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To Study the Trace Elemental Concentrations in Selected Medicinal Plants by EDXRF Technique

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Abstract— **Energy Dispersive X-ray Fluorescence (EDXRF) technique was one of the most powerful and quick multi elemental analyses with high sensitivity has been used to detect characterized for trace elements. To determine the trace elemental concentration in selected medicinal plants namely, *Eclipta prostrate, Acalypha indica, Andrographis paniculata, Datura metal, Alstonia scholarisis, Aloe vera* and *Acacia catechu* which are traditionally used by the natives of the Telangana State. From the present study, thirteen elements (P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb and Sr) and their elemental concentrations were determined in these plant samples. In this we are found to contain significant amount of the S, Fe, Cu, Mn and Rb, which can used to a new standard of prescribing the dosage of herbal drugs prepared from the plant material to integrate their medicinal values in the modern system of the medicines. It is hoped that, this elemental data will be a useful lead for phytochemists and pharmacologists for further study.**

Keywords— **Trace elements, Medicinal plants, Skin diseases, EX-3600 spectrometer.**

Introduction

The use of medicinal plants was an old practice dating back to ancient times. More than 70% of world population uses herbal plants to cure a variety of diseases [1]. The World Health Organisation (WHO) has estimated that 80% of the world population depends on traditional medicine for their primary health needs [2]. In recent times medicinal plants occupy an important position for being the paramount source of drug discovery [3]. People living in the developed or developing countries rely quite effectively on traditional medicine for primary health care [4]. Many hundreds of medicinal plant species worldwide are used in the traditional medicine as a treatment for various diseases. In that we have to chosen some medicinal plants which are used to cure the skin diseases. Skin disease is caused by bacteria, fungi and viruses [5]. Natural products are formulated to generate different types of effective drugs to enhance anti-skin disease activities. Medicinal plants are widely used to treat many human diseases due to of their minor side effects [6]. Most of the medicinal plants are found to be rich in one or more individual elements, thereby providing a possible link to the therapeutic action of the medicine [7].

Human skin is the most extensive, diverse and the largest organ of the body, which protects animals from the external environment and unfavourable external factors [8]. Skin forms the first guard line and plays a key role in protecting the body against pathogens [9] and it contains many specialized cells and structures [10]. Its three main layers are epidermis, dermis and hypodermis, each layer offers a distinctive role in the homeostasis of the skin [11]. They vary in thickness throughout the body and from person to person [8]. Skin having other functions are insulation, temperature regulation, sensation, storage and synthesis of vitamin-D by action of ultraviolet (UV) and the protection of vitamin B folates, absorption of oxygen and drugs [12] and water resistance. There are two general types of skin, hairly and glabrous skin [13]. However, the skin can be dry, sensitive, pale, sagging and tired. The people deficient in some essential nutrients such as beta-carotene, B-complex vitamins and vitamins C and E often suffer from the different skin disorders [8] and some of the skin diseases like ecne, hives, chickenpox, eczema, rosacea, seborrheic dermatitis, psoriasis, vitiligo, impetigo, warts, scabies, pruritus (itch) and skin cancer etc...[13]. Many skin disorders are treatable to common treatment methods for skin conditions include, antihistamines

medicated creams, ointments, antibiotics, vitamin or steroid injections, laser therapy and targeted prescription medications. Many of the allopathic drugs available in the market which are effective against skin diseases, but due to of high cost, problems of drug resistance and toxic levels of allopathic drugs, we have to choose alternative methods.

Elements are present in different forms in the nature and these trace elements are very essential for the body to perform different functions. Elements are very important for cell functions at biological, chemical and molecular levels. Human body requires a number of elements in order to maintain good health. A number of elements essential to human nutrition are accumulated in the different part of plants as it accumulates minerals essential for growth from the environment [14]. Macro and micro elements influence biochemical processes in the human organism [15]. Study of elements with respect to indigenous medicinal plants reveals that major and trace elements play a significant role in the formation of the active chemical constituents and combating a variety of human ailments and diseases [16]. A literature survey revealed that trace elements play a significant role in curing various diseases [17-22].

So this present study aimed to determine the concentrations of some macro and trace elements in selected medicinal plants i.e, Eclipta prostrate (w. plant), Acalypha indica (leaves), Andrographis paniculata (leaves), Datura metal (leaves and seeds), Alstonia scholarisis(leaves), Aloe vera (leaves) and Acacia catechu (bark). These medicinal plants are extensively used in the preparation of herbal drugs, cure skin diseases and find a presumed correlation between their curative properties.

Experimental

Sampling and sample preparation

Seven different medicinal plants were collected from Ramagiri khilla forest is located in Karimnagar district, Telangana, India. Table 1 gives the list of medicinal plants selected for present study, their botanical name, common name and the corresponding parts of the plants used for this analysis and while photographs of the parts of plants is shown in figure 1.

Table 1: List of anti-skin disease medicinal plants used in this present study.

S. No	Scintific name	Common name	Part used
1	*Eclipta prostrata*	Guntagalagara	w. plant
2	*Acalypha indica*	Muripinda	Leaves
3	*Andrographis paniculata*	Neela vemu	Leaves
4	*Datura metal*	ummetta	Leaves
5	*Alstonia scholarisis*	Adakula leaf	Leaves
6	*Aloe vera*	Kalabandha	Leaves
7	*Acacia catechu*	Tella tumma	Bark

Fresh plant samples of Eclipta prostrate (w. plant), Acalypha indica (leaves), Andrographis paniculata (leaves), Datura metal (leaves and seeds), Alstonia scholarisis (leaves), Aloe vera (leaves) and Acacia catechu (bark) (shown in Figure 1) were collected and washed with tap water and rinsed thoroughly with double distilled water in order to remove surface contamination, dried in an oven at about 60^0C overnight (24 hours) and subsequently powdered by using agate mortar.

A quantity of pure 150 milligrams of each powder sample was weighted and compressed using a 150 ton hydraulic press and made in to pellets of 13mm diameter and about 1mm thickness. Triplicates of each sample were done. These pellets were used as targets for the EDXRF experiment.

Experimental system

Present study reports the trace elemental analysis of medicinal plant samples was carried out at trace elemental laboratory, UGC-DAE CSR Kolkata centre, Kolkata, India. The setup consist of Xenemetric (previously Jordan valley) EX-3600, energy dispersive X-ray fluorescence (EDXRF) spectrometer, which consist of an oil cooled Rh anode X-ray tube (maximum voltage 50kV, current 1mA). The measurements were carried out in vacuum chamber using different filters (between the source and sample) for optimum detection of elements. Si (Li) detector with a resolution of 143eV at 5.9keV and 10 samples turret enables mounting and analysing 10 samples at a time.

The targets were positioned at an angle of 45^0 to the beam direction. The X-ray beam was collimated to a diameter of 4mm and was made to fall on the targets. The detector was kept at an angle of 45^0 to the target position and at an angle of 90^0 to the X-ray beam direction. The characteristic X-rays emitted from each sample were recorded with a high resolution Si (Li) detector. The spectra were collected for a sufficiently long time so that good statistical accuracies can be achieved. The generated data were analysed by out by nEXT software and our results were checked against the certified values from the standard reference materials (NIST 1515 Apple leaf).

Results and Discussion

The thirteen elements P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb and Sr were determined by using Energy Dispersive X-ray Fluorescence (EDXRF) in present selected medicinal plants i.e. Acacia catchu (bark), Eclipta prostrate (whole plant), Acalypha indica (leaves) and Aloe vera (leaves), and their concentrations estimated in ppm (parts per million) level. Fig 2 shows the graphical representation of the essential trace elements of the present study. The average concentrations of the trace elements detected in the samples of various medicinal plants analysed and corresponding standard deviations for each element are reported in table 2. Analysis of present data revealed that the elements K (18940.69±2596), Ca (26031.12±476), S (6537.69±470ppm), P (2097.67±89.5ppm) and Fe (3027.41±50ppm) were found in major level of concentrations. The highest concentration of Se (0.46±0.61ppm), Mn (126.97±4.6ppm) and Br (57.50±1.58ppm) were found in Eclipta procera. Aloe vera had the highest concentration of Cl (14452.01±948ppm), Zn (244.06±11.15ppm) and Sr (300.87±15.21ppm) respectively.

a. Eclipta prostrate

b. Acalypha indica

c. Andrographis paniculata

d. Datura metal

e. Alstonia scholarisis

f. Aloe vera

g. Acacia catechu

FIG. 1 Photographs of selected medicinal plants in present study.

Table 2 Elemental concentrations (in ppm) of the medicinal plants with standard deviation

Sample Name	P	S	Cl	K	Ca	Mn
Ecliptа procera	2015.92±134	2909.94±43	6247.4±365	12134.31±276	7984.19±249	126.97±4.6
Acalypha indica	2097.67±89.57	2100.85±46.6	4091.31±301	17064.05±722	16776.39±880	31.06±1.6
Andrographis paniculata	768.31±378	1652.43±366	6317.72±2015	18940.69±2596	20413.66±2905	22.06±0.08
Datura metal	1464.34±135	6537.69±470	4916.51±270	19786±624	6772.73±115	24.33±0.7
Alstonia scholarisis	500.23±18.24	1282.25±43.93	2537.34±61	8722.50±63	26031.12±476	27.28±1.39
Aloe vera	1423±61	452.17±26	14452.01±948	16537.94±417	24695±916	3.36±0.57
Acacia catechu	143.53±70	927.83±114	1078±138	5546.59±607	12644.05±1490	67.33±5.89
NIST 1515 apple leaf	1590	1800	579	16100	15260	54.00

Table 2 continued....

Sample Name	Fe	Cu	Zn	Se	Br	Rb	Sr
Eclipta procera	3027.41±50	10.11±0.4	16.45 ±0.61	0.46 ±0.61	57.50±1.58	16.4 ±0.53	74.53 ±0.89
Acalypha indica	204.25±8.7	8.98 ±0.83	16.94 ±1.42	0.12 ±0	3.23 ±0.84	3.7± 1.26	156.76±6.07
Andrographis paniculata	250.39±6	4.49 ±0.31	37.74 ±2.35	0.41 ±0.25	7.14 ±0.6	14.81±0.7	93.20 ±5.2
Datura metal	264.06±16.2	10.60±0.5	41.16 ±1.05	0.39 ±0.4	14.25±1.46	20.23±2.09	27.76 ±2.7
Alstonia scholarisis	221.4 ±6.97	60.66±2.15	8.69± 1.36	0.213±016	49.6 ±2.24	8.95 ±0.94	132.67±2.02
Aloe vera	54.77 ±6.11	3.20 ±0.75	244.06±11.15	0.32 ±0.34	5.32 ±0.15	13.87±1.9	300.87±15.21
Acacia catechu	181.54±11.96	27.77±3.8	7.44± 0.58	0.33 ±0.29	4.22 ±0.18	4.48 ±2.02	96.54 ±3.96
NIST 1515 apple leaf	83	5.64	12.50	0.05	1.80	10.20	25.00

However it can be observed from table 2 that the trace elements present in these medicinal plants shows a significant variation in their concentration. In general the beneficial characteristics of these elements may play a role either directly or indirectly in the therapeutic value of the plant samples. The amount of elemental content present in the medicinal plants is very important since many trace elements play a key role in the formation of active constituents responsible for their curative properties. In this present study the results revealed that Potassium and Calcium are the most abundant detected elements compared to other trace elements in the different/various medicinal plant samples, here first presented

Phosphorus (P): The highest concentration of P is found in Acalypha indica (2097.67ppm), lowest concentration of P is found in Acacia catechu (143.53ppm) and sufficient P is found in Datura metal and Aloe vera (1464.34ppm and 1423ppm) plant samples, which are compared to standard values of NIST 1515 apple leaf (1590ppm). DRI (Dietary Reference Intake) for P is 700mg/d for adults [23]. P is works with calcium to make our bones strong and makes up about 1 percent of our total body weight. Excessive intake of P supplements may worsen conditions such as heart disease, pancreatitis, high blood pressure.

Sulphur (S): The high amount of S is found in Datura metal (6537.69ppm) and minimum observed in Aloe vera (452.17ppm). S has a long history of use for variety of dermatological disorders [24], S aids in healing of wounds via keratin and folklore usage as a remedy of skin rashes [25]. Although there is no official RDA for S, it is critical nutrient. Daily intake is usually 800 to 900mg/d of S, certain health conditions such as arthritis and liver disorders may be improved by increasing the intake of S to 1500mg/d in supplemental form. The S deficiency can lead to a number of health issues and without sufficient S you may experience joint pain.

Chlorine (Cl): The highest concentration of Cl was found in Aloe vera (14452.01ppm) and in this present study all plant samples had maximum concentration of Cl, Which is compared to standard reference material of NIST 1515 apple leaf. RDA (Recommended Dietary Allowances) of Cl is 1.5 g/d. Cl is mostly present in the form of inorganic chlorides both inside the cells as well as in the extracellular fluids. It acts as antiseptic for wounds, anti malaria, disinfectant lie measles and has wound healing property [26]. In adults Cl deficiency may results in dehydration and associated symptoms and excessive chloride levels on the other side can results in water retention and the associated elevated blood pressure as well as increased risk of developing cancer.

Potassium (P): The high concentration of K in plants is needed for many essential processes including enzyme activation, water use efficiency, starch formation and photo synthesis [6]. K concentration levels ranged from 19786ppm to 5546.59ppm, Datura metal plant sample had the highest concentration of K (19786ppm) and low concentration of K (5546.59ppm) is found in Acacia catechu plant. RDA of K is 4.7 g/d for adults. Low level of K can cause weakness as cellular processes are affected. Hyperkalemia is a condition that occurs when your blood contains too much K.

Calcium (Ca): Ca is the main constituent of the skeleton and is important for regulating many vital cellular actions, helps prevent the accumulation of too much acid or too much alkali in the blood, assists in normal blood clotting and cellular mortality [6]. The high amount of Ca is found in Alstonia scholarisis (26031.12ppm). In this present study all plant samples had maximum concentration of Ca which is compared to standard value of NIST 1515 apple leaf (1526ppm). RDA of Ca is 1000 mg/d for adults [23]. Excess Ca can also cause muscle pain, mood disorders, abdominal pain and kidney stones.

Manganese (Mn): It is mainly located in the mitochondria, is a constituent of many important metalloenzymes and Mn is to enhance the process of aggregation of platelets and thus help in the coagulation of blood [26]. The highest concentration of Mn is found in Eclipta procera (126.97ppm), Acacia catechu (67.33ppm) and very low concentration is present in Aloe vera (3.36ppm). RDA of Mn is 1.2 mg/d for females and 2.3mg/d for males [27]. Deficiency of Mn can causes for humans myocardial infarction and other cardiovascular diseases [28]. Toxic exposure to Mn containing dust produces hallucinations, psychosis and neurological symptoms resembling Parkinson's disease [27]. Mn overload is generally due to industrial pollution and significant rises in manganese concentrations have been found in patients with severe hepatitis and possthepatic cirrhosis.

Iron (Fe): The highest concentration of Fe was found in Eclipta procera (3027.41ppm) and minimum Fe is found in Aloe vera (54.77ppm). In these present results all medicinal plants had maximum concentration of Fe except Aloe vera plant sample. Fe is the most abundant trace element in our body and it facilitates oxidation of carbohydrates, protein and fat to control body weight [26]. A high iron diet may increase the risk of development of colorectal and liver cancer. RDA of Fe is 8 mg/d for males and 18 mg/d for females [23]. Fe deficiency is associated with impairment in the immune function and increased by physiological requirements [24] and Fe overload occurs in case of high dietary intake, excessive intestinal absorption or repeated parenteral administration.

Copper (Cu): Cu is the third largest trace element found in human body (after Iron and Zinc). It is a major component of the oxygen carrying part of blood cells [29]. The maximum concentration of Cu is found in Alstonia scholarisis (60.66ppm) and similarly minimum in Aloe vera (3.20ppm). RDA of Cu is 900µg/d for adults [27]. Cu deficiency is rare except in malnutrition, prolonged parenteral nutrition,

malabsorption disorders, depigmentation of hair and skin [29] and mostly associated with liver damage.

Zinc (Zn): The highest concentration of Zn was found in Aloe vera (244.06ppm) and lowest concentration is found in Acacia catechu (7.44ppm) except this plant sample all remaining medicinal plants had higher concentration than that of standard reference material of NIST1515 apple leaf (12.50ppm). This trace element is crucial to play vital processes, playing a unique role in growth, development, release and use of hormones in the body and also Zn is known to play an important role in wound healing or prevention and the reduction of skin irritation [30, 31]. It helps developing foetuses grow correctly and our brain to work right [29]. Whereas RDA of Zn is 15 mg/d for adult men and 12 mg/d for adult women [26]. Zn deficiency can causes growth retardation, skin rash like dermatitis, alopecia, diarrhoea, night blindness, hypogonadism, ageusia and acrodermatitis enteropathica and toxicity of Zn leads to copper deficiency , swelling, gastritis fever, nausea and vomiting [27].

Selenium (Se): Se is a component of many enzymes]. Se is used in the synthesis of ascorbic acid and also contributes to maintenance of normal immune function. RDA of Se is 55µg/d for adults [23]. In this present study Se is present in all the medicinal plants at trace levels with concentration that varies between 0.46ppm to 0.12ppm. The maximum concentration of Se is found in Eclipta procera (0.46ppm) plant sample and all the plant samples had higher concentration than that of standard reference material of NIST 1515 apple leaf (0.05ppm). Se deficiency can cause keshan's disease, kashin-beck desease and a form of hypothyroidism with mental retardation [24].

Bromine (Br): The highest concentration of Br is observed in Eclipta procera (57.50ppm) and all the medicinal plants had higher concentration than that of standard reference material of NIST 1515 apple leaf (1.80ppm). Br is considered as a non-essential element for living organisms [32]. Br is obviously harmful to human health.

Rubedium (Rb): The maximum concentration of Rb is found in Datura metal (20.23ppm) and minimum concentration of Rb is found in Achalypha indica (3.7ppm). Rb reacts with skin moisture to form rubidium hydroxide which causes chemical burns of eye and skin.

Strontium (Sr): Finally high level of Sr is found in Aloe vera (300.87ppm) and all the medicinal plants in this present study had higher concentration than the standard reference material of NIST 1515 apple leaf (25ppm). n the present study we have discussed with their maximum and minimum concentrations of selected medicinal plant samples and also discussed their significances of the trace elements on their own or as important constituents of metalloenzymes. Thus, the trace elements like S, Fe, Mn, Zn, Cu and Rb are responsible for the defence from pathogen and recovering quickly from the serious skin infections and diseases, as evident from the present study where all the trace elements present in drugs with appreciable amount in the selected medicinal plants traditionally used for curing skin diseases by the local people of Telangana state.

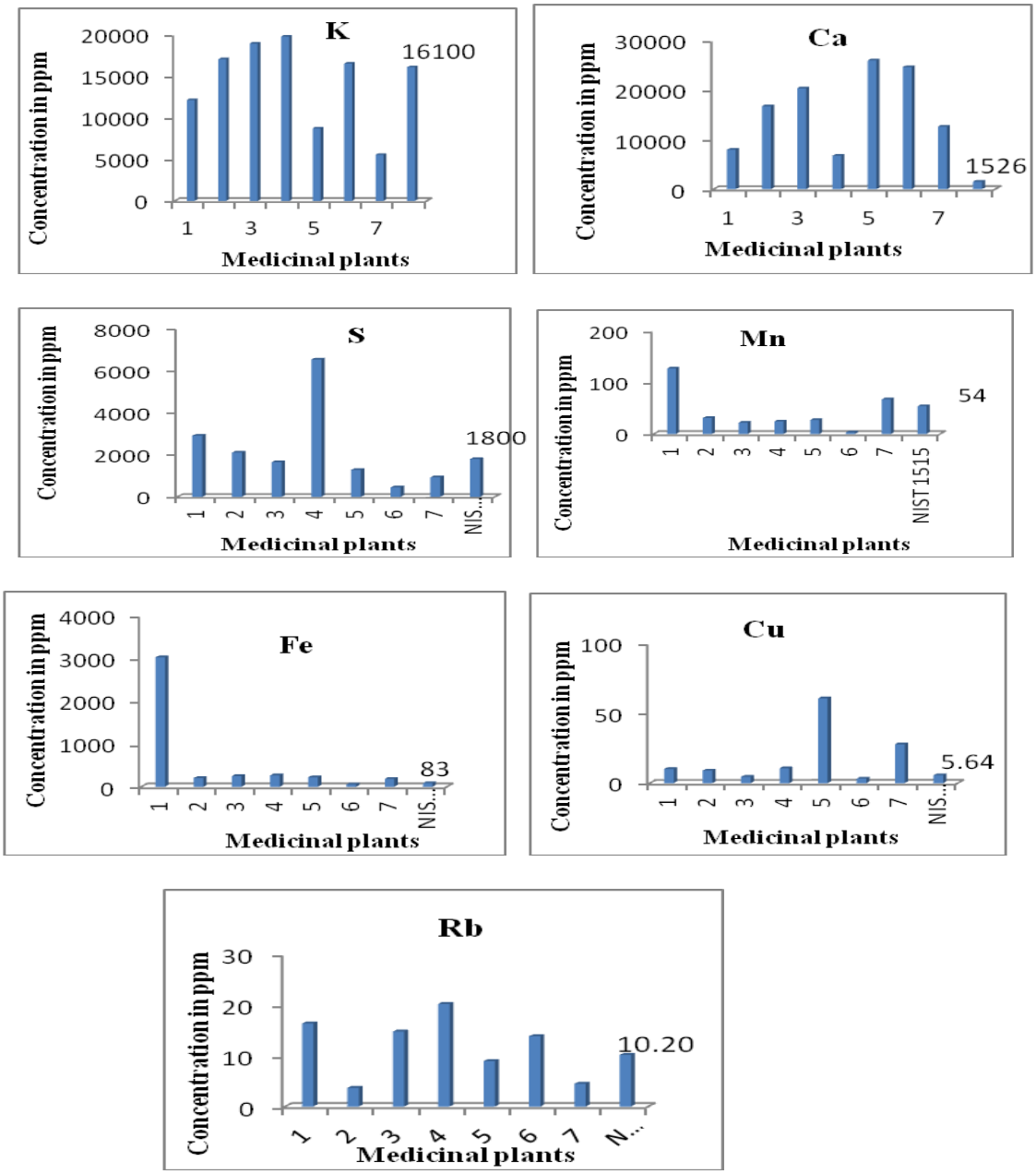

Figure 2. Graph of S, K, Ca, Mn, Fe, Cu and Rb concentrations in studied plant samples with NIST 1515 standard.

I. Conclusions

In this present study, EDXRF technique was employed for the trace elemental concentration of the selected 7 medicinal plants. The elements P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb and Sr were detected and their concentrations determined to ppm level. Which are widely used in the preparation of traditional anti-skin disease drugs and herbal creams or antiments. Instead of administering these beneficial metals in the form of drugs prepared from chemicals, administering

them in the form of plant based preparations is thought to be more effective. It is evident that various elements present in these medicinal plants have either direct or indirect role in the control and management of the skin diseases. We have concentrated on the curative property of these plants against to skin disease they also form a part of medicinal preparations for some other ailments also. No toxic heavy elements such as As, Pb and Hg are detected in the specimens studied. Since they are found to contain appreciable amount of Fe, Zn, Cu, Mn and Rb which can used to a new standard of prescribing the dosage of herbal drugs prepared from the plant material to integrate their medicinal values in the modern system of the medicines. The conventional therapies for skin disease have many short coming in terms of side effects and high rates of secondary failure on the other hand herbal extracts are expected to have similar efficacies without the side effects of conventional drugs. It is hoped that, this information will be very useful lead for phytochemists, pharmacologists and ayurvedic clinical who would like to pursue further study in the area of herbal and alternative medicines. Once the efficiency of herbal drugs in treating skin disease is scientifically established, the popularisation of these remedies can be recommended in Indian health system for wider application, since these plants are well within the reach of rural masses.

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