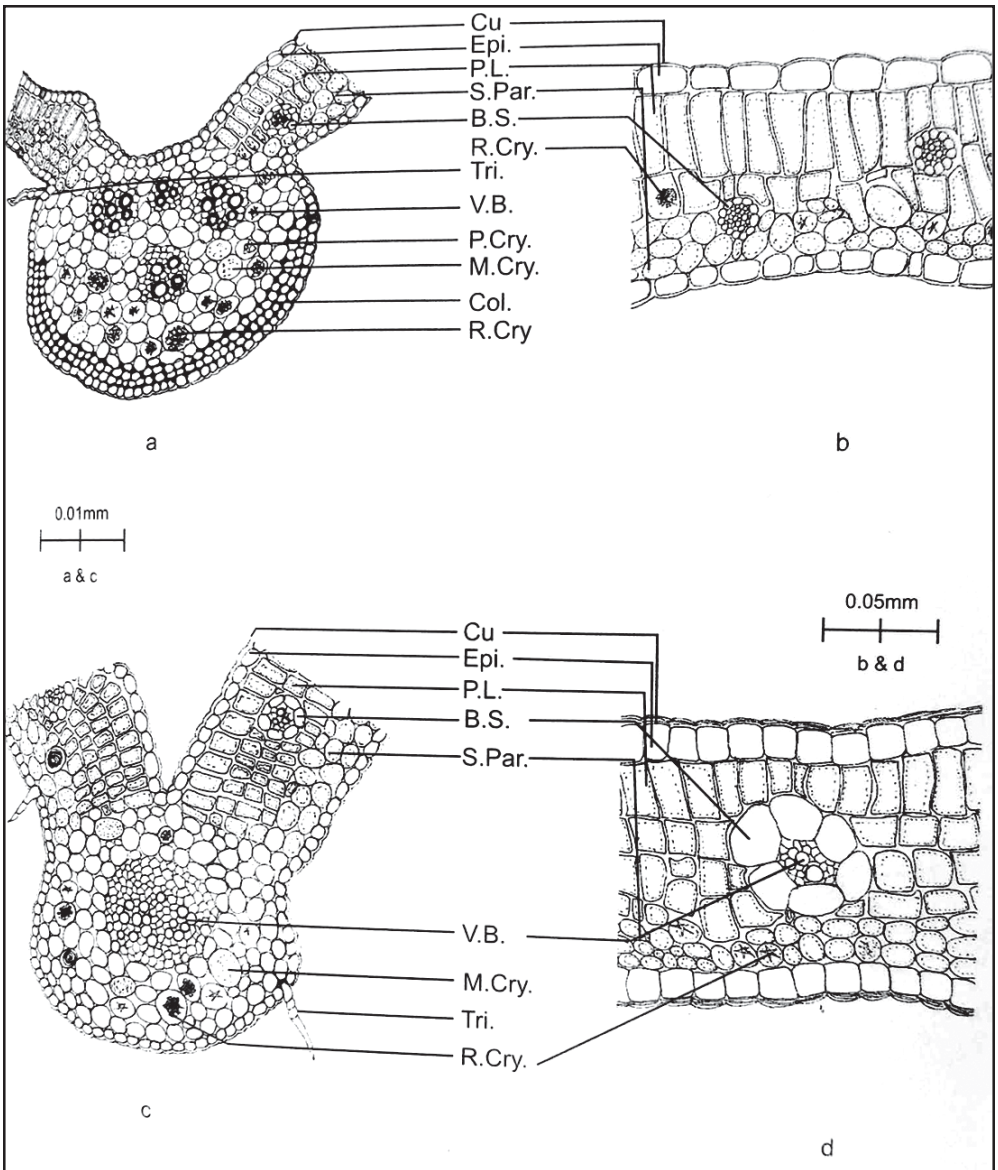

PLATE-3

Anatomical differences in the Leaf of *Amaranthus spinosus* Linn. Growing in non-polluted and polluted areas.

Fig.a-T. S. of Leaf through midrib (non-polluted).

Fig.b-T. S. of Leaf through Lamina (non-polluted).

Fig.c - T. S. of Leaf through midrib (polluted).

Fig.d- T. S. of Leaf through Lamina (polluted).

Abbreviations: A. Cry-acicularcrystals; B.S.-bundlesheath; Ca-cambium; Chl-chlorenchyma; Col-collenchyma; Cu-cuticle; En-endodermis; Epi-epidermis; M.Cry-microcrystals; M.V.B.-medullary vascular bundle; Par-parenchyma; P.Cry-prismatic crystals; Peri-pericycle; Ph-phloem; P.L.-palisadelayer; Pro-prosenchyma; R.Cry-rosettecrystals; S.Par-spongy parenchyma; Tri- trichomes; V.B.-vascular bundle; X.V.-xylem vessel.

Table-3. Colour reaction test of *Amaranthus spinosus* Linn. growing in non-polluted and polluted areas.

			Degree of Changes	
			Non-polluted	Polluted
Dragenorff's reagent {Cromwell (1955)}	Alkaloid	Orange ppt	++++	+
Mayer's reagent	Alkaloid	Brown	++	+
Wagner's reagent (Trease & Evans (1983))	Alkaloid	Brown	+++	++
Tannic Acid	Alkaloid	Turbidity	+++	++
Hager's Reagent	Alkaloid	Yellow	+++	++
Plant powder + H_2O + Shake	Saponin	Froth (W)	+++	++
$FeCl_3$	Tannin	Black	+++	++
Molisch Test after hydrolysis	Glucoside	Yellow	++++	++
Bendict's Reagent after heating	Sugars	Negative	-	-
Phloroglucinol + HCl	Lignin	Dark Red	+++	++
Millon's Reagent	Protein	Red ppt	+++	+
Xanthoprotic test	Protein	Yellow	++++	+++
Molisch test	Carbohydrates	Red	++++	++
Sample + heating with strong KOH + H_2So_4	Subernin	Red Black	++++	++
Mg Powder + conc. HCL	Flavanoids	Negative	-	-
Oils	Sudan IV	Red	++	+
Steroids	Libermann's Buchard reagent	Violet	++	+

Table-4. Fluorescence nature of the plant drug.

	Visible Light		UV Light (264nm)		UV Light (365nm)	
	NP	P	NP	P	NP	P
Water	Brown	Brown Yellow	Green	Green	Dark Green	Dark Green
Benzene	Pale Yellow	Yellow	Green	Green	Orange	Grayish Orange
Chloroform	Yellowish	Light Yellow	L. Green	Brown	Light Browhish	Light Brown
Acetone	Brown	Brown	Brownish	Brownish Green	Red Brown	Brownish Red
P. Ether	Light Green	Light Green	White Green	White Green	Pink Violet	Violet
E. Acetate	Green	Leafy Green	Green	Dark Green	Green Red	Green Red
Methanol	Green	Green	Green	Green Dark	Green Orange	Greenish Orange
E. Alcohol	Black	Dark Green	Black Green	B. Green	Black Orange	Brick Red

Table-5. Ash values of *Amaranthus spinosus* Linn. growing in non-polluted and polluted areas

Ash Value*		
Parameters	Non-polluted	Polluted
Total ash value	12.745 ± 0.950 cv = 7.454	17.745 ± 0.955 cv = 5.382
Acid insoluble	4.547 ± 1.410 cv = 31.009	6.501 ± 1.021 cv = 15.705
Sulphated ash	3.391 ± 0.837 cv = 24.682	4.991 ± 0.698 cv = 13.985

Table-6. Extractive value of *Amaranthus spinosus* Linn. growing in non-polluted and polluted areas.

Extractive value *		
Parameters	Non-polluted	Polluted
Water soluble	18.272 ± 0.052 cv = 0.284	14.582 ± 0.019 cv = 0.130
Alcohol soluble	26.267 ± 0.978 cv = 3.723	2.3625 ± 0.712 cv = 3.013
LOD	33.028 ± 0.512 cv = 1.550	23.482 ± 0.546 cv = 2.325

* The values are in percentage

Table-7

	Non-Polluted	Polluted
	RF values (mm)	Rf values (mm)
Sunlight	0.24, 0.29, 0.33, 0.37, 0.90	0.33, 0.38, 0.93,
UV Light (264 nm)	0.24, 0.26, 0.30, 0.33, 0.37, 0.40, 0.56, 0.90, 0.93	0.26, 0.30, 0.33, 0.38, 0.93
UV Light (365 nm)	0.26, 0.30, 0.33, 0.37, 0.56, 0.90	0.38, 0.93

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