Anti-inflammatory Activity of Root of *Dalbergia sissoo* (Rox.b) in Carrageenan-Induced Paw Edema in Rats

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ABSTRACT

Dalbergia sissoo Roxb commonly known as Indian rosewood found throughout India, Bangladesh, Pakistan and Nepal up to 900 m. The root of *Dalbergia sissoo* Roxb was collected and then dried. The phytochemical analysis of hexane, chloroform and methanol extract revels presence of different phytochemical constituent's. The methanolic extract showed the presence of Alkaloids, Carbohydrates, saponins, flavonoids, glycosides (Cardiac glycosides, anthraquinone glycoside and saponin glycosides) and steroids.

The methanolic extract of *Dalbergia sissoo* Roxb was investigated for anti-inflammatory activity in experimental animal models. Treatment with 70% methanolic extracts of *Dalbergia sissoo* demonstrate a diminished inflammation in rat hind paw when challenged with carrageenan induced paw edema. The methanolic extract of *Dalbergia sissoo* root at 1000 mg/kg showed the most potent anti-inflammatory activity compared to the other groups (100 and 500 mg/kg) throughout the observation period. *Dalbergia sissoo* Roxb was devoid of ulcerogenic effect on the gastric mucosa of rats in acute and chronic tests. It was concluded that the *Dalbergia sissoo* root extract possessed significant anti-inflammatory activity without any side effect on gastric mucosa.

Keywords: *Dalbergia sissoo*; phytochemical screening; Anti-inflammatory; methanolic extract

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INTRODUCTION

Dalbergia sissoo Roxb (Fabaceae) is a medium to large sized gregarious and deciduous having thick rough grayish brown bark. Commonly known as Indian Rose wood found throughout India. It is used as timber or fire wood and for the treatment of a variety of ailments by different ethnic groups.[1–6] *Dalbergia sissoo* Roxb has been used as indigenes system of medicine are reported to be useful in the treatment of arthritis, gonorrhoea and rheumatic pains. *Dalbergia* genus has provided a large number of compounds, which include flavonoids and neoflavonoids have been reported from *Dalbergia sissoo*.[7–9] Although several drugs are used to treat inflammatory disorders but their prolonged uses may cause serve adverse effect. Consequently there is a need to develop new anti-inflammatory agents with minimum side effects. Several plants are being used in traditional medicine for treating there disorder which are inflammatory in nature.[10]

MATERIALS AND METHOD

Plant Material

The fresh roots of the plant (*Dalbergia sissoo*) were shade dried at room temperature (25^0–35^0 C) for 20–25 days. The dried roots were powdered in a grinder and weighed before used for calculating the yield. The plants were authenticated by comparison with the herbarium and voucher specimen was lodged in the departmental herbarium of Botanical Research survey of India Dehradun. The voucher specimen has *ID-BSD* 112718.

Preparation of Extracts

The dried powdered roots (300gm.) were subjected to extraction with different solvent as per the decreasing order of polarity, Hexane, Chloroform and methanol with the help of soxhlet apparatus. The plant material

was separated by filtration and different polarity extracts were concentrated (by Rotavapour, Büchi, Switzerland) and lyophilized to preserve it. The percentage yield was calculated. The yields of different extracts were 21.6, 17.3 and 35.5%. Preliminary phytochemical screening was carried out on the extract using the standard screening method.[11] Dilutions of the methanolic extract were made in 2% gum acacia for the pharmacological studies.

Phytochemical Screening

The individual fractions were subjected to the identification of different compounds.[11, 12] The molish's test and fehling's test were carried out for carbohydrate. Mayer's test and Wanger's Test for alkaloids, Aq. Sodium hydroxide test, concentrated sulphuric acid test and shinoda's test were carried out for flavonoids. Foam test for saponins, Salkowiski test and Libermann burchard test for phytosterol, Biuret test, Ninhydrin test and Million's test were carried for proteins and amino acid.

Animals

For anti-inflammatory activity Wistar rats (200–300 g) were kept in the Animal House at the College of pharmacy, GRD (PG) IMT, Dehradun (U.K.) was used. The animals were housed in groups of 6–10 under environmentally controlled conditions with free access to water and standard food. Food was withheld overnight prior to experiments while water was still provided. The handling and use of animals were in accordance to the Guidelines of Institute Animal Ethics Committee were followed while using live animals. All the animals were acclimatized to the laboratory environment for 5 days before the experiment. Six animals per group were used in each experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water.

Anti-inflammatory Activity

In present study anti- inflammatory activity was determined in wistar rats of either sex according to the previously described method.[14] Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gam acacia in normal saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile) at '0' and '3' hours after the carrageenan injection. Indomethacin 100 mg/kg, p.o. suspended in 2% gum acacia was used as the standard drug. The inhibitory activity was calculated according to the following formula

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{control} - (C_t - C_0)_{treated}}{(C_t - C_0)_{control}} \times 100$$

Where C_t = paw circumference at time t, C_0 = paw circumference before carrageenan injection

Statistical analysis

Results are expressed as mean ± S.E.M. statistical evaluations were made using ANOVA followed by t-test (GraphPad InStat software) and P values less than 0.001 were considered significant. Data are represented as mean ± S.E.M.

RESULT

The extraction with different solvent as per the decreasing order of polarity, Hexane, Chloroform and methanol with the help of soxhlet apparatus, the yields of extracts were 21.6, 17.3 and 35.5% (Table 1). Phytochemical screening showed that the alcoholic extract of *Dalbergia sissoo* contains proteins, amino acids, carbohydrates, tannins and flavonoids (Table 2). The effects of methanolic extracts of *Dalbergia sissoo*, on paw edema induced by carrageenan are shown in Table 3. Methanolic extract of *Dalbergia sissoo* possessed anti-inflammatory activity at the 1000 mg/kg dose. A dose of 1000 mg/kg elicited a greater percent inhibition of inflammation after 4 hr. than other groups. These results showed that test drug at the dose level of 1000 mg/kg have the most potent anti-inflammatory activity.

DISCUSSION

Treatment with methanolic extract of *Dalbergia sissoo* produced a diminished inflammation in rat hind paw when challenged with carrageenan. Non-steroidal anti-inflammatory drugs act by inhibiting cyclooxygenase and the production of prostaglandins. Indomethacin offers relief from inflammation by suppressing the production of prostaglandins and bradykinin. The presence of flavonoids has been reported in Dalbergia species,

Table 1: Percentage yield of extracts (Successive extraction) from *Dalbergia sissoo Rox.b* roots

Part of Plant	% Yield		
	Hexane	Chloroform	Methanol
Roots	21.6	17.3	35.5

Table 2: Phytochemical screening of Root extracts of *Dalbergia sissoo Rox.b*

Phytoconstituents	Test Performed	Hexane	Chloroform	Methanol
Alkaloids	Mayer's Test	–	–	+
	Hager's Test	–	–	–
	Wanger's Test	–	–	–
	Dragendroff's Test	–	–	+
Carbohydrates	Molisch Test	–	+	+
	Fehling Solution	–	+	+
Glycosides	Keller killanis test	–	–	–
	Legal's Test	–	+	+
Saponin	Foam Test	–	–	+
Sterols	Salkowski's Test	–	+	+
Phenolic compounds and tannins	Lead Acetate Test	–	–	–
	1% Gelatin Test	–	–	–
Proteins and Amino Acids	Million's test	–	+	+
	Biuret Test	–	+	+
	Ninhydrin Test	–	–	–
Flavonoids	Aq. NaOH Test	+	+	+
	Conc. H_2So_4 Test	+	–	+
	Shinoda's test	+	–	+
Gum and mucilages	Gum and mucilages	+	–	–
Resin	Resin	+	+	+
Fixed oil and fats	Saponification Test	+	–	–

Where (+) = Present; (–) = Absent

Table 3: Effect of Dalbergia sissoo root extracts on carrageenan-induced paw edema in rats

Groups	Dose orally (mg/kg)	Change In Mean Paw Volume (ml)					Inhibition (%) on at 4h
		30 min	1h	2h	3h	4h	
Control	---	0.79±0.013	0.83±0.017	0.84±0.016	0.88±0.018	0.97±0.058	-----
D.sissoo I	100	0.80±0.020	0.81±0.016	0.79±0.034	0.82±0.015*	0.86±0.010*	11.34%
D.sissoo II	500	0.78±0.016	0.80±0.014	0.75±0.029**	0.71±0.016**	0.73±0.018**	24.74%
D.sissoo III	1000	0.66±0.049**	0.56±0.011**	0.59±0.023**	0.52±0.016**	0.49±0.017**	49.48%
Indomethacin	10	0.48±0.015**	0.53±0.019**	0.52±0.020**	0.49±0.010**	0.47±0.018**	51.54%

Values expressed as MEAN ± S.E.M, n = 6 in each group
**P<0.01.
*P <0.05 compared with control

and flavonoids are known to inhibit prostaglandin synthetase.[15, 16]

CONCLUSION

The methanolic extract of *Dalbergia sissoo* root contained carbohydrates, phenolic compounds, flavonoids and tannins. Its methanolic extract shows the most effective anti-inflammatory activity at a dose of 1000 mg/kg throughout the observation period. The results shows in the present study provide evidence that the methanolic extract of *Dalbergia sissoo* root possesses anti-inflammatory activity.

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Uncovering Negative Results: Introducing an open access journal *"Journal of Pharmaceutical Negative Results"*

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"Once you replace negative thoughts with positive ones, you'll start having positive results."
- *Willie Nelson*

Drug discovery nowadays is a highly innovative process by which new drugs are discovered and/or designed. It is expected that in the year 2010, at least 50 percent of all new approved drugs will come from the biopharmaceutical sector. The discovery and validation process is mostly carried out by new innovative and medium-sized companies. But despite advances in technology and greater understanding of biological systems, drug discovery is still a lengthy process. Specialized drug discovery companies need an ideal business climate, that is, political, economic and social conditions, to be able to cope with the entrepreneurial risks of drug development.

Global Pharmaceutical Industry is at the crossroads. On one side, market demand for novel and better medicines is getting redefined and growing due to demographic, economic and epidemiological trends, but at the same time, pharmaceutical companies are finding it difficult to survive since they are unable to innovate and discover new molecules. The pipeline of the new drug molecules is drying fast due to poor R&D productivity, high failure rate and generic competition.[1]

Scientific research proceeds with an infinite variety in the availability of data, the maturity of conceptions, and the mix of conviction and uncertainty. Often a major discovery is the culmination of a process with numerous blunders, wrong turns, false hypotheses, missed opportunities, persistent hard work, lucky breaks, rational arguments, insightful conjectures, and accumulating knowledge over decades. But none of failures are being reported or published in the journals and other resources. This part is always ignored because of publishing style of most of the journals in pharmaceutical field.

Welcome to the *Journal of Pharmaceutical Negative Results(PNR)*. We are proud to introduce you to a new home for negative results focused on all aspects of Pharmacy. *Journal of Pharmaceutical Negative Results*[2] represents the first open access source for research concerning negative results and will be a valuable resource for researchers all over the world, both to those who are already experts and also to those entering the field.

Journal of Pharmaceutical Negative Results is a peer reviewed journal developed to publish original, innovative and novel research articles resulting in negative results. This peer-reviewed scientific journal publishes theoretical and empirical papers that report negative findings and research failures in pharmaceutical field. Submissions should have a negative focus, which means the output of research yielded in negative results is being given more preference. All theoretical and methodological perspectives are welcomed. We also encourage the submission of short papers/communications presenting counter-examples to usually accepted conjectures or to published papers.[2-3]

The main aim of the journal is to publish negative results, so that newer generation of researchers should not waste their time and money repeating the same studies and finding the same unpublishable results. Most of the journals have published only the positive results and findings, however they do not publish negative results. We believe that negative results are also a part of quality research. Thus, the journal focus is not limited to negative issues but will also address advances in pharmaceutical research lead to failures. This Journal will provide multilateral coverage of numerous applied and basic aspects of current pharmaceutical research.[4]

Taking into account the growing amount of pharmaceutical journals available, one can argue whether we need another one. However, any scientist working on theoretical and practical aspects of pharmaceutical field would undoubtedly agree that there are few or no

journals covering or boldly accepting negative results, and that there are no journals providing immediate open access. We performed an informal analysis (Figure 1 and 2) that shows the consistent interest of researchers to publish their original data in peer-reviewed pharmacy journals. A simple pubmed query resulted in more than 61674 for significant reports and just 885 for insignificant results. However, to read the largest proportion of articles (over 90%) related to pharmaceutical field has published positive results, It looks like research is always successful, is this a right statement? Therefore, numerous scientists all over the world, especially those in the developing countries, miss the opportunity to keep abreast with the failures in Pharmaceutical research. Since there are no open access journals in the pharmaceutical field covering negative results, the present journal fills this gap in the current pharmaceutical publishing.

Journal of Pharmaceutical Negative Results aims to become a significant international participant in this field. The purpose of the journal is, therefore, to publish timely and high-quality articles [See author information at http://pnrjournal.com/content/author-info] concerning failures in techniques and negative or unexpected results

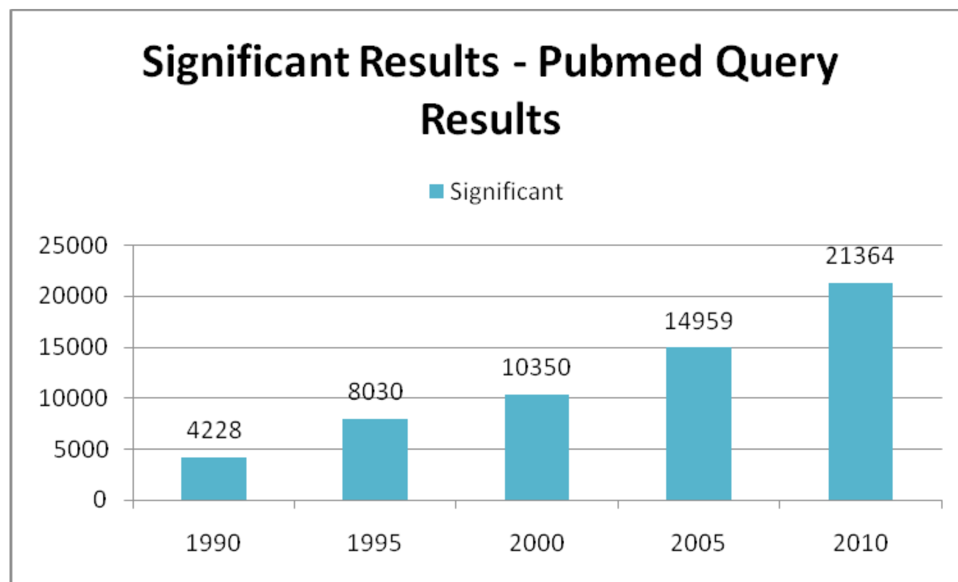

Figure 1: *Represents number of pubmed queries results with the year limits for the MESH term "Significant, Pharmaceutical"*

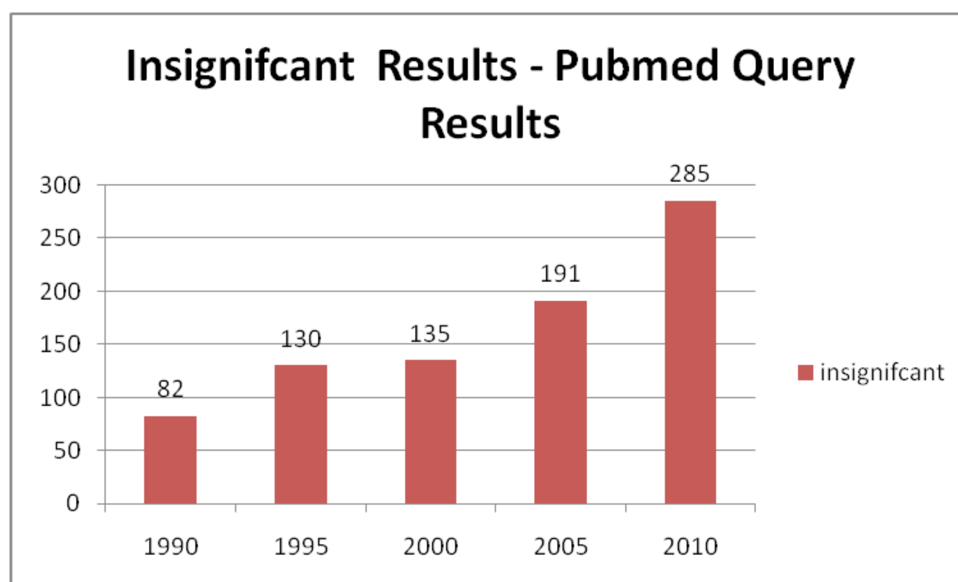

Figure 2: *Represents number of pubmed queries resulted with the year limits for the MESH term "Insignificant, Pharmaceutical"*

in all areas of Pharmacy. To achieve this, the Editorial Board of PNR Journal gathers well-known experts from all over the world.[4] To make a decision whether a manuscript fits the format of publishing in *journal*, the Editor-in-Chief or corresponding specialists from the Editorial Board will immediately screen a submission and appropriate manuscripts will be sent for peer review to two independent reviewers. Based on their reports and scoring, a final decision will be made regarding the acceptance of manuscripts. Editors-in-Chief reserves the right to make final decisions concerning each manuscript submitted to the journal. At first sight, our peer review system does not significantly differ from that proposed by other journals. However, we encourage our Editorial Board members as well as reviewers to be motivated critical advisers for improving a manuscript rather than suggesting rejection, which is unfortunately a common experience for anyone who has ever tried to publish his or her original negative research results.

Journal of Pharmaceutical Negative Results is published by the open access publisher, Medknow Publications and Media Pvt. Ltd, India[5]. When submitting a manuscript, authors will be required to declare competing interests. In addition, for all the manuscripts accepted for publication an article-processing charge will be levied, to cover the costs incurred by open access publication. We believe that authors' ability to publish their negative research should be governed only by the quality of the work and not financial limitations.

Considering all the advantages of this journal, we believe this journal to be of great benefit to the field of pharmacy. We invite you to work together with us to create this new and high-visibility open-access forum for the pharmaceutical sciences.

Competing interests

Dr. Vipra Kundoor is the Editor-in-Chief of Journal of Pharmaceutical Negative Results; Dr. Mueen Ahmed KK is a member of the Editorial board. The authors declare that they have no competing interests.

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This editorial may appear in other journals supporting Journal of Negative Results [www.pnrjournal.com]

Ethnobotanical study of medicinal plants used as anti-obesity remedies in Nkonkobe Municipality of South Africa.

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ABSTRACT

Introduction

Obesity is one of the world's leading preventable causes of death, diabetes, cardiovascular diseases and cancer. In South Africa, the number of people suffering from excess body weight is believed to be rising steadily. An ethnobotanical study of medicinal plants used for body weight reduction was carried out in the Nkonkobe Municipality of the Eastern Cape of South Africa.

Method

Structured questionnaire was administered to the informants.

Results

A total of 20 plants belonging to 18 families were identified for the management of obesity in the area.

Dicussion/Conclusion

Three medicinal plants namely, *Cissaempelos capensis, Curtisia dentata* and *Schotia latifolia* were repeatedly mentioned by the traditional healers and the local dwellers to have weight-reducing properties. Roots, leaves, whole plant and barks are the common parts of the plants used while decoctions and infusions are the main methods of preparation. There was a general belief on the efficacy of the prepared extracts; though there is still the need for further phytochemical and pharmacological investigations to validate the uses of the plants for the treatment of obesity.

Keywords: *obesity, herbal remedies, plant survey, medicinal plants*

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INTRODUCTION

Obesity is the sixth most important public health problems in both developed and developing countries. About 1.6 billion adults and 10% of children are now classified as overweight or obese.[1,2] Individuals with a body-mass-index (BMI) of equal or greater than 25.0kg/ m^2 are classified as overweight and when the BMI is greater than 30.0kg/ m^2; the individual is obese. The fundamental cause of this condition is the chronic energy imbalance in the excess energy intakes which is more than expenditure.[3] This is traceable to increased high fat and sugar intakes, reduced physical exercise, genetic susceptibility, hormonal abnormalities and socio-cultural factors.[2] Generally, this it is due to easy access to palatable diet, increased reliance on cars, and mechanized manufacturing.[4] Obesity is characterised by metabolic syndrome which usually manifests in insulin resistance, hypertension, dyslipidaemia, type-2 diabetes and cardiovascular risk factors that cluster within the

individuals. It could even lead to death. Recently, it has been observed that the risk of various diseases increases from a BMI of 21.0kg/m^2 and decreases life expectancy by seven years at the age of 40 years.[5]

In South Africa, at least, 25% of the adult population of all races are overweight and about 20% of men and women aged 20–30 are obese, a figure which increases to more than 50% in age 50–60. In recent times, the number has increased progressively such that obesity in South Africa is highly prevalent in all sectors[3], especially among children and urbanised black women. Interestingly however, overweight body type has positive connotations within the black South African community; symbolising happiness, beauty, affluence, health and negative HIV/AIDS status.[6,7] Unfortunately, obesity and its burden of diseases negatively affect the life of many South Africans, promote poverty and contribute to the increasing cost of health care.[8]

Recently, there has been a renewed interest in the search for herbal remedies for weight reduction.[9] Medicinal plants continue to play a role as abundant reservoir of bioactive compounds that could have beneficial effects on weight loss. This is consequent upon the fact that drug treatment of obesity, despite short-term benefits, is often associated with rebound weight gain with side effects after the cessation of drug use.[10] Preventative efforts at the societal and individual levels to address excessive caloric intake are currently not been met. Worse still, surgical interventions used in some cases to treat obesity are not appropriate.[11] There is the need to continue the search for medicinal plants for possible discovery of novel drugs for the management of obesity and related complications. The present study therefore, is a documentation of plants and plant parts that posses anti-obesity potential which are used by traditional healers in the Nkonkobe Municipality.

MATERIALS AND METHODS

Study area

Nkonkobe Municipality in the Eastern Cape Province of South Africa is situated between 32° 47′ S and 26° 50′ E. The area is bounded by the sea in the east and drier Karroo in the west. The altitude is approximately 1300 m above sea level and the vegetation is veld type 7.[12] The people of the region use herbal medications either alone or in combination with orthodox medicines for the treatment of several diseases. Majority of the people in the area are rural dwellers and use plants for the treatment of obesity, especially secondary complications of obesity such as diabetes, hypertension, heart problems and arthritis. They do not however, consider overweight or obese body mass as health risk.

Methodology

This study was carried out in March 2010 using a well structured questionnaire, interviews and general conversations with the herbalists and rural dwellers. A total of 15 informants were selected for the interview and four among them were women. Prior to the administration of the questionnaire, conversations were held with the assistance of a local interpreter to facilitate communication on the objective of the study and to build trust that the common goal was to document and preserve the knowledge on medicinal plants. The set questions contained the diagnosis of obesity, the names of plants used to treat the condition, methods of preparation, duration of treatment, adverse effects (if any) and the method of administration of the plant materials. Traditional healers and herbalists interviewed consisted of women and men between 40 and 60 years of age. Generally, they have low education qualification. Identification of the plants collected was done using relevant literature.[13] The vouchers of the plants were deposited at the Giffen Herbarium of the Botany Department, University of Fort Hare.

RESULTS AND DISCUSSION

The results of this study revealed 20 plant species belonging to 18 families that are frequently used for treatment of obesity by herbalists, traditional healers and people of the study area (Table 1). This appears to be the first mention of the plants for the treatment of obesity except *Rosmarinus officinalis* which is packaged and sold as a *slimtea* for weight management.[14] It was observed that, some of the plants are not taken solely, rather in mixture of vinegar, camphor, methylated spirit, salt and cayenne pepper as in *Agathosma apiculata* and *Cissampelos capensis*. Infusion from crushed *Kedrostis africana* and *Vernonia mesphilifolia* is also taken for its synergistic effect. Most of the plants mentioned are also employed in the treatment of other related diseases such as diabetes[15,16] hypertension, arthritis, body weakness and stomach ache, constipation.[16-17] Patients who reported such disease conditions were mostly with clinical signs of obesity such as excessive body mass with unflattering appearance, visceral fat and discomfort in the stomach, aching and swollen legs, psychological complications, unhappiness, dizziness and disordered eating. The plant extracts are usually orally administered for a long period of time, depending on the severity of the ailment.

"Table 1: Medicinal plants used for the management of obesity in Nkonkobe Municipality, South Africa".

Plant name	Family	Local/English name	Part used	Purpose of use	Preparation/ administration
Agathosma apiculata G.Mey	Rutaceae	Ibuchu/ Buchu	Roots	Used to reduce body weight and fluid retention	A mixture of powdered root, vinegar and camphor is taken ½ glass cup twice daily.
Alepidea amatymbica Eckl. & Zeyh	Apiaceae	Igwili / Umvuthuza/ Larger tinsel flower	Roots	Used for weight loss, stomach pain and wound healing	The powder root is soaked in water and infusion taken orally twice daily
Aloe ferox Mill	Aloaceae	Ikhala- lasekoloni/Bitter aloe	Leaves	Used for weight loss and as anti-diabetic. It enhances body healthiness, and treats arthritis and constipation	The liquid from the boiled leaves is taken ½ glass cup daily
Asparagus africana Lam	Asaparagaceae	Umthunzi/Climbing asparagus	Leaves	Used to reduce body weight, to increase urination and to treat diabetes	The leaves are crushed and soaked in water. ½ glass cup of the extract is taken twice daily
Bulbine alooides(L.) Willd	Asphodelaceae	Irooiwater	Roots	Used for weight loss, as anti-hypertensive, treats heart problems, and skin burns	2 litres of boiled root infusion is taken ½ glass cup twice daily for 2weeks
Cannabis sativa L.	Cannabaceae	Isangu/Marijuana	Leaves	It is used for weight loss, as psychoactive and stimulates energy.	Fresh leaves are crushed, soaked in water and extract is mixed with vinegar.
Catharanthus roseus L G.Don.	Apocynaceae	Epinkie/Madagascar periwinkle	Leaves	Used for body weight loss and to treat diabetics	Infusion from boiled leaves is usually taken ½ glass cup twice daily for 2weeks.
Cucumis africanus L.f.	Curcubitaceae	Ithangazana/Scaret guord	Whole plant	Used for body weight loss and wound healing	Cold Infusion of whole plant is taken ½ glass cup three times daily
Cissampelos capensis L.f.	Menispermaceae	Umayisake/David root	Roots	Used for weight loss, stimulates body energy and arrests stomach aches.	2 litres of mixture called "isiwasho" is made from crushed root and vinegar, rooi pepper,cayane salt and methylated spirit. It is taken ½ glass cup daily or two spoonfuls twice daily.
Curtisia dentata (Burm.f.) C.A.Sm	Cornaceae	Umlahleniselefile/ Capelancewood	Bark	Used to reduce body weight, as anti-diabetic, anti-hypertensive and to treat stomach ailments	Powered root is boiled in water and taken ½ glass cup for a start and two spoonfuls twice daily for a period of 1½ weeks

Plant name	Family	Local/English name	Part used	Purpose of use	Preparation/ administration
Exomis microphylla (Thunb.) Aellen	Chenopodiacae	Umvawenyathi/ Sugarbeet	Leaves	Used for body weight loss, as anti-diabetic and for wound healing	Decoction is taken ½ glass cup three times daily. healing
Kedrostis africana L.Cogn	Cucurbitaceae	Uthuvishe/Uthuvana/ Baboons cucumber	Bulb	Used for body weight loss	Decoction from crushed fresh bulb is taken twice daily
Leonotis leonurus L.R.Br	Lamiaceae	Umunyamunya/Wild dagga	Whole plant	Used for body weight loss, as anti-diabetic and for wound healing	A fresh plant is crushed, boiled in water and infusion is taken ½ glass cup twice daily.
Leonotis ocymifolia Burm.f Iwarsson	Lamiaceae	Umuncwane/wild dagga	Whole plant	Used for body weight loss, and to treat stomach ache	A fresh plant is crushed, boiled in water and infusion is taken ½ glass cup twice daily.
Mimosops obovata Nees ex Sond.	Sapotaceae	Umntunzi/ Red milkwood	Bark	Used for body weight loss, and to treat stomach ache	It is crushed and soaked in water. Infusion is taken twice daily.
Phytolacca dioca L.	Phytolaccaceae	Idolo lenkonyane/ Phytolacca	Leaves	Used for body weight loss and as purgative	A mixture is made from boiled leaves and vinegar which is taken ½ glass three times
Rosmarinus officinalis L	Lamiaceae	Roosmaryn /Rosemary	Leaves	Used for weight loss, reduces body fluid, as digestive, and anti-hypertensive. It is also used for flavour	Decoction is made from boiled fresh leaves and taken severally
Rubia petiolaris D.C	Rubiaceae	Impendulo/madder	Roots	Used to reduce body weight, treat stomach-ache and body weakness	The infusion is made from root decoction and taken ½ glass cup twice daily
Schotia latifolia Jacq	Fabaceae	Umaphipa/Forest boer-bean	Bark	Used for body weight loss, as anti-diabetic and antihypertensive. Used in the treatment of chest pain and arthritis	The bark is crushed to power and 2 spoonfuls of infusion is taken orally twice daily for 2weeks
Vernonia mesphilifolia Less	Asteraceae	Uhlunguhlungu/Iron weed	Whole plant	Used for weight loss, as anti-hypertensive, and removes body liquid	Decoction from ground fresh plan is taken ½ glass cup twice daily

Whole plant = root, stem and leaf.

However, the dosages and frequency of treatment are not standardized; they depend on the decision and folk experience of the herbalist. Decoctions and infusions were the most frequently used methods of preparation from roots and leaves (31.3%), whole plant (21%), bark (10.53%) and bulb (5%). There was no report on the use of seeds, fruits, and latex in any of the herbal preparations. The effect of the treatment on the patients following prolong administration of the extract, according to the traditional healers, reduces the body weakness, removes excess body fluid by increasing frequency of urination and induces purging of the stomach to reduce abdominal fat.

However, the mechanisms of action of the plant remedies are yet to be examined.

All the traditional healers reported the practice of combining two or more medicinal plants to treat obesity. In recent animal and human studies, a combination of plants or compounds has shown efficient decrease in weight gain and body fat.[18, 19] Three of the plant species namely, *Cissaempelos caenensis, Curtisia dentata* and *Schotia latifolia* were repeatedly mentioned by the traditional healers in the management of obesity in the area. Information from literature revealed that the plants are highly-valued and widely used by the community for the treatment of many other diseases besides obesity. For example, decoction from the root of *Cissampelos capensis* has been used topically for the management of glandular swelling, gall stones, menstrual problems, prevention of miscarriage and difficult labour, headache, pains, diabetes, tuberculosis, purgative, stomach and skin cancers.[20-22] The traditional uses of *Curtisia dentata* include the treatment of stomach ailments, diarrhoea, as a blood strengthener and as an aphrodisiac.[23] Other uses of the plant include the treatment of heartwater in cattle in the Eastern Cape[24] and the treatment of pimples.[25] The antifungal and antibacterial activities of the leaves of *C. dentata* showed five-fold inhibition more than the bark according to the recent studies.[26] The bark of *Schotia* species has been used in tanning and the decoction is taken for heartburn and to alleviate hangover. Both the bark and root are also used to treat diarrhoea.[20] *S. latifolia* has also shown growth inhibition of a number of bacterial and fungal species and it is used by rural livestock owners for the treatment of livestock diseases.[27] Although, there are a number of scientific investigations documented on the plants, unfortunately none of the studies revealed their anti-obesity properties.

Interestingly, responses from the four local healers revealed that excessive weight gain is not considered a health risk; rather it is culturally acceptable to the people as a sign of good living and healthy life. Consequently, the social perception and high caloric diet habit have predisposed several people to high risk morbid obesity, diabetes, hypertension, and arthritis in the area.[28]

CONCLUSION

The results of this study again have revealed that medicinal plants still play vital role in the primary healthcare of the people of this community. Further ethnopharmacological and phytochemical investigations of these plants are in progress to explore possible novel agents in the plants.

ACKNOWLEDGEMENT

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Intellectual property agreement

All the elderly and the traditional healers who contributed one information or the other during our ethnobotanical survey of medicinal plants used for the management of diabetes mellitus in the Nkonkobe Municipality were adequately financially rewarded with further verbal agreement that this research shall not be for commercial purposes but to serve as an enlightenment information to the community and the entire Eastern Cape, Province on the plants use for the management of obesity.

Compliance statement

No part of this study in any form has been commercialized, instead it is meant to be used as a tool for information dissemination on the medicinal plants used for the management of obesity in Nkonkobe Municipality and the entire Eastern Cape Province of South Africa.

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Pharmacognostic studies on *Alangium salvifolium* (Linn.f.) Wang. root bark

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ABSTRACT

Root bark of *Alangium salvifolium* (Linn.f.) Wang. (Family Alangiaceae) is a reputed drug mentioned in the ancient books of Ayurveda and Siddha for the treatment of epilepsy, jaundice, hepatitis etc. Root bark of the plant was subjected to macro-microscopic, photomicrographic, physico-chemical, fluorescence, preliminary phytochemical, TLC and HPTLC to fix quality standards for this drug. Microscopic studies have shown stratified phellem, rhytidome, cluster crystals of calcium oxalate and uni- to triseriate medullary rays in the root bark. Chloroform, ethyl acetate, ethanol extracts and alkaloid fraction revealed characteristic chromatographic patterns with presence of alkaloids in varying concentrations. This study would be useful in the identification and authentication of the raw drug.

Keywords: Alangiaceae, *Alangium salvifolium*, alkaloid fraction, HPTLC.

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INTRODUCTION

Alangium salvifolium (Linn.f.) Wang. (Family Alangiaceae) syn. *Alangium lamarckii* Thw. is a common tree growing in India. If is known as Sageleaved alangium in English. Ankola, Akoda, Dhera in Hindi, Ankota in Sanskrit and and Aliñcil in Tamil. It is a small tree growing up to 10 meter in height, with more or less spinescent branches; stem bark pale brown colored with shallow cracks; young parts pubescent; root yellow, strong with brown bark.[1,2] The tree is widely distributed throughout India, Ceylon, South China, Malaya, Philippines. The plant flowers during February to April and bears fruits during May – August.[3,4] Root bark of this tree is bitter, purgative, anthelmintic, astringent, pungent, efficacious in leprosy, has emetic properties and useful in fever, skin diseases and as a purgative, antipoisonous against rat, snake and insect bites, antipyretic, anti-inflammatory, analgesic, diuretic, anthelmintic, antidiarrhoeal, useful in insanity, epilepsy, biliousness, syphilitic and other skin diseases, antihemorrhagic, expectorant in cold and cough, rabies, jaundice and hepatitis and effective remedy for blood disorders.[5,6] In the Siddha system of medicine it is used in the preparations of Pataic Cankāran and Ayapirunka Rāja Karpam.[7] Root bark of *A. salvifolium* is reported to contain alangine A, alangine B, alanginine, ankoline, lamarkine, emetine, cephaeline, psychotrine, tubulosine, alangicine, desmethylpsychotrine, desmethyltubulosine, myristic, palmitic, oleic, linoleic acids, myricyl alcohol, stigmasterol and β-sitosterol.[8-13] As there is no detailed pharmacognostical data reported on the root bark of this plant, present study attempts to develop pharmacognostical data on the drug essential for its standardization and authentication.

MATERIALS

Plant materials were collected from Anna Hospital Campus, Chennai, identified with the help of regional floras.[3,4] The dried specimen was deposited in the crude drug museum of CSMDRIAS (J/RB3). For microscopical study properly washed plant material was cut in to desirable size and preserved in FAA.

METHODS

Free hand sections were taken, stained with the reagents used in pharmacognosy studies.[14,15] Photomicrography was done using Olympus Trinocular microscope attached to Olympus digital camera, drawings for powder analysis were made with the help of camera lucida.

Powder of the dried root bark of *A. salvifolium* was used for chemical analysis. Physico- chemical studies like total ash, water soluble ash, acid insoluble ash water and alcohol solubility, loss on drying at 105°C, heavy metals and successive extractive values by Soxhlet extraction method were carried out as per the WHO guidelines.[16] Preliminary phytochemical tests were done as per the standard methods.[17] The fluorescence behaviour of the powdered plant material in the ordinary light and ultraviolet light were observed by treating the powder in different reagents and viewing under the light of required wave length in a UV chamber.[3] The alkaloid content was estimated using standard methods.[18] Four grams of powdered drug was extracted in *n*-hexane in Soxhlet apparatus for consecutively three times. Then the marc was successively extracted with chloroform, ethyl acetate and ethanol. The combined extracts were concentrated separately under reduced pressure and made upto 10 ml in standard flasks with respective solvents. These solutions were used for both TLC and HPTLC analysis. The HPTLC finger print profile of *n*-hexane, chloroform, ethyl acetate, ethanol extracts and alkaloid fraction of *A. salvifolium* root bark were performed on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (E. Merck) as adsorbent and employing CAMAG Linomat IV applicator. The mobile phase used was toluene: ethyl acetate: diethylamine (5:3.5:0.5 v/v). The plate after air drying was scanned using CAMAG TLC Scanner 030618 with WINCATS 4.05 version software at a wavelength of UV 254 and 366 nm using deuterium and mercury lamps respectively.[19-22]

RESULTS

Macroscopy

Root bark is 3 to 4 mm in thickness, externally rough, light brownish yellow, exfoliating into very thin papery phellem, easily peeling in layers; exfoliated bark looks light copper in colour, occasionally lenticels can be seen. Surface of the transversely cut root bark shows an outer light copper coloured phellem, pale cortex and pale brownish phloem. Fracture is fibrous, odour characteristic and taste bitter.

Microscopy

TS of the root bark shows outer phellem, a broad zone of irregular phellem showing development of rhytidome, a narrow band of cortex and phloem (Figure 1). Phellem is well developed and 0.1 to 0.15 mm wide. Phellem tissue is sometimes seen in 5 or more successive layers with alternating parenchymatous tissue in between them due to the formation of phellogen at different levels in the outer phloem region of the root. Some of these phellem layers have 10-20 rows of thin-walled tangentially elongated regularly arranged cells measuring 15-30-45-60 µ tangentially and 12-15 µ radially. The parenchymatous tissue in between two phellem layers is composed of slightly obliterated phloem elements and parenchyma cells. Some of the cells contain druse crystals of calcium oxalate measuring upto 18-30 µ in diameter. The phloem extends from the cambial zone up to the phellem tissue. The cells are usually uniformly thin-walled and regularly arranged except that the cells towards the phellem show radial divisions and are larger in size.

The phloem elements are arranged in narrow radial strips with alternating phloem rays. Sieve elements and companion cells are distinct. The phloem parenchyma cells contain solitary druse crystals of 9-15-27 µ in diameter. The phloem parenchyma cells are thin walled measuring 20-25-40 µ tangentially and 20-27 µ radially. Phloem rays are usually uniseriate, few biseriate rays are also seen. The cells are nearly circular to polygonal, thin-walled and become slightly larger towards the periphery. The cells near the cambial zone measure 30-36 µ tangentially and 25-30µ radially and the cells towards the phellem measure 45-60 µ tangentially and 25-30 µ radially. Most of these cells especially those of the inner half of the phloem are filled with simple round starch grains of 2-3-6 µ in diameter. Sclerenchyma cells (phloem fibres) are absent in the phloem region. Cambium is a narrow zone composed of 4-5 rows of thin-walled

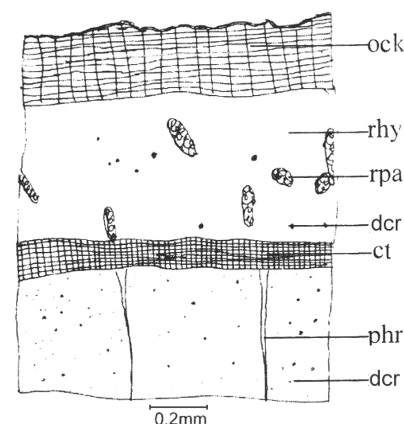


Figure 1. Diagrammatic TS of *A. salvifolium* root bark.
ct, cortex; ock, outer phellem; pr, phloem ray; dcr, druse crystals of calcium oxalate; rhy, rhytidome; rpa, radially dividing parenchyma.

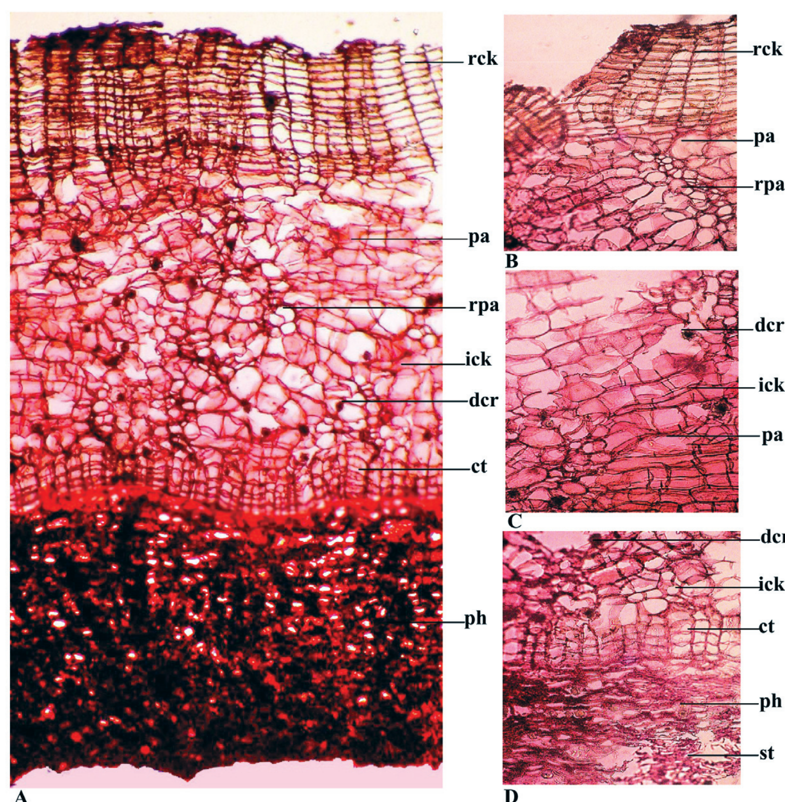


Figure 2. Microscopy of *A. Salvifolium* root bark.
A. Detailed TS,
B. A portion enlarged to show dividing phellem,
C. A portion enlarged to show irregular phellem,
D. A portion enlarged to show cortex and phloem.
ct-cortex; dcr-druse crystals of calcium oxalate; ick-irregular phellem; pa-parenchyma; ph-phloem; rpa-radially dividing parenchyma; st-sieve tube.

regularly arranged cells measuring 12-27 μ tangentially and 6-9 μ radially (Figure 2).

Powder analysis

Powder drug is pale yellow with slightly bitter taste and unpleasant odour. Microscopic examination of the powder showed cluster crystals of calcium oxalate, phellem in surface view, obliquely cut phellem, elongated tubular phellem cells in sectional view, 2-3 seriate medullary rays, thin-walled phloem parenchyma embedded with druse crystals of calcium oxalate. Rows simple elongated thin-walled parenchyma each embedded with a druse crystal of calcium oxalate, fragments of radially dividing parenchyma from the rhytidome and numerous druse crystals of calcium oxalate (Figure 3).

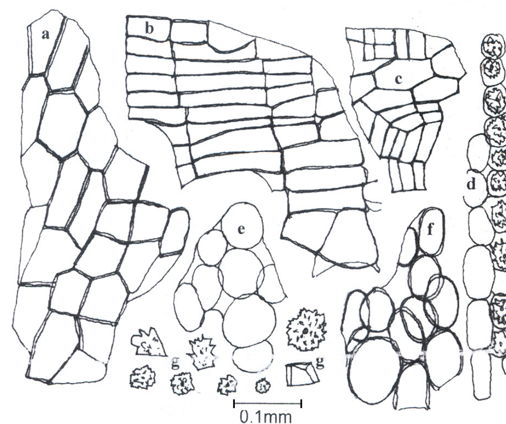


Figure 3. Powder microscopy of *A. salvifolium* root bark.
a, phellem in surface view; b, transversely cut phellem; ck, obliquely cut phellem; d, parenchyma containing druse crystals of calcium oxalate; e, medullary ray cells; f, parenchyma from rhytidome; g, druse crystals of calcium oxalate.

Preliminary phytochemical study

Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the root bark of

A. salvifolium. All the extracts showed the presence of steroid. Chloroform extract showed the presence of phenol and alkaloid. Ethyl acetate and alcohol extracts gave positive results for flavonoid, phenol, tannin and alkaloid and did not answer for quinone, coumarin, iridoid and terpenoids (Table 1). The results of fluorescence analysis have been presented in Table 2.

Physio-chemical study

Physio-chemical parameters of the root bark of *A. salvifolium* are shown in Table 3. The chloroform and ethyl acetate extractive (hot successive) values were indicative of the presence of various phyto-constituents (Table 3).

Table 1. Preliminary phytochemical tests for different solvent extracts of root bark of *A. salvifolium*

Test	n-Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Ethanol Extract
Alkaloid	–	+	+	+
Quinone	–	–	–	–
Coumarin	–	–	–	–
Flavonoid	–	–	+	+
Steroid	+	+	–	–
Phenol	–	+	+	+
Tannin	–	–	–	–
Glycoside/Sugar	–	–	+	+
Iridoid	–	–	–	–
Terpenoid	–	–	–	–

Table 2. Fluorescence analysis of root bark of *A. Salvifolium*

Powder	Daylight	UV 254 nm	UV 366 nm
Powder	Pale yellow	Brownish yellow	Yellow
Powder+1N HCl	Yellow	Fluorescent yellow	Greenish yellow
Powder+50% H_2S0_4	Pale brown	Fluorescent yellow	Greenish yellow
Powder+1N NaOH	Dark brown	Dark brown	Bluish yellow
Powder+Alcoholic 1N NaOH	Dark brown	Dark brown	Bluish yellow

Table 3. Physico-chemical parameters of root bark of *A. salvifolium*

Parameters	Mean value (% w/w, n=3) ± SD
Loss on drying at 105°C	10.81 ± 0.04
Total ash	11.28 ± 0.69
Water soluble ash	3.52 ± 0.045
Acid-insoluble ash	2.48 ± 0.059
Solubility values (Cold)	
a. Water	24.74 ± 0.36
b. Ethanol (95%)	13.88 ±0.33
Extractive (Hot successive) values	
a. n-Hexane	0.73
b. Chloroform	3.30
c. Ethyl acetate	9.31
d. Ethanol	8.62
Alkalinity (cc of 0.1N HCl/g)	0.12 ± 0.06
Alkaloid content	1.743
Cadmium	BDL (< 0.2 ppm)

SD-Standard Deviation; BDL-Below Detection Limit

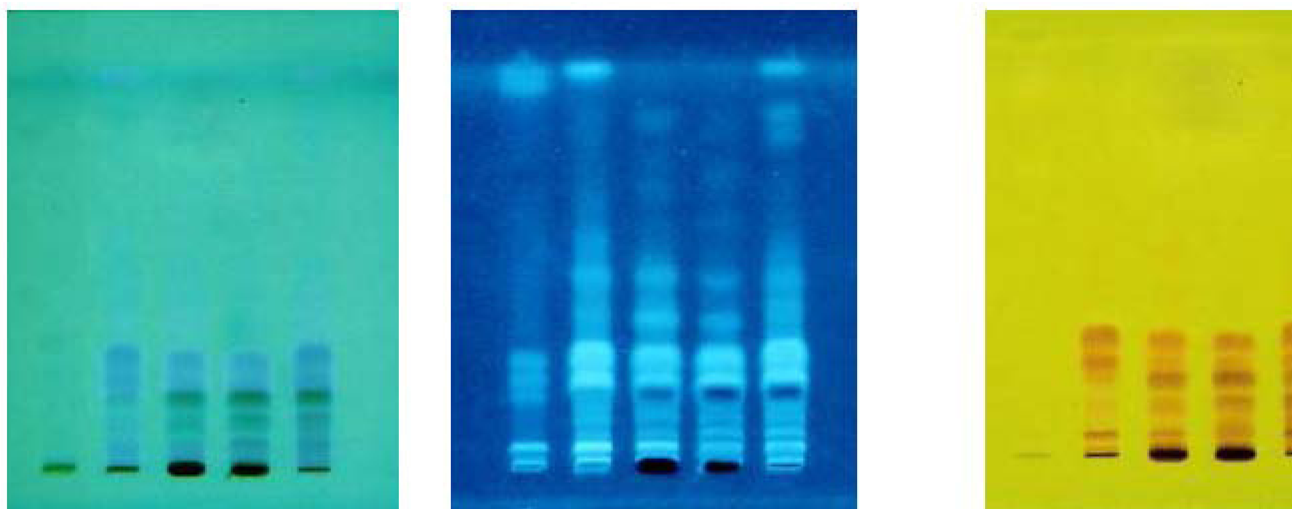


Figure 4. Thin Layer Chromatography of extracts of *A. salvifolium* root bark
Track 1: Hexane extract; Track 2: Chloroform extract; Track 3: Ethyl acetate extract; Track 4: Ethanol extract; Track 5. Alkaloid fraction

TLC/HPTLC Analysis

TLC and HPTLC finger print profile of *n*-hexane, chloroform, ethyl acetate, ethanol extracts and alkaloid fraction are shown in Figure 4, 5 & 6.

DISCUSSION

Stratified phellem, development of rhytidome, druse crystals of calcium oxalate and uni- to triseriate medullary rays are salient microscopic features of the drug.

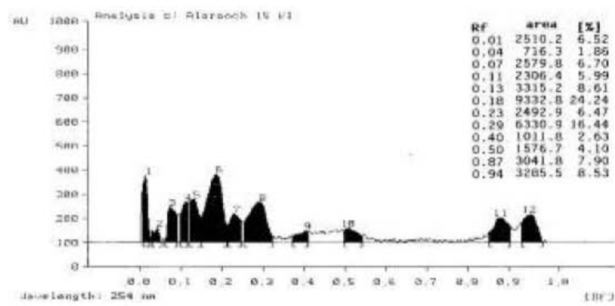
Deterioration time of the barks depend upon the amount of water present in them. If the water content is high, the bark will deteriorate due to fungal attack. The loss on drying at 105°C in root bark was found to be 10.8 %. Total ash value indicates the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value and water-soluble ash content were 11.28 % and 3.52 % respectively. The amount of acid-insoluble siliceous matter present in the plant was 2.5 %. The water-soluble extractive value (24.74%) indicated the presence of sugar, acids and inorganic compounds. The successive hot extractive value with ethanol (8.62%) was found to be less than that of ethanol soluble extractive value (13.88%) by cold extraction method. The alcohol soluble extractive values (13.88%, 8.62%) indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. *n*-Hexane (hot) extractive values indicated the non-polar secondary metabolites present in the plant.

The finger print profile of *n*-hexane extract under UV light at 254 nm showed no peaks; but under 366 nm showed 5 spots at R_f value 0.05, 0.18, 0.21, 0.26 and 0.82 (all blue colour); after derivatization with Dragendorff's reagent *n*-hexane extract showed no spot indicating the absence of alkaloid. The chloroform, ethyl acetate, ethanol extracts and alkaloid fraction under UV 254 nm showed 8 spots at R_f value 0.07, 0.14 (both blue), 0.19, 0.26, 0.36 (all green), 0.53,0.72 and 0.94 (all blue); under UV 366 nm showed 8 spots at R_f 0.04, 0.09, 0.15 (all blue), 0.21 (dark blue), 0.24, 0.32, 0.39 and 0.49 (all blue); and after derivatization with Dragendorff's reagent showed 5 spots at R_f 0.07, 0.14, 0.19, 0.21 and 0.26 (all orange). The finger print profile of chemicals in chloroform, ethyl acetate and ethanol extracts were found to be similar and hence either of the solvent can be used for extraction and also for identification of root bark.

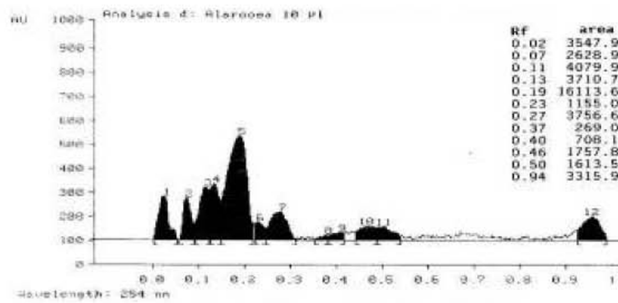
CONCLUSION

Morphology as well as various pharmacognostic characters of the root bark was studied and preliminary phytochemical, physico-chemical, TLC and HPTLC finger print analyses were also done for authentication and quality control of the drug. The study showed stratified phellem, rhytidome, druse crystals of calcium oxalate and uni- to triseriate medullary rays are the characteristic microscopic features of the drug. Chloroform, ethyl acetate, ethanol extracts and alkaloid fraction revealed characteristic chromatographic pattern with varying concentrations of alkaloids. Chromatogram has shown a common spot

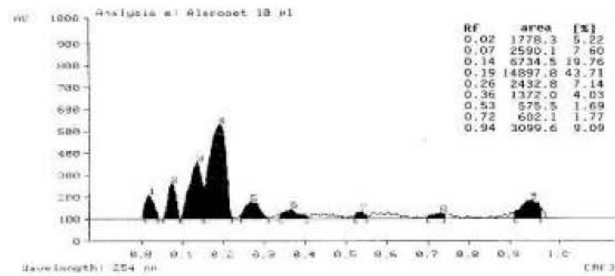
5A. Chloroform Extract 10 μl



5B. Ethylacetate Extract 10 μl



5C. Ethanol Extract 10 μl



5D. Alkaloid Fraction 10 μl

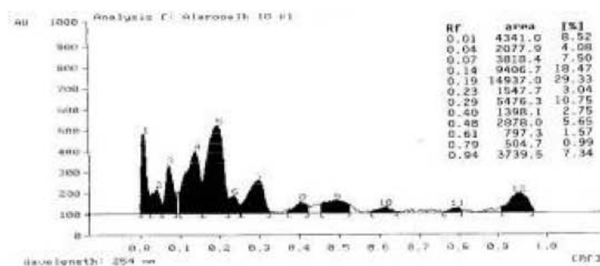
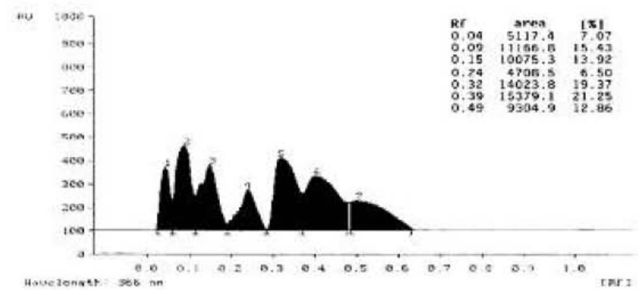
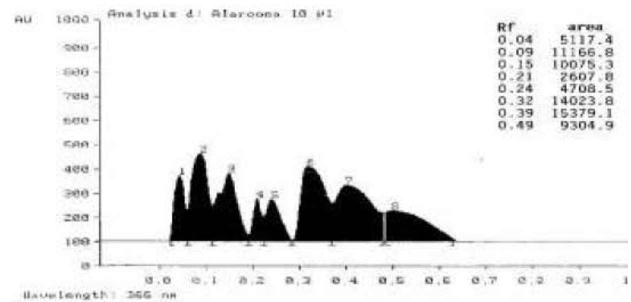


Figure 5. A-D, HPTLC finger print profile of extracts of *A. salvifolium* under UV 254 nm

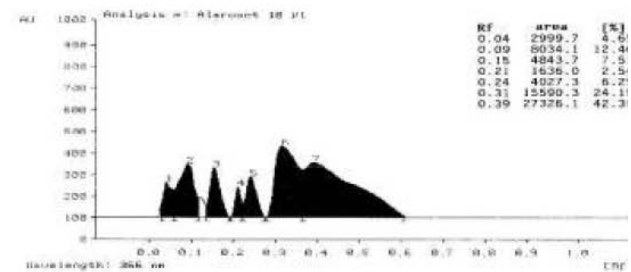
6A. Chloroform Extract 10 μl



6B. Ethylacetate Extract 10 μl



6C. Ethanol Extract 10 μl



6D. Alkaloid Fraction 10 μl

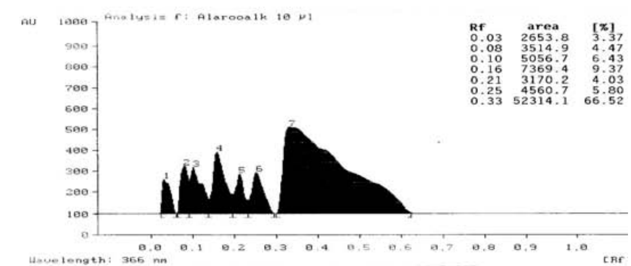


Figure 6. A-D, HPTLC finger print profile of extracts of *A. salvifolium* under UV 366 nm

(R_f 0.19) for ethyl acetate and ethanol extracts when visualized under UV at 254 nm which is also detected in the alkaloid fraction indicating a common constituent to all the three fractions. This study would be highly useful for identification and standardization of this raw drug.

ACKNOWLEDGEMENT

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HPTLC Fingerprinting of different leaf extracts of *Tylophora indica* (Burm f.) Merill.

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ABSTRACT

Tylophora indica is very popularly used for the treatment of asthma based on its traditional use for asthma. *Tylophora* is perennial climbing plant native to the plains, forests, and hills of southern and eastern India. A method has been developed for different extracts of *Tylophora indica* for HPTLC fingerprinting analysis for identification and quantification of marker compound. For chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5) v/v, Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) v/v and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v. The HPTLC fingerprinting profile developed for different extracts of *Tylophora indica* will help in proper identification and quantification of marker compound.

Keywords: *Tylophora indica*, HPTLC fingerprinting, TLC, Tylophorine.
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INTRODUCTION

Tylophora indica (Burm f.) Merill. (Family: Asclepidaceae) commonly known as Antmul is a twining perennial plant distributed throughout southern and eastern part of India in plains, forests, and hilly places.[1] The plant is found growing normally in Uttar Pradesh, Bengal, Assam, Orissa, Himalayas and sub Himalayas in India.[2] It is a branching climber or shrub that grows up to 1.5 meters, leaves are obvate-oblong to elliptic-oblong, 3-10cm long and 1.5-7cm wide.[3] Roots Long fleshy with longitudinally fissured light brown, corky bark. Flowers minute, 1-1.5 cm across, in 2-3 flowered fascicles in axillary umbellate cymes. Calyx divided nearly to the base , densely hairy outside; segments lanceolate, acute. Corolla greenish yellow or greenish purple; lobes oblong, acute. Fruit a follicle, up to 7 × 1cm, ovoid lanceolate, tapering at apex forming fine micro, finally striate, glabrous, Seeds 0.6-0.8 × 0.3-0.4cm long.[4]

The plant has been reported to contain 0.2-0.46% alkaloids viz. Tylophorine, tylophorinine, tylophorinidine, (+)septicine, isotylocrebrine, tylophorinicine, sterols, flavanoids, wax, resins, and tannins.[5] The plant has been traditionally used for the treatment of bronchial asthma, jaundice and inflammation.[3,6] Its antitumor, immunomodulatory, antioxidant, antiasthmatic, smooth muscle relaxant, antihistaminic, hypotensive, antirehumatic activities are scientifically proven. In Ayurveda, the plant has been used in treatment of asthma, dermatitis and rheumatism.[1,6] Although the leaf and root of this plant are widely used for treating jaundice in Northern Karnataka, there is a paucity of scientific evidence regarding its usage in liver disorder[3]. The other reported activities include immunomodulatory activity, anti-inflammatory activity, anticancer activity and antiamoebic activity.[6, 7, 8, 9, 10]

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Tylophora indica* is collected in the month of August-September (2009) from Herbal Garden of JAMIA HAMDARD, New Delhi was identified and authenticated at Raw material, Herbarium and museum NISCAIR, CSIR, New Delhi, India and sample was submitted in museum for future reference. Ref. NISCAIR/RHMD/consult/-2009-10/1361/163.

Preparation of extract

For development of HPTLC fingerprints the different extracts of *Tylophora indica* were preparared by taking 5 gm of dried leaves powder in 250 ml of conical flask and added 100 ml of corresponding solvents such as methanol Chloroform and Pet. ether and heated on water bath for one hour. Filtered the extract and evaporated to dryness and prepared the samples 20 mg/ml by reconstitute with same solvents and developed solvent systems for their separation by thin layer chromatography.

TLC/HPTLC Profile

Developing solvent system

A number of solvent systems were tried, for extracts Methanolic extract, Chloroform extract, and Petroleum ether extract For chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5) v/v, Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) v/v and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v.

Sample application

Application of bands of each extract was carried out (5mm in length and 2 µl in concentration) using spray technique. Sample were applied in duplicate on pre-coated silica gel $60F_{254}$ aluminium sheets (20x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of chromatogram

After the application of spots, the chromatogram was developed in Twin trough glass chamber 20× 10 cm saturated with solvents developed for each extracts before puting the plates for 20 mins.

Detection of spots

The air-dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 366 nm. The R_f values and finger print data were recorded by WIN CATS software. The data obtained are summarized in Table (1, 2 &3) for where as the developed chromatogram can be seen at 366 nm.

RESULTS AND DISCUSSION

Different solvent systems were tried by hit and trial method, for extracts: Methanolic extract, Chloroform extract, and Petroleum ether extract. Satisfactory resolution was obtained in solvent systems developed, for chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5) v/v, Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) v/v and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v. The solvent system tried for Pet. Ether extract 12 peaks is observed, in methanolic extract 12 peaks is obtained too. However in Chloroform 8 peaks is observed. The RF values obtained are calculated through WINCATS HPTLC software supplied with the instrument (Table 1, 2 &3).

For Petroleum Ether:

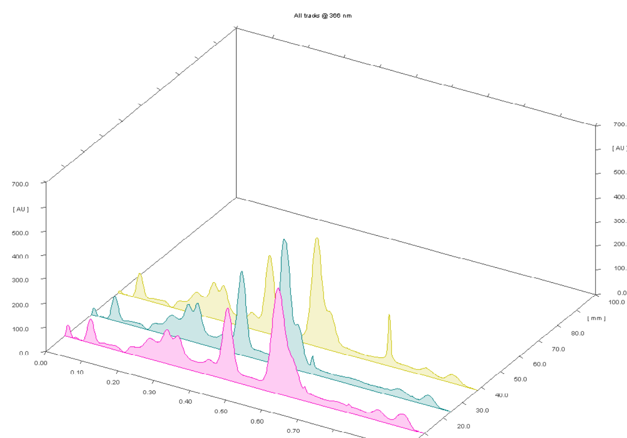

Figure 1 Pet. Ether Ext. 3-D View at 366nm.

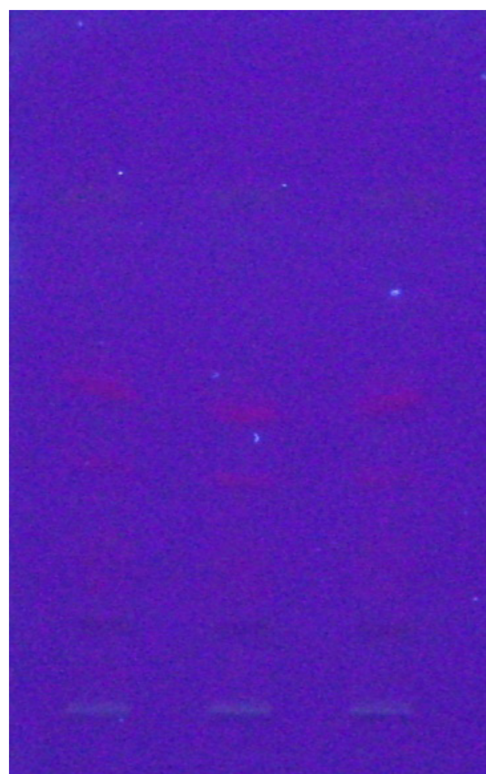

Figure 2 *Developed TLC plate of Pet. Ether extract of Tylophora indica* at 366nm.

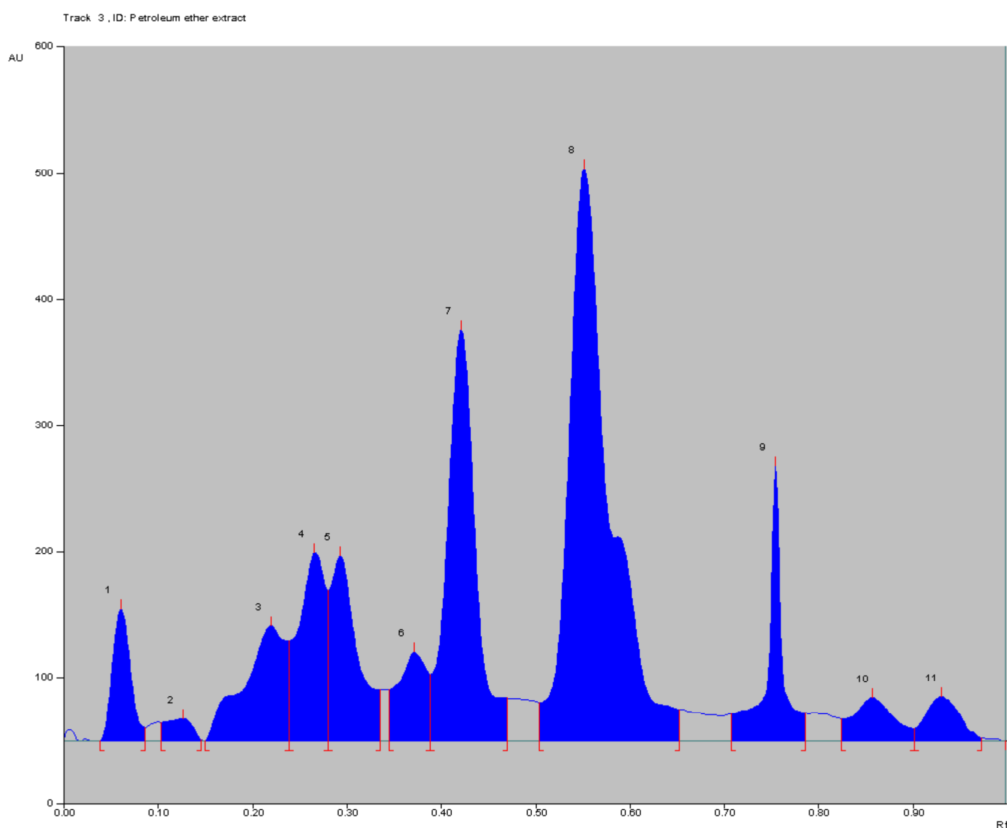

Figure 3 Pet. Ether Ext. peaks at 366nm.

Table 1 Thin layer chromatography of Pet. Ether ext.

Sample	Solvent System developed	No. of peaks and Rf values
Pet. Ether	Hexane(40):ethyl acetate(60)	**(12)** 0.01, 0.07, 0.13, 0.22, 0.27, 0.29, 0.37, 0.42, 0.53, 0.61, 0.85, 0.94

For Methanolic extract:

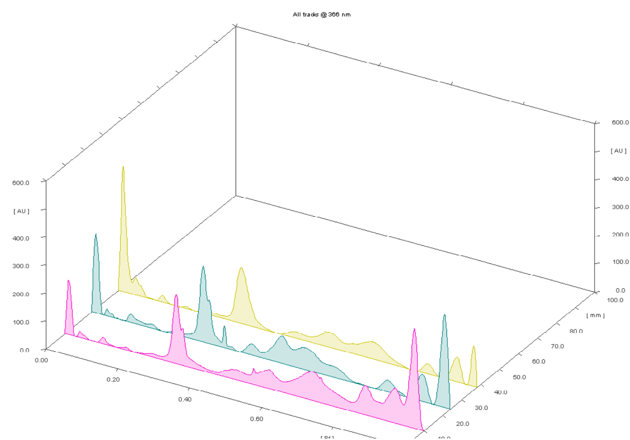

Figure 4 Methanolic Ext. 3-D View at 366nm.

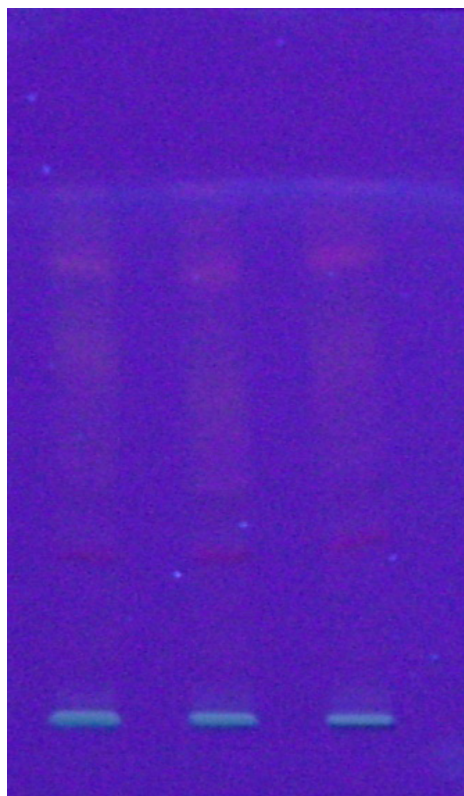

Figure 5 Developed TLC plate of methanolic extract of *Tylophora indica* at 366nm

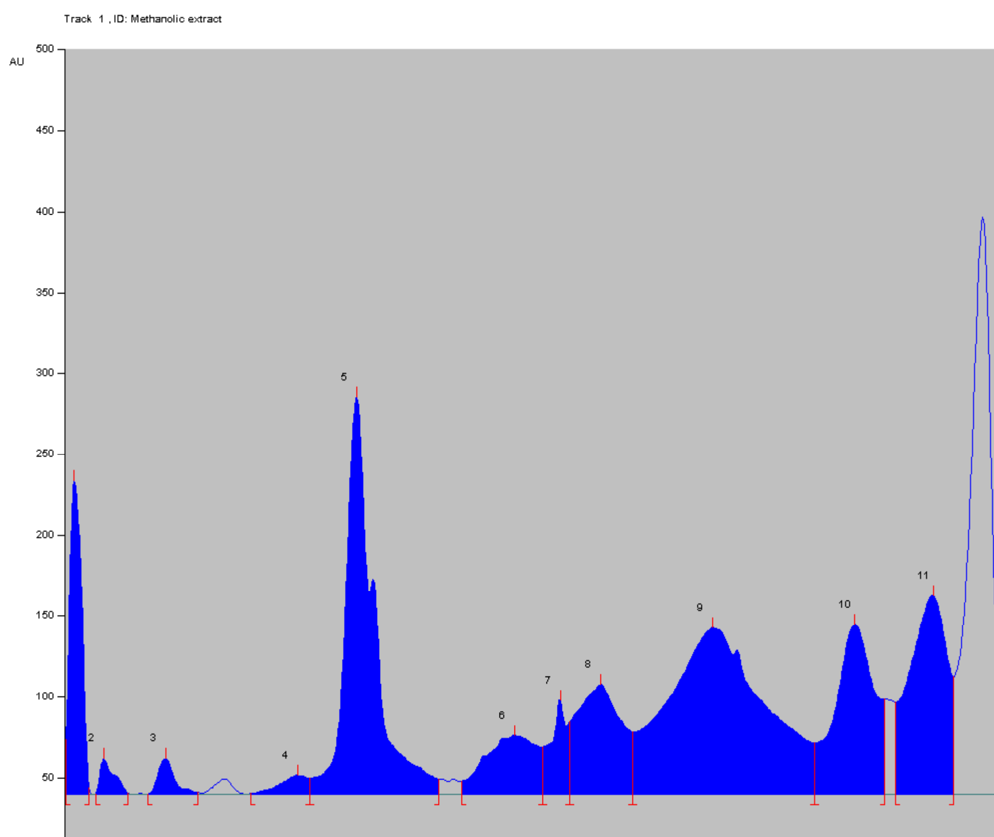

Figure 6 Methanolic Ext. peaks at 366nm.

For Chloroform Extract:

Table 2 Thin Layer chromatography of methanolic ext.

Sample	Solvent system developed	No. of peaks and Rf values
Methanolic Extract	Toluene(5):Chloroform(90):ethylacetate(5)	**(12)** 0.01, 0.04, 0.11, 0.17, 0.24, 0.31, 0.37, 0.45, 0.53, 0.60, 0.83, 0.92

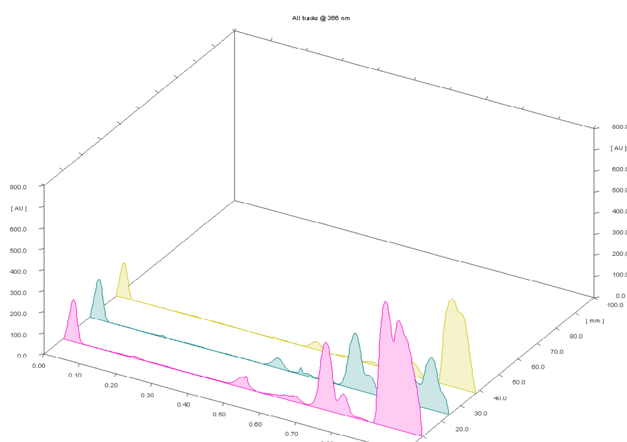

Figure 7 Chloroform extract 3-D view at 366nm.

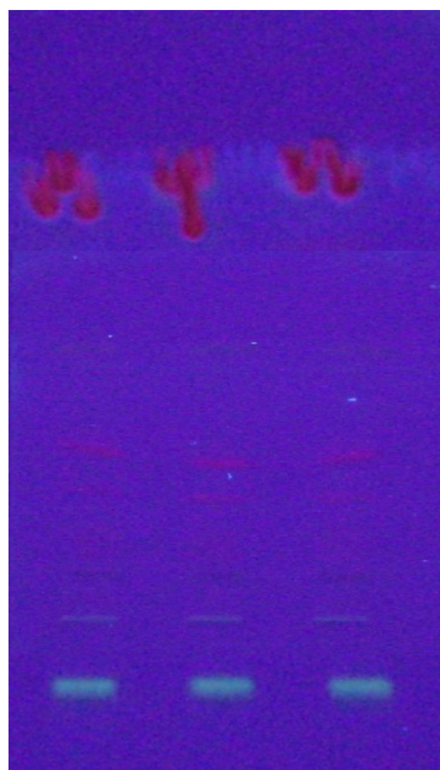

Figure 8 Developed TLC plate of chloroform extract of *Tylophora indica* at 366nm

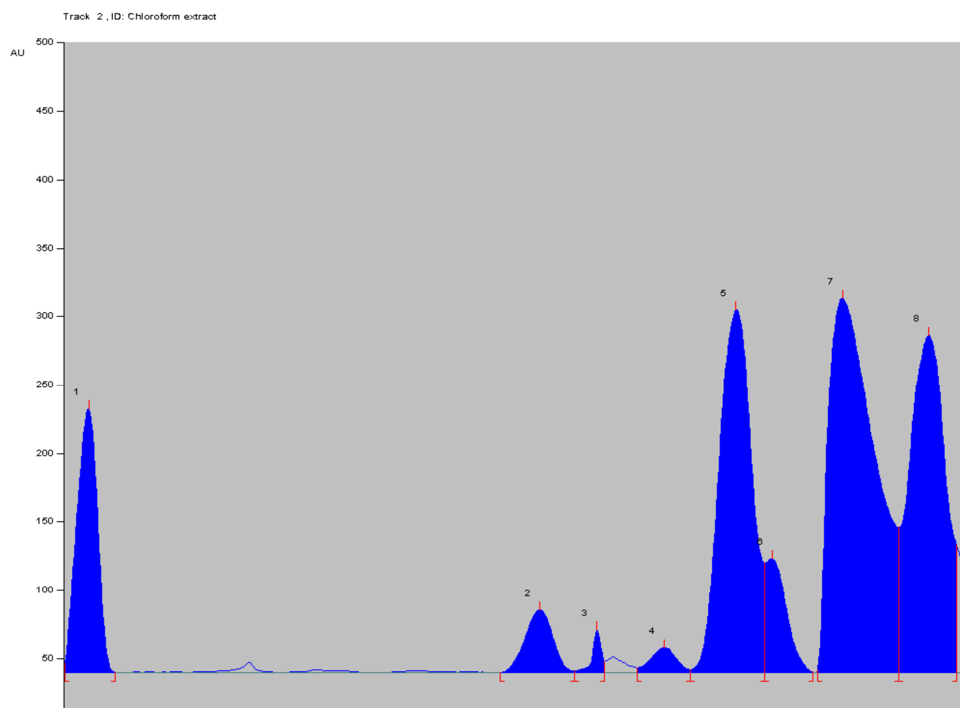

Figure 9 Chloroform Ext. peaks at 366nm

Table 3 Thin Layer chromatography of Chloroform ext.

Sample	Solvent system developed	No. of peaks and Rf values
Chloroform Extract	Chloroform (90): methanol (5) : ethyl acetate (5)	**(8)** 0.03, 0.52, 0.59, 0.66, 0.74, 0.78, 0.86, 0.95

CONCLUSION

Medicinal Plant material is obtained from different heterogeneous sources which may lead to variation in therapeutic values and variation in phytochemistry. The HPTLC-Fingerprinting profile is very important parameter of herbal drug standardization for the proper identification for medicinal plants. This parameter can also be very important tool if adulteration is suspected in medicinal plant material. The present HPTLC-fingerprinting profile can be used as a diagnostic tool for the identity and to determine the quality and purity of the plant material in future studies. Also the present study on *Tylophora indica* leaves will help in identification and quantification of chemical marker compound.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Sayeed Ahmad, Assistant professor, Department of Pharmacognosy, Faculty of Pharmacy, Jamia Hamdard, New Delhi, for providing laboratory facilities and reagents and chemicals.

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Pharmacognostical Standardization of *Withania coagulans* Dunal.

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ABSTRACT

Pharmacognostical standardization of fruits of *Withania coagulans* Dunal. (Solanaceae) has been carried out in the present study. The study includes macroscopical and microscopical evaluation along with estimation of its physicochemical parameters such as ash and extractive values, preliminary phytochemical screening and fluorescence analysis. It also includes quantification of some of the active constituents such as withanolides (withaferin-A) by HPTLC, total phenolic, tannin, flavonoids and flavonols. The present study reveals standardization profile for drug like *Withania coagulans*, which would be of immense value in botanical identification and authentication of plant drug and may help us in preventing its adulteration.

Keywords: *Withania coagulans*: Standardization: Pharmacognosy: HPTLC Quantification: Withaferin-A.

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INTRODUCTION

Pharmacognosy at present has become one of the pillars of areas like pharmacy, medicine, natural product chemistry and many others allowing them to recognize the importance of plants as sources of medicines. This approach has initiated in various active research programmes either to isolate new lead compounds or to produce standardized extracts[1]. For this it is very necessary to evaluate various qualitative and quantitative parameters, which may be helpful in setting standards for particular medicinal plant/parts of the plant. With the help of this standards one can easily identify and characterize an individual drug, which may play a major role in maintaining quality and purity of that particular drug and its formulation and prevent it from being adulterated by drug of same or other genius having low potency[2].

Thus, the present study deals with standardization of one of such medicinal plant i.e. *Withania coagulans* Dunal. (Solanaceae) distributed in the east of the Mediterranean region and extends to South Asia. It shows the presence of esterases, lignan, alkaloids, free amino acids, fatty oils, essential oils and withanolides[3]. The drug has shown to have anti-inflammatory[4], cardio tonic activities[5], hepatoprotective[6], anti-fungal[7], hypoglycemic, free radical scavenging activity[8], hypolipidemic[9] and wound healing activity[10]. Recent investigation has shown that withanolides isolated from the aqueous extract of fruits possessed a good antihyperglycemic and antidyslipidemic activity[11]. Due to its wide therapeutic importance it is worthwhile to obtain various qualitative and quantitative standards of drug to prevent its adulteration.

MATERIAL AND METHODS

Macroscopical and Microscopical studies

The fruits of *Withania coagulans* Dunal. (with persistent calyx and pedicle) were purchased from the local market of Varanasi and were authenticated by Prof. V.K. Joshi, Department of Ayurveda (Banaras Hindu University) and Mr. Narayanappa, Chief botanist TAMPCOL (Chennai). The voucher specimen (SM/WC/02) has been deposited in the department. The fruits were then examined macroscopically with reference to its colour, shape, size, odour and taste etc. Hand section of the petiole, calyx, pericarp and seeds of the overnight soaked fruits

were taken stained and mounted following usual micro techniques[12]. Micro chemical test for cell wall and cell content were performed according to Johansen (1940)[12], Kay (1938)[13] and Trease and Evans (1983)[14]. Schultz's fluid was used to study the characteristics of the individual cells and tissues of pedicel, calyx, pericarp and seeds by mounting with glycerin.

Physicochemical standards

Physicochemical parameters of the powdered drug such as total ash, acid insoluble ash, water and alcohol soluble extractive values were determined according to the procedure mentioned in Indian Pharmacopoeia (1996)[15].

Fluorescence characteristics

Air dried coarse powder of fruits was examined under ultraviolet light according to the method described by Kokaski et al., (1949)[16].

Preliminary phytochemical screening

The fruits of *Withania coagulans* (with persistent calyx and pedicle) were coarsely powdered and extracted with methanol using soxhlet. To the extract water was added (1: 1) and fractionated using water immiscible solvent i.e. chloroform (2: 1) in a separating funnel. Both the fractions chloroform and hydroalcoholic were concentrated and dried in a Rota evaporator initially and then in vacuum desiccator. Preliminary phytochemical screenings of methanolic extract along with chloroform and hydroalcoholic fractions were done for the presence of various phytoconstituents by using standard procedure[17].

Quantitative estimations

Total phenolic and tannin contents were estimated according to the method described by Hagerman et al., (2000b)[18], whereas estimation of total flavonoid and flavonol content were done following the standard procedure proposed by Kumaran & Karunakaran (2007)[19].

Identification and quantification of withaferin-A by TLC and HPTLC method

Identification and quantification of withaferin-A in the two fractions i.e. chloroform and hydroalcoholic along with the parent extract was carried out by following two steps:

1. *Confirmative test for the presence of withaferin-A by TLC and preparative TLC:* The parent methanolic extract along with its chloroform and hydroalcoholic fractions of fruits were compared with standard withaferin-A (Ranbaxy) by TLC method using toluene: ethyl acetate: formic acid (5: 5: 1) as solvent system and Liebermann-burchard as spraying reagent. Depending upon the result obtained from TLC analysis preparative TLC plate of chloroform fraction was prepared. The wavelength of the compounds obtained as bands were then compared with the wavelength of standard withaferin-A.

2. *Quantification of withaferin-A by using HPTLC method:* Quantification of withaferin-A was done on both chloroform and hydroalcoholic fraction along with methanolic extract by using solvent system toluene: ethyl acetate: formic acid in the ratio of (5: 5: 1).

Instrumentation

Executed by: anchrom (Mumbai), Applicator: CAMAG Automatic TLC Sampler, Scanner: CAMAG TLC Scanner, Wavelength used: 580nm, Plates used: Silica Merk 60F plates.

RESULTS

Macroscopical description

The fruits are superior, indehiscent, many seeded berry type. It is pedicellate, round to globous in shape, 4–6mm in diameter, yellow to brown in colour and closed in leathery persistent calyx mostly with pedicel. The fruits have an indistinct odour with a slightly bitter taste (Figure. 1).

Figure 1: Fruits of *Withania coagulans*.

Microscopical description

The transverse section of the pedicel shows a single layered epidermis covered with a large number of branched and unbranched trichomes, followed by cortex constituting 6–10 layers of collenchymatous cells. The pericycle shows the presence of pericyclic fibers with intervening parenchymatous cells, whereas the central region represents a continues narrow band of phloem encircling the xylem beneath which is a ring of intraxylary phloem. The centre most region is consist of hollow pith surrounded by parenchymatous cells with a few thick walled lignified fibers towards the intraxylary phloem. Calyx shows a single layer of thin walled cells in upper and lower epidermis with a few branched and more unicellular covering trichomes similar to the pedicel which are present only in the upper epidermis, followed by the presence of few candelabra-type trichomes. The mesophyll is represented by spongy parenchyma traversed by a number of small veins covered with a bundle sheath of thin walled parenchymatous cells (Figure 2).

The transverse section of fruit shows the presence of exocarp which represents a single layer while mesocarp shows a wide zone of parenchymatous cells with strong cellulosic thickening. The endocarp is similar to that of exocarp but at some places the cells are flattened and collapsed. The seeds in transverse section show a single layer of epidermis followed by a layer of highly flattened thin walled sub-epidermal cells. Beneath the sub-epidermis there is a layer of highly lignified palisade-like cells with narrow lumen. The inner epidermis of the seed coat comprise of 1–2 layer of thin-walled parenchymatous cells which at places are collapsed showing hyaline–like structure. The endosperm is represented by cells showing strong cellulosic thickening filled with aluerone grains without any globoide. The cotyledon shows thin-walled radially elongated cells enclosing a wide zone of round to oval to polyhedral parenchymatous cells (Figure 3).

The powder characteristic of the fruits of *Withania coagulans* is demonstrated in Figure 4-A, which shows a large number of parenchymatous cells of cotyledons (a), fragments of pericarp showing parenchymatous cells (b-c), thick walled endosperm cells showing aluerone grains (d-e), epidermal cells of calyx with unicellular covering trichomes (f) and few xylem vessels with spiral thickening (p). Figure 4-B represents the isolated elements and Table 1 represents the measurements of different cells of fruit of *Withania coagulans* in microns.

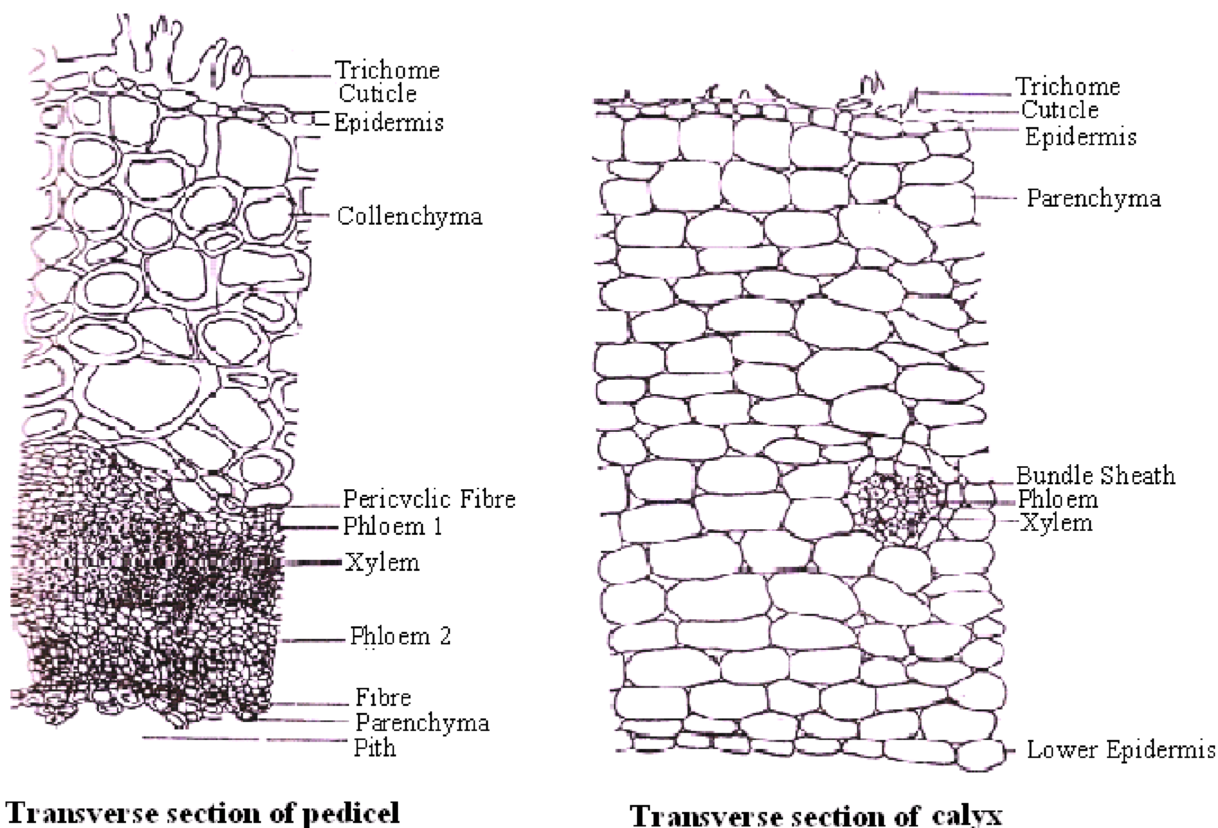

Figure 2: Microscopical characters of pedicel and calyx of fruit of *Withania coagulans*.

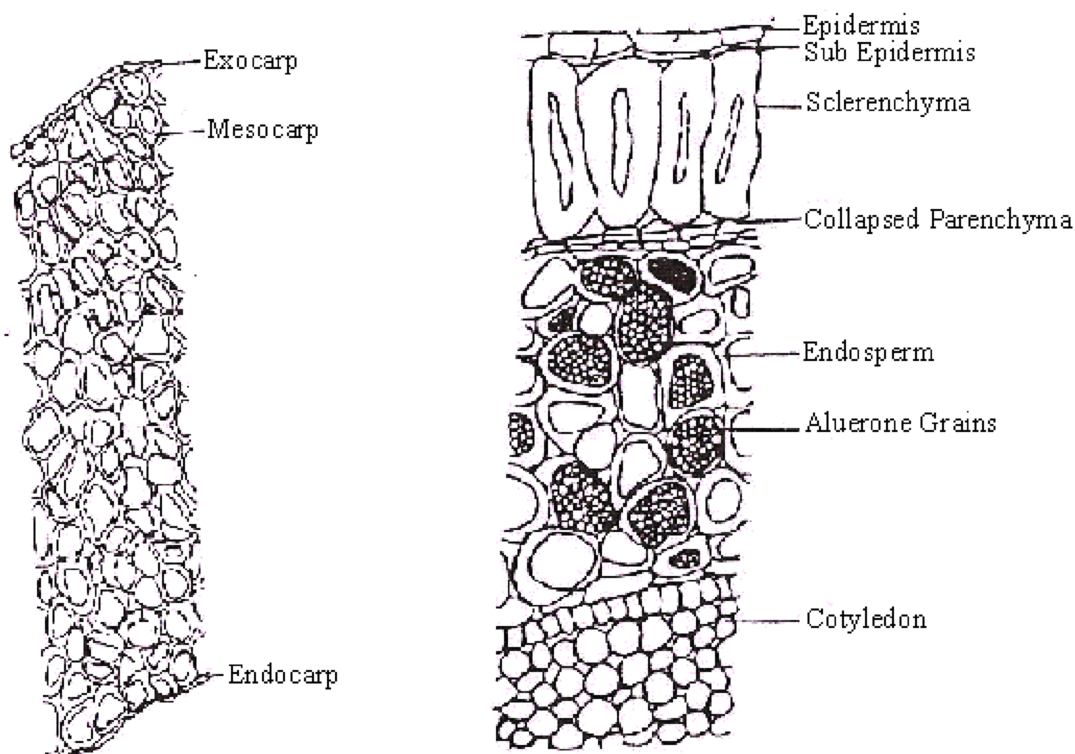

Figure 3: Microscopical characters of pericarp and seed of fruit of *Withania coagulans*.

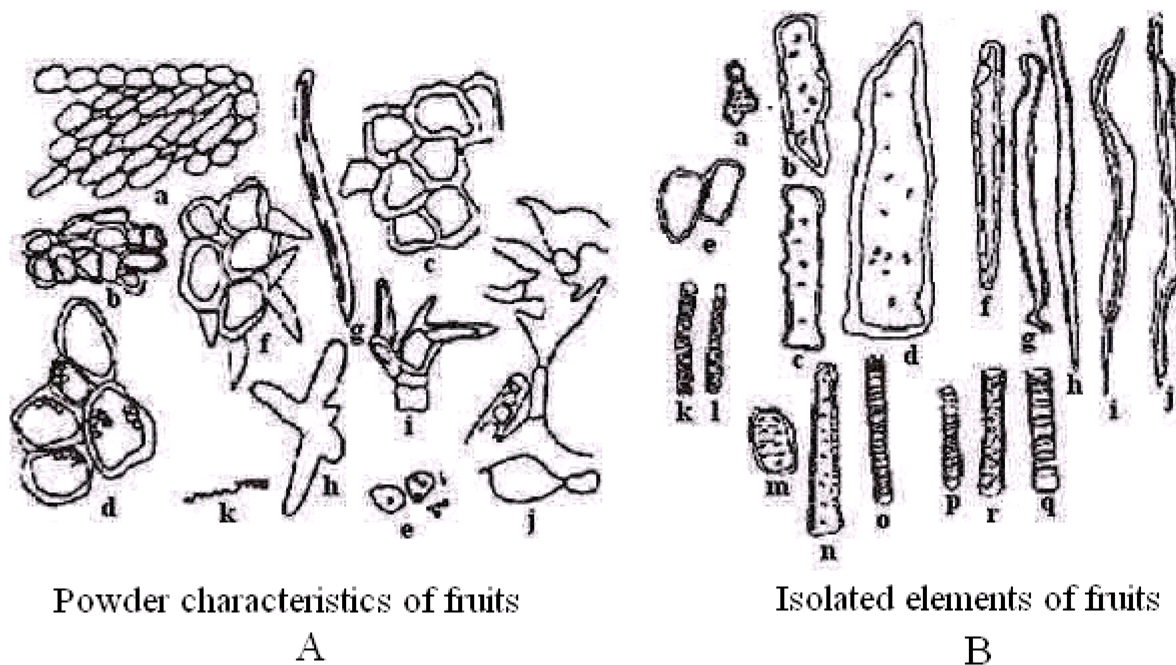

Figure 4: Powder characteristics (A) and Isolated elements (B) of fruits of *Withania coagulans*. Note: In Fig 4 (A) a: Portion of cotyledon, b-c: Pericarp in surface view, d-e: Endosperm cells with aluerone grains, f: Upper epidermis of calyx, g: Fibre, h-j: Trichome, k: Xylem vessel. In Fig 4 (B) a-d: Tracheids, e: Parenchyma, f-j: Fibre, k-r: Tracheidal vessels.

Table 1: Measurements of different cells of fruit of *Withania coagulans* in microns

Different cells of fruits	Measurements in microns
Pedicel:	
Epidermal cells	15–19–23×7–11–15
Spongy parenchyma cells	34–45–56×23–38–45
Xylem	11–15–19×4–8–11
Phloem	8–11–15×4–8–11
Sclerenchymatous cells	26–30–34×15–19–23
Trichomes	113–188–262×23–26–30
Pericarp:	
Parenchymatous cells	19–27–30×11–15–19
Seed:	
Epidermal cells	45–53–60×15–19–23
Scelerenchymatous cells	75–86–98×30–38–41
Endosperm cells	38–45–52×23–26–30
Embryo cells	11–15–23×8–11–15
Calyx:	
Epidermal cells	23–30–38×15–19–23
Spongy parenchyma cells	83–94–101×23–30–38
Vascular bundle	15–19–26×11–15–19

Table 2: Physicochemical standards

Physicochemical standards	%W/W
Total ash	19.0
Acid insoluble ash	12.10
Water soluble extractive value	21.20
Alcohol soluble extractive values	5.10

Values are taken as mean of triplicate.

Physicochemical standards

Various physicochemical standards such as total ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive values are reported in Table 2.

Fluorescence characteristics

Fluorescence analysis helps us in fulfilling the inadequacy of physical and chemical methods for identification of plant drug. Fluorescence analysis of powdered drug on treatment with various reagents was studied under ultraviolet light and their observations are expressed in Table 3.

Preliminary phytochemical screening

Phytochemical screening of hydroalcoholic fraction showed the presence of steroids, alkaloids, phenolic

Table 3: Fluorescence characteristics

Treatment	Fluorescence
Powder as such	Light brown
Powder treated with nitrocellulose in amyl acetate	Dark brown
Powder treated with 1N NaOH in methanol	Reddish green
Powder treated with 1N NaOH in methanol, dried and mounted in nitrocellulose in amyl acetate	Dark green
Powder treated with 1N HCL	Light green
Powder treated with 1N HCL, dried and mounted in nitrocellulose in amyl acetate	Dark green
Powder treated with 1N NaOH in water	Yellowish green
Powder treated with HNO_3 (1:1)	Light green
Powder treated with H_2SO_4 (1:1)	Light green

Table 4: Preliminary phytochemical screening

Test	Methanolic extract	Hydroalcoholic fraction	Chloroform fraction
Steroids	+	+	+
Alkaloids	+	–	+
Tannins	+	+	+
Phenolic	+	+	+
Cardiac glycosides	–	–	–
Anthraquinone glycosides	–	–	–
Saponin	+	+	–
Amino acids	+	+	–
Proteins	+	+	–
Carbohydrate	+	+	–
Organic acids (oxalic acid)	+	+	–

+ indicate presence and – indicate absence.

compounds, tannins, saponins, carbohydrates, proteins, amino acids and organic acids, whereas chloroform fraction showed the presence of mainly steroids and alkaloids (Table 4).

Quantitative estimation

The quantitative estimation of total phenolic, total tannin, total flavonoids and total flavanol content in the parent extract along with the two fractions are given in Table 5. The results showed that the total phenolic content was higher in case of hydroalcoholic fraction as compared to chloroform fraction and was expressed as mg/g equivalent to gallic acid (w/w). The total tannin content in the samples was expressed as mg/g equivalent to tannic acid (w/w) which was observed to be higher in case of chloroform fraction. Total flavonoid and flavanol content as observed in the result was found to be higher in hydroalcoholic fraction as compared to chloroform fraction and was expressed as mg/g equivalent to rutin (w/w).

Identification and quantification of withaferin-A by TLC and HPTLC method

The results obtained from the TLC studies (Figure 5) showed the presence of withaferin-A in both methanolic extract and chloroform fraction (having similar R_f values to that of withaferin-A), whereas it was found absent in hydroalcoholic fraction. Results obtained from the preparative TLC (Table 6) showed five bands among which wavelength of band 4 (209 nm) was found to be similar to that of withaferin-A (208 nm).

Peaks of withaferin-A are expressed in Figure 6 which shows its presence in methanolic extract and chloroform fraction, whereas it was not detected in hydroalcoholic fraction. Their quantification data are given in Table 7.

Table 6: Preparative TLC data for withaferin-A

No of Bands	Wavelength (λ max) (nm)	Absorbance (nm)
Standard withaferin-A	208	2.26
Band 1	(a) 220	1.87
	(b) 266	0.29
Band 2	212.5	2.76
Band 3	(a) 220	1.74
	(b) 280	0.16
Band 4	(a) 280	2.31
	(b) 209	4.00
Band 5	219	1.80

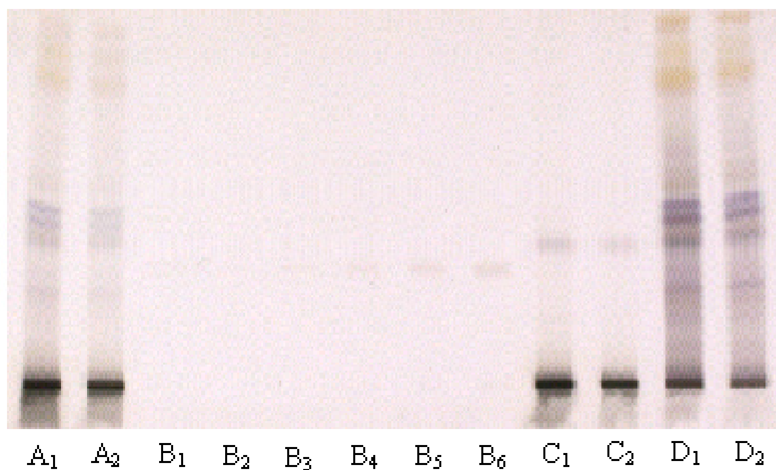

Figure 5: Image of TLC plate at 366nm after derivatization. Note: A_1, A_2 represent methanolic extract, B_1 to B_6 represents standard Withaferin-A, C_1, C_2 represents hydroalcoholic fraction and D_1, D_2 represents chloroform fraction.

Table 5: Quantitative estimation of various phytoconstituents

Phytoconstituents	Methanolic extract	Hydroalcoholic fraction	Chloroform fraction
Total phenolic content mg/g Equivalent to Gallic Acid (w/w)	55.9	43.9	33.1
Total tannin content mg/g Equivalent to Tannic Acid (w/w)	76.6	32.6	48.0
Total flavonoid content mg/g Equivalent to Rutin (w/w)	0.88	0.21	0.07
Total flavonol content mg/g Equivalent to Rutin (w/w)	0.25	0.02	0.013

Values are taken as mean of duplicate.

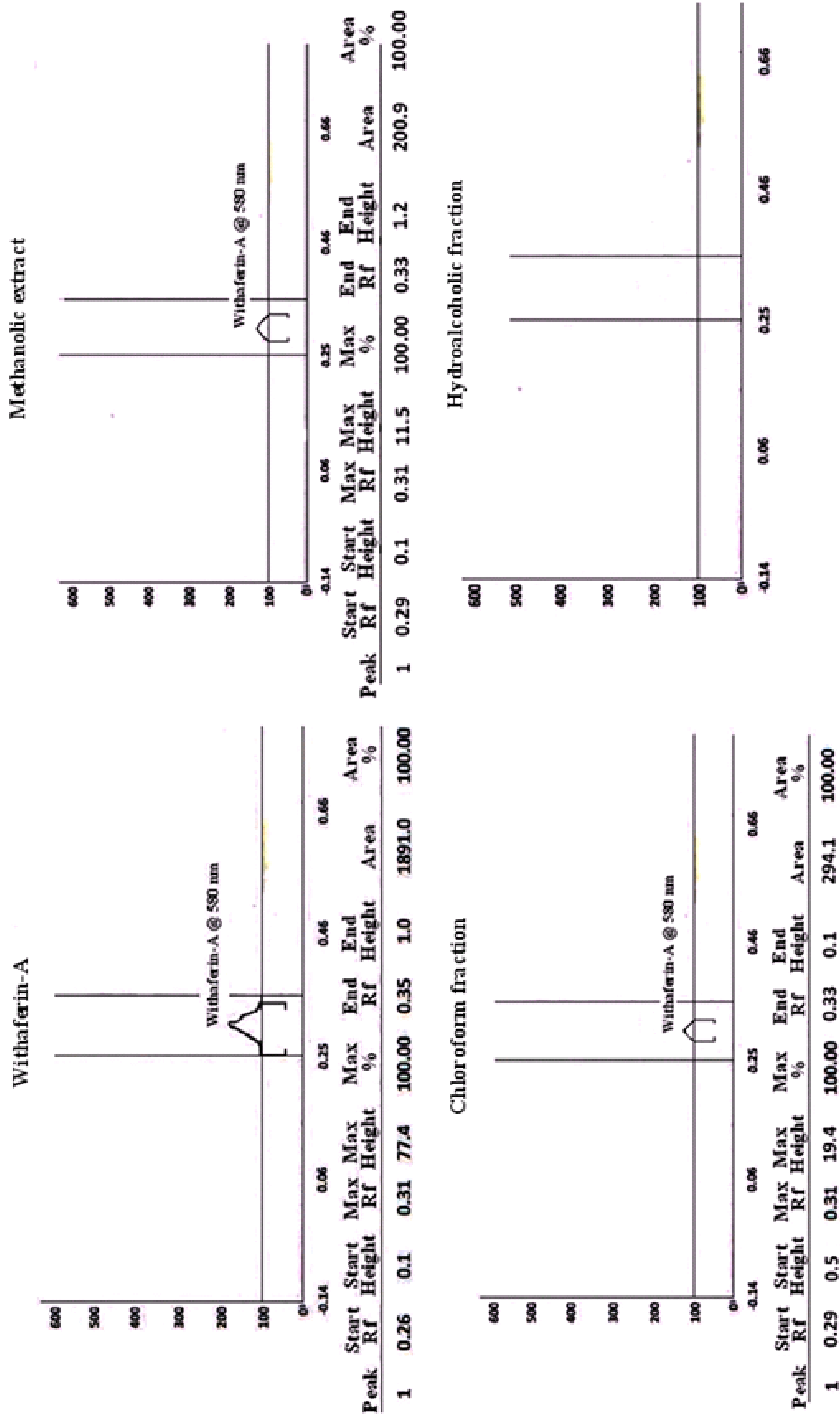

Figure 6: Peak area for withaferin-A

Table 7: Quantification data for withaferin-A by HPTLC

Sample	Concentration	Volume	Results (mg/g of sample)
Withaferin-A	0.2 mg/ml	8 µl	–
Methanolic extract	4.0 mg/ml	10 µl	3.67
Chloroform fraction	10 mg/ml	10 µl	2.10
Hydroalcoholic fraction	9.97 mg/ml	10 µl and 20 µl	Not detected

DISCUSSION

It is assumed that macroscopic and microscopic evaluation of any plant drug are considered to be the primary steps for establishing its quality control profile and according to WHO, botanical standards should be proposed as a protocol for the diagnosis of the herbal drug. The histochemical studies give a preliminary idea about the type of compounds and their accumulation in the plant tissues. Thus, helps us in selecting the particular part or tissue of that plant where the compounds of interest are located[20].

Physicochemical standards such as total ash value helps us in determining both physiological ash (plant tissue) and non-physiological ash (extraneous matter like sand and soil), whereas acid insoluble ash gives an idea about the amount of silica present, especially as sand and siliceous earth. Extractive values help us in determining the amount of active constituents and is done on plant materials for which as yet no suitable chemical or biological assay exists[21]. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like Phenolic compound as a good anti-oxidants[22], tannins having protein precipitating property[23], whereas flavonoids and flavonols possess a good anti-inflammatory[24, 25] and anti-oxidants activity[26]. The quantified values of the above phytoconstituents can be used as a major tool for obtaining a quality control profile for a drug.

Withanolides i.e. withaferin-A has been previously quantified from the roots and leaves of *Withania coagulans* by HPLC method[27]. In the present study withaferin-A has been quantified from the fruits of *Withania coagulans* with the help of HPTLC method which may act as a chemical marker for standardization of *Withania coagulans*.

The results obtained from the present study may play a major role in setting particular standards for the plant, which might broaden its pharmacognostical, pharmacological, botanical and economical importance. These parameters may also prove beneficial in authentication of the plant. Thus, with the help of these standards we can minimize the adulteration of *Withania coagulans* which will be of great use for the future workers in selecting the correct herbal specimen.

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Preparation and characterization of *Beta vulgaris* pulp powder as a pharmaceutical excipient

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ABSTRACT

Mouth dissolving tablet is the highly growing and highly accepted drug delivery system. In the present study, an attempt had been made to prepare fast dispersible tablets of diclofenac sodium using *Beta vulgaris* pulp powder as a pharmaceutical disintegrant. In the present investigation, wet granulation technique was used to prepare tablets, using microcrystalline cellulose as filler. Wet granulation technique is one of the most convenient and acceptable technology for preparation of fast dispersible tablets. Dispersible tablets of Diclofenac sodium were prepared with different concentrations viz; 2.5, 5, 7.5 and 10 % (w/w) of *Beta vulgaris* pulp powder and corn starch, and evaluated for physical parameters such as thickness, hardness, friability, weight variation, drug content, disintegration time and drug dissolution behavior. The formulated tablets had good appearance and better drug release properties. Studies indicated that the *Beta vulgaris* pulp powder is a good pharmaceutical adjuvant, specifically a disintegrating agent.

Keywords: *Beta vulgaris*, disintegrant, pharmaceutical excipients, diclofenac sodium

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INTRODUCTION

The tablet is the widely used dosage form because of its convenience in terms of self-administration, low cost and ease in formulation. However, geriatric and pediatric patients experience difficulty in swallowing conventional tablets, which leads to poor patient compliance. "Mouth dissolve (MD)" tablets are novel types of tablets that disintegrate/ dissolve/disperse in saliva within 15 to 60 s, without the need of water.[1,2] This characteristic advantage leads to their suitability for geriatric and pediatric patients at anytime, anywhere. The benefits, in terms of patient compliance, rapid onset of action, increased bioavailability, and good stability make these tablets popular as a dosage form of choice in the current market. Different technological techniques, such as freeze drying, moulding, direct compression, are currently employed to prepare the formulations of this type present in the pharmaceutical market.[3,4,5] *Beta vulgaris* (Chenopodiaceae) is an important plant found in India. Pulp powder of this plant was used to prepare fast dispersible tablet. This type of natural plants plays an important role as pharmaceutical excipient. These are easily available, biodegradable and having low cost. Bio compatibility of these natural polymers promotes their use as in pharmaceutical formulations.

Present work used direct compression technique to prepare tablets. In present study Diclofenac sodium, a non-steroidal anti-inflammatory drug was selected as model drug. It is an acetic acid nonsteroidal antiinflammatory drug (NSAID) with analgesic and antipyretic properties. Diclofenac sodium is used to treat pain, dysmenorrhea, ocular inflammation, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and actinic keratosis.[5,6]

MATERIAL AND METHODS

Preparation of Beta vulgaris pulp powder

Beta vulgaris was purchased from local market of Meerut (Uttar Pradesh) India. The fruit was clean with water to

remove dust from surface and further peel was removed. Pulp was cut into small pieces and put into grinder to form paste. This was further lyophilized to get solid porous mass. Size reduction was done and powder was collected. The collected powder was passed through 80 # sieve and stored in the air tight container for further study.[1]

Characterization of Beta vulgaris pulp powder

Bulk density

Apparent bulk density (g/ml) was determined by placing pre-sieved bulk powder blend into a graduated cylinder via a large cylinder and measuring the volume and weight of powder blend.[1,7,8]

Bulk density =weight of powder blend / volume of powder blend

Tapped density

It was determined by placing a graduated cylinder, containing a known mass of powder on mechanical tapping apparatus, which was operated for fixed number of taps (around 50). Using the weight of powder in a cylinder and its tapped volume, the tapped density was computed.[1,7,8]

Tapped density =weight of powder blend/ tapped volume of powder blend

Carr's index

It is an important parameter to study compressibility behavior of powder blend. Carr's index was calculated, from the results of bulk density and tapped density.[1,7,8]

Carr's index = (bulk density-tapped density)/ tapped density

Bulkiness

It is reciprocal of bulk density, and calculated as[1,7,8]

Bulkiness= 1/ bulk density

Angle of repose

For the measurement of angle of repose, a glass funnel was taken with its tip at a given height (H), above a piece of graph paper placed on a horizontal surface. Powder was poured through the funnel until the apex of the conical pile touched the tip of the funnel. The angle of repose was calculated with the formula; $\tan \theta = H/R$, where θ is the angle of repose and R is the radius of the conical pile.[1,7,8]

Swelling index

The swelling index is defined as the volume (in milliliters) taken up by the swelling of 1 g of powder material under specified conditions. 1 gm of the pulp powder was introduced into a 25 ml glass-stoppered measuring cylinder. Twenty five milliliters of water was added and mixture was shaken thoroughly for 10 min. It was then allowed to stand for 24 h at room temperature. Then the volume occupied by the pulp powder was noted.

Drug and other excipients procurement

Diclofenac sodium was obtained as gift sample from Cadila Pharma, Gujarat. Other materials used include cellulose microcrystalline (fine powder), talc and magnesium stearate of analytical grade were purchased from RANKEM limited, New Delhi India.

Methods

Preparation of tablets

The formula for the work was designed as per the table1

Table 1: Formula to prepare tablets containing Diclofenac sodium as model drug.

Ingredients	Formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
Diclofenac sodium	50	50	50	50	50	50	50	50
Beta vulgaris pulp powder	_	_	_	_	10	20	30	40
Corn starch	10	20	30	40	-	-	-	-
Magnesium stearate	9	9	9	9	9	9	9	9
Sodium saccharine	12	12	12	12	12	12	12	12
Talc	9	9	9	9	9	9	9	9
Menthol	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Micro crystalline cellulose	113.6	109.6	105.6	101.6	113.6	109.6	105.6	101.6
Total weight of tablet	200	200	200	200	200	200	200	200

The preparation of tablets were carried out in two different steps-

Granulation

Weighted quantity of diclofenac sodium, *Beta vulgaris* pulp powder and microcrystalline cellulose was added according to formula as per table 1. All the ingredients are mixed properly with the help of mortar and pestle and water was used as granulating agent. The wet mass was passed through sieve No 20 to prepare granules. Granules were dried at 45° C for 5 hours. Talc and magnesium stearate was added accordingly in granules of all batches and stored in air tight packets for further study.[1,9,10]

Compression

Defined amount of granules was used to prepare tablets of all batches. Powder was compressed using a single punch tableting machine (Cadmach Machinery Co. Pvt. Ltd., India) equipped with 8 mm punch at 0.5 ton pressure.[1,10]

Technological parameters

Weight variation

All prepared matrix tablets were evaluated for weight variation as per USP XXIV monograph. Twenty tablets of each batch were used to evaluate weight variation among tablets and standard deviation was calculated.[1,10,11]

Friability

Tablets of all batches were used to evaluate friability as per USP XXIV monograph. Friability testing was done by Roche friabilator with triplicate readings.[1,10,11]

Hardness

Hardness of all batches was determined using Digital Force Gauge (Model:EL=500, Electrolab). The test was carried out in triplicate for all batches as per USP XXIV monograph for uncoated tablets.[1,10,11]

Thickness

Thickness was measured by vernier caliper as per USP XXIV monograph. The readings were carried out in triplicate and average value was noted.[1,10,11]

Drug content

The tablets were powdered, and 50 mg equivalent weight of Diclofenac Sodium in tablet powder was accurately weighted and transferred into a 100 ml volumetric flask. Initially, 10 ml of phosphate buffer (pH6.6) was added and shaken for 10 min. then, the volume was made up to 100 ml with buffer. Subsequently, the solution in volumetric flask was filtered, and 1 ml of the filtrate was diluted and analyzed at 276 nm using ultraviolet/visible variable wavelength spectrophotometer at 276 nm (Shimadzu UV-2450, Japan). The drug content of the each sample was estimated from their standard curve.[1,10, 12]

In vitro dissolution study

Dissolution test was performed at 37°c using the paddle method at 100 rpm with 900 ml phosphate buffer (pH6.6) as dissolution medium. For this digital tablet dissolution test apparatus (Lab India Disso 2000, India) was used. At predetermined intervals, 5 ml of the medium was sampled and filtered. The filtrate was analyzed by ultraviolet/visible variable wavelength spectrophotometer at 276 nm.[1,10,12]

RESULTS AND DISCUSSION

Beta vulgaris pulp powder was characterized as a pharmaceutical excipient in terms of micromeritic properties and flow behavior. Bulk density, tapped density, bulkiness and angle of repose all are found to be good to use this plant based material as a pharmaceutical excipient. Swelling index was also studied with an aim to evaluate swelling behavior of polymer that also effect drug release from matrix tablets.

Bulk density, tapped density, compressibility index and flow behavior (angle of repose) were found to be good so this pulp powder can be act as a good candidate for pharmaceutical preparations (table 2). Relative study of physical parameters of tablets of each batch of *Beta vulgaris* pulp powder reveals that the tablets compressed using pulp powder as disintegrant are quite harder, so can be easily handled. The variation in the hardness, weight variation, friability and thickness values of all the fabricated tablets, in reference to average values for each parameter, were found within the official limits. Friability of tablets ranged from 0.62 to 0.82%, easily predict the fact that tablets were less friable and so provide ease of

Table 2: Characterization parameters of *Beta vulgaris* pulp powder.

Bulk density (gm/cm3)	Tapped density (gm/cm3)	Bulkiness	Angle of repose (°)	Compressibility index (%)	Swelling Index (%)
0.82 (0.21)	0.98 (0.19)	1.21 (0.17)	24.82 (0.11)	21.6 (0.30)	22 (0.18)

Table 3: Evaluation parameters of tablets containing Diclofenac sodium as model drug

Parameters	Formulations							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Diameter (mm)	9.48	9.78	9.67	9.81	9.78	9.70	9.72	9.72
Thickness (mm)	2.54	2.55	2.45	2.41	2.43	2.44	2.40	2.41
Wetting time (sec)	20	22	25	30	28	25	18	15
Wt. variation (mg)	195	194	195	196	194	197	195	196
Hardness (kg/cm2)	6.1	4.3	8.3	8.3	6.4	11.5	10.7	11.5
Friability (%)	0.67	0.72	0.68	0.75	0.68	0.62	0.76	0.82
Disintegration time (sec)	27	40	45	60	28	26	25	20
Drug content (mg/tablet)	49.2	49.4	49.3	49.5	49.7	49.6	49.7	49.9

handling. The comparative data of different studies of all batches has been tabulated in table 3.

Less weight variation and uniform drug content easily elicit the fact that this process of tablet formulation is reproducible and so easily adopted at industrial level. Findings of the results showed that as the concentration of pulp powder increases wetting time of tablets decreases in same proportion and so disintegrating time also go down in same manner.

In vitro dissolution study of formulations at phosphate buffer pH 6.8 reveals that Batch F8 gave better release than other batches and it was upto 98.99% in 60 minutes study. The comparative drug release from different formulations was shown in figure1.

Results easily predict the fact that formulations containing (F5, F6, F7, F8) *Beta vulgaris* pulp powder showed better release profile than the formulations containing (F1, F2, F3, F4) corn starch. As the concentration of pulp powder increases disintegrating time of formulations decreases in same proportion. Results also showed that tablets prepared using pulp powder have relatively more hardness and less friability in comparison of tablets of corn starch. So it is easy to handle formulations prepared using *Beta vulgaris* pulp powder.

CONCLUSIONS

The comparative study of various parameters clearly states the fact that the naturally obtained *Beta vulgaris* pulp powder stands as a good candidate to act as disintegrant and it is possible to design promising Fast disintegrating tablet using this polymer. On the basis of

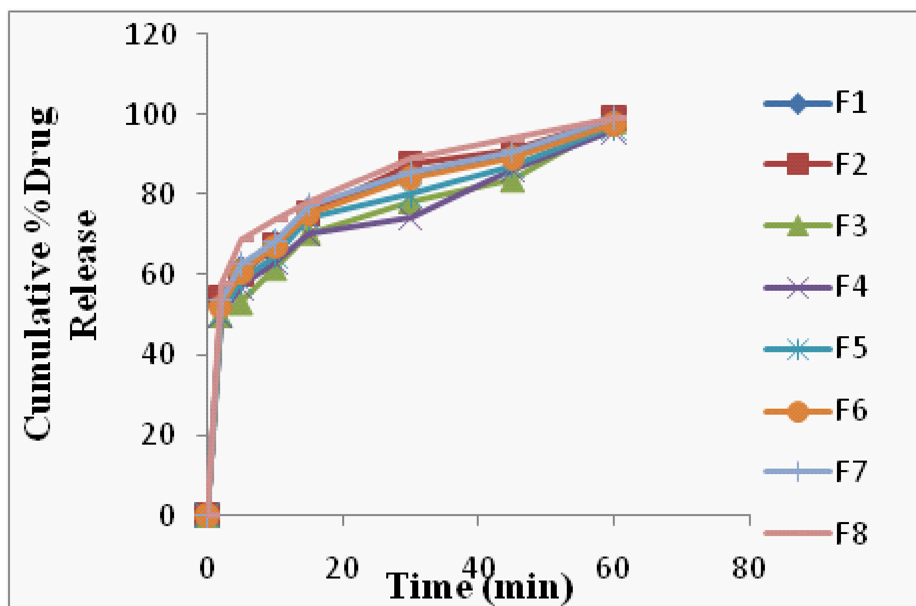

Figure1: drug release study from different formulations

results obtained it can be concluded that this polymer having good micromeritic properties and flow behavior and so may act as a pharmaceutical excipient.

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Anti-microbial, Anti-oxidant and Anthelmintic Activity of Crude Extract of *Solanum xanthocarpum*

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ABSTRACT

The aim of the present study was to evaluate the antimicrobial, anti-oxidant and anthelmintic potential of extracts of the herb *Solanum xanthocarpum*. The antimicrobial activity was tested against selected bacterial and fungal species by agar well diffusion method and the antioxidant activity was assessed using FRAP and reducing power scavenging assays. While that of anthelmintic activity was performed against the erthworms. The FRAP assay evaluates the ability of a substance to reduce Fe^{3+} to Fe^{2+}. Preliminary phytochemical screening revealed the presence of saponins; phytosterols and carbohydrates in the plant.

Keywords: Agar well diffusion method, anthelmintic activity, antimicrobial activity, anti-oxidant activity, FRAP, *Solanum xanthocarpum*

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INTRODUCTION

Dependence on herbs as medicine in the treatment of diseases is still much practiced by a large proportion of the rural population because of its availability and affordability. Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile. In every society, whether technologically primitive or advanced, there exist some sort of curative recipes for the health maladies.[1]

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc.[2]

Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) commonly known as Yellow Berried Nightshade (syn: kantakari), is a prickly diffuse bright green perennial herb, woody at the base, 2–3m height found throughout India, mostly in dry places as a weed on roadsides and waste lands.[3] The fruits are glabrous, globular berries, green and white strips when young but yellow when mature.[4] The fruits are known for several medicinal uses like anthelmintic, antipyretic, laxative, antiinflammatory, antiasthmatic and aphrodisiac activities.[5] The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions.[6] The hot aqueous extract of dried fruits is used for treating cough, fever and heart diseases.[7] The fruit paste is applied externally to the affected area for treating pimples and swellings.[8] The *kondh* tribes of Dhenkanal district of Orissa, India uses the hot aqueous extract of the matured fruits as a traditional medicine for the treatment of diabetes mellitus. The fruits are reported to contain several steroidal alkaloids like solanacarpine,[9] solanacarpidine, solancarpine, solasonine[7] and solamargine.[10] Other constituents like caffeic acid[10] coumarins like aesculetin and aesculin,[11] steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol were reported from the fruits.[12,13] The antispasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic and cytotoxic activities were also reported.[14-16] In the present communication we report the hypoglycaemic

activity of the aqueous extract of the fruits of *Solanum xanthocarpum*.

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen- derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage.[17] Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical compounds such as a-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione.[18] When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage.[17,19-21]

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^-), singlet oxygen ($1O_2$) and non-free radical species such as hydrogen peroxide (H_2O_2) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors.[21-23] ROS are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms.[24] There is a balance between generation of ROS and antioxidant system in organisms. In pathological condition, ROS are over produced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules.[25] Various endogenous antioxidant defence mechanisms play an important role in the elimination of ROS and lipid peroxides, and therefore, protect the cells against toxic effects of ROS and lipid peroxides.[24-26]

Vegetables and fruits are considered to be good sources of functional ingredients. Many studies have shown that antioxidants, present in plants at high levels, are the compounds responsible for these functionalities.[27] Antioxidants or molecules with radical scavenging capacity are thought to exert a potential protective effect against free radical damage. These biomolecules contribute to prevention of coronary and vascular diseases and of tumor formation by inhibiting oxidative reactions.[28] This oxidative damage is the result of free radical action on, for instance, lipids or DNA.[29]

The antioxidant activity of some of these molecules is based on their ability to donate a hydrogen atom to free radicals. Because these compounds are able to scavenge free radicals, they are believed to have potential in the prevention of cancer, atherosclerosis, and diabetes.[30] Nowadays there is considerable evidence that the antioxidants contained in fruits, vegetables and beverages play an important role in the maintenance of health and in prevention of disease.

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other diseases. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, *Ascaris, Nippostrongylus*, and *Heterakis*. Of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* because they resemble intestinal "worms" in their reaction to anthelmintics and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic.[31]

MATERIAL AND METHOD

Plant Material

The fresh whole plants of S*olanum xanthocarpum* were collected from the rural area. The plant was identified and authenticated by Dr. Ms. Sattarupa Rao, Professor and Head, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). Specimen Voucher no. HD/CHPY/1138.

Preparation of Extract

The plant was shade dried at room temperature and the dried whole plant was ground into coarse powder. The powder was sieved to have a uniform size. The aqueous, ethanolic and hydroethanolic extracts were obtained by cold maceration. The extraction procedure was repeated thrice in order to have optimum extraction. The extracts were filtered using a muslin cloth and concentrated at 40±5°C; dried extracts were refrigerated at 4 °C until use.

Phytochemical Analysis

The phytochemical analysis of the plant was carried out by the standard methods[32,33] (Table 1).

Determination of Anti-oxidant Property
Chemicals

Safranine, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid were purchased from CDH New Delhi. The other chemicals and reagents used were of analytical grade.

Ferric Reducing Antioxidant Potential (FRAP) Assay

Ferric reducing ability was evaluated using different concentrations of crude extract. The FRAP reagent contained 10mM of TPTZ solution in 40mM HCl, 20m M $FeCl_3$ $6H_2O$, and acetate buffer (300mM, pH 3.6) (1:1:10, v/v/v). A 100µL 50% aqueous methanol of the test compounds was added to 3mL of the FRAP reagent, and the absorbance was measured at 593nm after incubation at room temperature for 6min, using the FRAP reagent as a blank. Different dilutions of each of the test compounds were assayed and the results were obtained by interpolating the absorbance on a calibration curve. The FRAP value was defined as the milliequivalents of Trolox having the antioxidant power equivalent to a 1.0mM solution of the substance under study. Two independent experiments in triplicate were performed for each of the assayed compounds.[34]

Scavenging Activity against Hydroxyl Radicals

Different concentrations of extract were prepared and 1 ml of each aliquot were mixed with 1ml of 10 mMol/L phosphate buffer solution (pH 7.4), 1 ml of 40 µg/ml safranine T solution, 1 ml of 3ml/100ml of H2O2 and 1.0ml of 0.15 mol/L EDTA, FeNa. This reaction mixture was incubated at 37°C for 30 min. same procedure is repeated with control.

Determination of Anti-microbial Property
Microorganisms

Bacterial and fungal cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The bacterial strains were *Escherichia coil* MTCC 2960, *Pseudomonas auruginosssa* MTTC 4676, *Staphylococcus aureus* MTTC 3160, *Klebsiella oxytoca* MTTC 3030, *Bacillus subtilis* MTCC 1790, *Candida albicans* MTCC 183.

Preparation of Inoculums

All the microorganisms mentioned above were incubated at 37 ± 0.1°C, for 24 h in Nutrient broth, *C. albicans* in YEPD broth at 28 ± 0.1°C for 48 h.

Determination of Antimicrobial Activity

Nutrient Agar and YEPD Agar (20 ml) were poured into each sterilized Petri dish (10 X 100 mm diameter) after injecting cultures (100µl) of bacteria and yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried herb extracts were dissolved in dimethylsulfoxide to a final concentration of 20% and sterilized by filtration through a 0.22µm membrane filter.[35,36] Each sample (100 µl) was filled into the wells of agar plates directly. Plates injected with the fungal cultures were incubated at 28 °C for 48 h, and the bacteria were incubated at 37°C for 24 h. At the end of the incubation period, inhibition zones formed on the medium were measured in mm. Studies were performed in triplicate and the inhibition zones were compared with those of reference discs. Amphotericin B (10µg) and tetracycline (30µg) were taken as reference.

Determination of Anthelmintic Activity
Standard

Piperazine citrate (10mg/ml) was used as reference standard

Worm Collection and Authentication

The earthworm *Eisonia fatida* (African type) were collected and authenticated from Madhya Pradesh Pashu Chikitsa Vishwavidyalaya, Jabalpur (M.P.), India.

Anthelmintic Activity

The anthelmintic assay was carried using different concentrations of crude aqeous, ethanolic and hydroethanolic extracts (25, 50, and 100mg/ml in distilled water) were prepared, and three worms of nearly same type per concentration were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water(50°C).[37]

Piperazine citrate (10mg/ml) was used as reference standard and distilled water served as the control.

RESULT AND DISCUSSION

Anti-microbial activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay.[38] Many reports are available on the antiviral,

antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants.[39-45] The antimicrobial activity of extracts of *S. xanthocarpum* was tested under *in vitro* conditions by agar well diffusion method against three bacterial and one fungal pathogen. The process was performed in triplicates. The zone of inhibition of microbial growth with Hydroethanolic extracts is given in Table 2. The aqueous, hydroethaolic and ethanolic extract of *S. xanthocarpum* (50 µg/ml) inhibited the growth of Gram negative bacteria S. *aureus* (10, 15 and 13mm respectively) and *E. coli* (6, 8 and 7mm respectively). Solvent extracts *S. xanthocarpum* (50 µg/ml) exhibited mild to moderate inhibition over the growth of tested bacterial pathogens. Ethanolic extract was found to be having more potent anti-microbial activity than those of aqueous and hydro ethanolic extract (Table 2).

Anti-oxidant activity

The antioxidant activity of the *Solanum xanthocarpum* was assessed using FRAP and reducing power scavenging assays. The FRAP assay evaluates the ability of a substance to reduce Fe^{3+} to Fe^{2+}, which is measured by the formation of a coloured complex with TPTZ that can be read spectrophotometrically at 593 nm. Since the antioxidant activity of a substance is usually correlated to its reducing capacity, this assay provides a reliable method to evaluate the antioxidant activity.[34] The hydroethanolic extract of the herb was found to be having mild anti-oxidant activity.

These results may also be helpful to describe the various pharmacological activities like anti-infective, protective activities.

Anthelmintic activity

In this study we have evaluated the effect of *Solanum xanthocarpum* whole plant extracts on earthworms. The extract showed significant wormicidal activity. Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis followed by death of parasite. On introduction of extract to the worms there was slight excitory activity was observed but as the moment passes the worms were got fatigue and ultimately paralysed leading to there death. In the present study it was observed that the ethanolic extract was having more potent anthelmintic property than aqeous and hydroethanolic extract. 100mg/ml was having more potential against the worms (Table 3).

The predominant effect of Piperazine citrate on worm is to cause a flaccid paralysis which results in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.[46] The crude extracts of *L.siceraria* not only demonstrated paralysis but also caused death of worms especially at higher concentration of 100 mg/ml in nearly same time as compared to reference drug Piperazine citrate.

CONCLUSION

From this preliminary investigation it has been concluded that the herb *Solanum xanthocarpum* is having significant antimicrobial, anti-oxidant activity and anthelmintic activity, the constituent present in the herb might be a responsible for this activity. Further research is in necessary to isolate the compound responsible for this activity.

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Phytochemical and Antimicrobial Screening of the Leaf Extract of Diospyros barteri. Gurke.

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ABSTRACT

Diospyrous bateri Gurke family Ebenaceae, has been commonly used by traditional medical practitioners as anti-infectious agent. In this study, preliminary screening of phytochemical constituents of *D. barteri* was carried out in addition to evaluating the antimicrobial activity of four different extracts of this leaf on some common bacteria species.

The antimicrobial activity of methanol, chloroform, hexane and aqueous extracts of the leaves were tested using the agar diffusion method on two Gram positive, three Gram negative bacteria viz; *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae Escherichia coli,and Pseudomonas aeruginosa,*

The preliminary phytochemical screening revealed the presence of alkaloids, saponins and tannins while other secondary metabolites were absent.

The study amply demonstrated the broad-spectrum antimicrobial property of the polar extracts while the non polar extract exhibits no activity. The minimum inhibitory concentration of the methanol extract *on Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus* were 1.20mg/ml, 2.24mg/ml, 3.55mg/ml, 9.12mg/ml and 10.0mg/ml respectively, while the minimum inhibitory concentration of the water extract on *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* were 8.91mg/ml, 9.55mg/ml, 10.59mg/ml, 10.96mg/ml and 71.78mg/ml respectively.

Keywords: *Diospyros barteri*, antimicrobial, phytochemical screening
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INTRODUCTION

It is well known that misuse of antibiotics often leads to the appearance of resistant strains of micro-organisms[1] and such misuse is becoming a norm in our present day society. In view of this propensity of bacteria to drug resistance, the search for new antibiotic continues unabated.

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins[2].

Diospyros barteri belongs to the plant family, Ebenaceae. This is a family of trees and shrubs with alternate leaves which are always simple and have entire margins with no stipules. The fruit is a berry, surrounded at the base by the persistent and often enlarged calyx[3]. *D. barteri* is a forest shrub or a small tree 3 to 22 feet high. It is sometimes scrambling, young plants are covered with ferrugineous hairs. The leaves are glaucous (covered with white or bluish powder or bloom[4], beneath. The fruits are pale yellow or sometimes orange.

The thin fruit-pulp is edible and is a minor item of diet[5] the wood could also be used to make clubs, spear shafts, walking sticks and as house building materials[3]. Ethno botanical uses in various part of West Africa includes control of diarrhea, dysentery, leprosy, antiseptic washes for sores and wounds, boiled leaves are applied as poultices for treating vaginal discharges[6–7].

A decoction of the leafy stems in drought is a common treatment for fever in Gambia[8–9]. They are efficacious a chewing sticks in the Nigerian communities probably due to the presence of high fluoride (tannin) content in it[10]

Biological investigation of the methanolic extract of two species of *D. barteri*, indicate that the seed extract was active against polio virus type 2 in the 'post-treatment assay.[11]

This study is an attempt to determine the antimicrobial activity of the aqueous, hexane, chloroform and methanol extracts of *D. barteri,*

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Diospyros barteri* was collected at Olokemeji, Abeokuta and authenticated by Mr. T. K. Odewo of the Forestry Research Institute of Nigeria (FRIN), where voucher specimen was deposited.

Plant preparation and extraction

The leaves of *Diospyros barteri* were ground (Hammer mill). Measured quantity of it was extracted respectively in hexane (1000g), methanol (400g), chloroform (500g) and distilled water (800g) by maceration at room temperature (30^{0}C) for 72 hours respectively. The percentage yields of extracts in each solvent system were noted after removal of solvent.

Preliminary phytochemical screening

Air-dried and powdered plant materials were screened for the presence of tannins, alkaloids, anthraquinones, cyanogenetic glycosides, saponin glycosides, and steroidal nucleus flavonoids as described in literatures[12–14].

Microorganisms

All the bacteria used were clinical strain obtained from the laboratory stock of the Department of Pathology, Olabisi Onabanjo University Teaching Hospital, Sagamu. Human pathogenic bacteria made up of 3 Gram negative (*Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae*) and 2 Gram-positive (*Staphyloccocu*s *aureus, Proteus vulgaris*) bacteria were used for the antibacterial assay,

Media

Nutrient broth, Nutrient agar, (LAB M™) were used in the study.

Antimicrobial Agents

Gentamicin, 2mg/ml, was used as the standard reference drug for antibacterial assays.

Preparation of bacterial cultures

The agar cup diffusion method was used to test the fractions for antimicrobial activity and determination of the minimum inhibitory concentration of the crude plant extracts, from stored slopes of bacteria, 5ml single strength nutrient broth was inoculated. The tubes were well shaken and incubated at 37°C for 18–24.

Using sterile pipettes, 0.2ml of 1 in 100 dilution of the bacterial cultures were added to 20ml of the melted and cooled (45–50°C) nutrient agar. The contents were mixed by gentle swirling movements before being poured into clean, sterile Petri dishes. After agar in plates has solidified, wells (6 mm each) were bored in each plate using an aseptic cork borer. Thereafter, wells were dug into the agar plates with the aid of sterile cork borer (6mm in diameter). Extracts were then reconstituted with appropriate solvents (chloroform, methanol, distilled water, hexane).

The concentrations used for the aqueous, methanol, chloroform and hexane extract of the leaf sample were; 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.8125mg/ml, 3.9063mg/ml, 1.953mg/ml.

The plates were then allowed to stand at room temperature for an hour to allow for adequate diffusion before incubating at 37^{0}C for 24 hours for the bacteria.

The diameter of the zones of inhibition of the bacteria growth were measured to the nearest millimeter and recorded. The diameter of the zones of inhibition was measured along the two axes at 90 degree to each other and the mean of the two readings was calculated.

To determine the minimum inhibitory concentration MIC, the graph of the zone size (in mm) was plotted against the logarithm of concentration and the straight line obtained is extrapolated to appoint equivalent to the diameter of the cup bored. The antilog of the corresponding concentration obtained is the MIC[14].

RESULTS

The percentage yield of the methanol, chloroform, hexane and distilled water extract of D. barteri was 26.8%, 8.2%, 6.5%, and 9.5% respectively as shown in Table 1

The results of the phytochemical screening indicated the presence of alkaloids, saponin glycosides, and tannins. For the antimicrobial activity, the diameters of the inhibition zones were measured in duplicate and the

Table 1: Result of the phytochemical screening.

	D. barteri
Alkaloids	+++
Cardiac glycosides	----
Saponin glycosides	++
Anthraquinones	
Free	---
Combined	---
Cyanogenetic glycosides	---
Flavonoids	---
Tannins	+++

(-): Absent, (++): Fairly present, (+++): Abundant

result is presented as the mean of the two measurements as shown in Table 2. The MIC was determine graphically as a graph of zone of inhibition plotted and the log of concentration and the results is presented in Table 3

DISCUSSION

This study was designed to obtain preliminary information on the phytochemical constituents and antimicrobial activity of various extracts of *D.barteri* on five microorganisms. The cup plate diffusion method was used in this study.

Table 2: Result of the antimicrobial screening

Zone of inhibition in diameter

Test extract	Conc mg/ml	*Escherichia coli*	*Staphyloccocus aureus*	*proteus vulgaris*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Methanol	1000	22.5	20.5	18.5	17.5	22.5
	500	20.2	18.5	18.0	16.8	20.0
	250	19.5	15.25	15.8	15.0	15.5
	125	18.0	15.0	14.25	15.0	15.0
	62.5	13.0	12.5	13.0	14.75	14.25
	31.25	9.5	9.5	11.0	12.5	13.5
	15.63	8.0	7.5	10.0	9.5	10.27
	7.81	----	----	8.75	7.5	9.5
	3.9	----	----	7.75	7.0	9.5
	1.95	----	----	----	----	----
Water	1000	26.5	20.5	16.5	24.5	24.8
	500	20.0	15.5	15.0	21.0	23.0
	250	13.0	12.75	13.0	18.75	17.5
	125	12.8	9.0	10.25	13.25	15.0
	62.5	12.5	----	10	13.25	15.0
	31.25	10.0	----	10.0	10.5	11.0
	15.63	9.0	----	8.0	9.5	10.28
	7.81	----	----	----	----	----
	3.9	----	----	----	----	----
	1.95	----	----	----	----	----
Gentamycin	2mg/ml	22.0	26.0	24.0	25.0	22.0

Table 3: Minimum inhibitory concentration

Test Organisms	MIC value in mg/ml	
	Water extract	Methanol extract
Escherichia coli	10.96	9.12
Staphylococcus aureus	71.78	10.00
Proteus vulgaris	9.55	1.20
Pseudomonas aeruginosa	10.59	2.24
Klebsiella pneumoniae	8.91	3.55

The preliminary phytochemical screening revealed the presence of alkaloids, saponins and tannins while other secondary metabolites were absent. Earlier study indicated that the tannin present in the plant is hydrolysable tannin[15]. The water and methanol extract of *D. barteri* were seen from the result to demonstrate broad antimicrobial action which are dose dependent.

There is an indication that the active antimicrobial constituent of the plant is a polar compound since the hexane and chloroform extract showed no activity at all on all the organisms.

In general, the methanol extract was found to have the best antimicrobial effect on the organisms, the solubility of active antimicrobial constituents of the extract in methanol must be greater than that of water considering the fact that they are both polar

The control experiment carried out using Gentamicin 2mg/ml gives the comparison of the activity of the extract and gentamicin [table 2]. The result shows that both the water extract and methanol extract at 1000mg and 500mg give activities that are quite comparable to that of gentamicin on most of the organism, also both extracts were able to inhibit *Proteus vulgaris, Pseudomonas aeruginosa, and Klebsiella Pneumoniae* at concentrations that are as low as 15mg/ml. this is an indication of the potency of this crude extract.

Test organisms used for this experiment have been implicated in diseases such as diarrhea, oral and dental infection, urinary tract infection, wound sepsis, dysentery and so on. These results therefore justify the local usage of this plant in the treatment of the various diseases. As earlier mentioned, the preliminary phytochemical screening revealed the presence of tannins, saponins and alkaloids. Tannin-containing drugs will precipitate protein; this could probably be responsible for the strong antibacterial activity exhibited by the plant[16].

The efficacy of a given herbal drug in the treatment of an infectious disease relates to the presence of one or more active agent in the herb that has antimicrobial activity of inhibiting the processes involved in the infection. There is therefore the need for bioactivity-guided fractionation and isolation of the active components in the plant extracts.

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Formulation and Evaluation of Moisturizer Containing Herbal Extracts for the Management of Dry Skin

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ABSTRACT

Background

Formulating a moisturizer using all natural raw materials is a formidable task.
The Objective
present paper focuses on the formulation of completely herbal moisturizers, their evaluation and comparison with commercial moisturizer.

Methods

Three of the herbal moisturizers [M1–M3] formulated by using in varied concentrations [0.139–0.9%w/w] of herbal extracts, juice and gel. Further all of the prepared formulation and selected commercial moisturizer [M4] evaluated for its physicochemical and safety parameters by applying on the forearm of 20 volunteers.

Results

The physicochemical parameters of formulations i.e., pH, acid value, saponification value, viscosity, spreadability, layer thickness, microbial count and skin sensitivity were found to be in the range of 5.19±0.3–6.80±0.5, 6.80±0.5–9.2±1.3, 16.10±1.0–23.00±1.4, 5950±10–6600±15cps, 65±2.0–98±1.5%, 28.40±1.5–30.00±1.5m,31 ±4–48±3 colony forming units. Formulation M1 and M4 shown an increase in percentage of skin hydration, firmness and viscoelasticity after the 3 weeks study period. Comparison study of evaluated parameters justified that formulated herbal moisturizer M1 [$p<0.01$] possess almost same performance characteristics when compare to M4.

Conclusion

Herbal ingredients are not only efficacious to treat skin dryness as compare to synthetic one but also capable to substitute synthetic base to some extent. It is up to the cosmetologist to motivate and encourage the development and use of truly herbal cosmetics.

Keywords: herbs, extracts, formulation, moisturizer, dry skin, evaluation

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INTRODUCTION

The appearance and function of the skin are maintained by an important balance between the water content of the stratum corneum and skin surface lipids.[1–2] The skin represents the most superficial layer of the body and so it is constantly exposed to different environmental stimuli.[3] Exposure to external factors as well as endogenous factors[4–6] may disrupt this balance. In addition, frequent use of soaps, detergents and topical irritants such as alcohol and hot water can remove the skin surface lipids.[7] Disruption of skin barrier led to various type of skin problems most common condition is loss of water content which lead to dryness of skin such as roughness, scaling, cracks, redness and an uncomfortable feeling of tightness, sometimes with itching and stinging.[8] Treatment with moisturizers aims at maintaining skin integrity and the well-being by providing a healthy appearance of the individual.[9]

Today's skin-care consumer is presented with a wide array of available products to treat dry skin, the choices for the individual consumer seem endless.

The poly herbal cosmetic formulations are popular all over the world, as they convey the better impression of purity, safety and efficacy. Numbers of moisturizers are available under the label of natural, safe, organic, herbal, while the basic properties of humectancy, occlusivity and emolliency are consistent across all moisturizers.[10] Most of the available moisturizers use synthetic adhesives, emulsifiers, perfuming agents, pigments, surfactants and thickeners to form the base. There is extensive need to replace toxic synthetic agent from base using natural agents.[11–12] Table I summarized the toxic effects associated with the some of the synthetic ingredients used in commercial moisturizers. Formulating cosmetics using completely natural raw materials is a cumbersome task. Challenge not only lies to substitute synthetic base from naturals but also to get same functional effects acquiring from synthetic one. The selected herbs described in the present investigation have been utilized medicinally in crude aqueous and ethanolic extracts were well described in the literature for their potential cosmetic benefits.[13–14] These herbs have been selected on the basis of a traditional system, ethnobotanical survey and scientific justification with modern uses of *Glycerrizha glabra, Emblica officinale, Cucumis sativus, Trigonella foenum graecum, Triticum sativum, Cocos nucifera, Prunus amygdalus, Oleum olivae, Santalum alba, Azadirachta indica* and *Aloe barbadensis*.[15–18] Therefore, an attempt has been made in the present study to utilize various herbal ingredients' properties for substituting synthetic base [Table II] with functional benefits [Table III]. Our endeavour has been to formulate the almost complete herbal moisturizer with bare use of synthetic ingredients and to evaluate its efficacy and safety parameters as compare to available commercial moisturizer.

MATERIALS AND METHODS

Materials

The plant materials like dried barks of G. *glabra, E. officinale*, fruits of *C. sativus*, seeds of *T. foenum graecum,* oils of *T. sativum, C. nucifera, P. amygdalus, O. olivae and S. alba* and honey were procured from a local authentic herbal distributor of Raipur, Chhattisgarh [C.G.].

Table I: Synthetic ingredients used in moisturizers

S.no	Ingredients	Side effects	Used as/in
1	Propylene Glycol	Allergic reactions, hives and eczema	Humectant
2	Petrolatum	Dryness and chapping	Emollient and occlusive agent
3	Dimethicone	Harsh to skin or cause tumor	Adhesives and Emollient
4	Paraben	Allergic reactions and skin rashes	Antimicrobial agent
5	Diethanolamine (DEA), Triethanolamine (TEA)	Allergic reactions, eye irritation, dryness of hair and skin	Emulsifiers
6	Diazolidinyl Urea, Imidazolidinyl Urea, benzalkonium chloride	Contact dermatitis	Preservatives
7	Synthetic Colors	Carcinogenic	Colouring agent
8	Synthetic Fragrances	Headaches, dizziness, rash, hyperpigmentation, violent coughing	For fragrance

Table II: List of ingredients used to formulate natural base for herbal moisturizer

S.no	Ingredients	Used as
1	Soy lecithin, Glycerin, *Aloe barbadensis* (Aloe vera)	Humectant
2	Triple distill water, *Triticum sativum* (wheat germ) and *Trigonella foenum graecum (Methi)*	Emollient and occlusive agent
3	*Cucumis sativus* (Cucumber)	Adhesives or emollient
4	Acacia	Emulsifiers
5	Azadirachta indica (Neem)	Preservatives
7	Santalum Alba (Sandal oil)	For fragrance
8	Rose water	Cooling effect and fragrance

Table III: List of herbs with its chemical constituents and functional properties use to formulate herbal moisturizer

S. no	Herbs	Chemical constituents	Functional properties
1	*Aloe barbadensis* (Leaf extract)	Barbaloin, aloe-emodin, aloesin, amino acid, enzymes, vitamin	Moisturizing agent and impart elasticity
2	*Glycerriza glabra* (Bark extract)	Estragole, anethole, flavonoids	Astringent
3	*Cucumis sativus* (Main fruit juice)	Silica, vitamin C, folic acid	Moisturizing and firming agent
4	*Trigonella Foenum Graecum*(Seed extract)	Carbohydrates, lipids, flavonoids, free amino acids	Softening and soothing agent
5	*Triticum sativum(oil)*	Vitamin E, carbohydrate	Nourishing and occlusive agent
6	*Cocos Nucifera*(oil)	Lauric oils	Soothing agent
7	*Prunus Amygdalus*(oil)	Amandin, folic acid, alpha tocopherol and zinc	Hydrating and firming agent
8	*Oleum olivae*(oil)		Prevent drying and chafing
9	*Azadirachta indica*(Leaf extract)	Nimbin, nimbinin and nimbidin	Rejuvating and extrafoliating agent
10	*Santalum Alba*(oil)	Santalol	Alleviate itching and cooling agent
11	*Emblica officinale*	Vitamin c	Antioxidant

Leaves of *A. indica* and *A. barbadensis* were collected from the medicinal garden of the Institute of Pharmacy, located in Chhattisgarh State, India. All plant materials were identified [from the Herbarium, Dept. of Pharmacognosy of Pt. Ravishankar Shukla University, Raipur, India] and tested for percent purity [99.7%] by microscopic methods Soy lecithin and glycerin was obtained from S.D Fine Chem. Ltd., Mumbai, India. Commercial herbal moisturizer was purchased from cosmetician.

Instruments

Instruments were used for analysis are pH meter [335, Systronic, India], Brookfield viscometer

[DV-I, LV-I spindle, Brookfield Engineering Laboratories, USA], Colony counter [M-37, Rolex, India], Muffle furnace [77 S8HT8, Tempo, India], Homogenizer [R220, Iyca, Japan], Micro centrifuger [RM-12CDX, Remi, India], Deep freezer [RQF 650, Remi, India], Cutometer [MPA 580, Courage and Khazaka, Koln, Germany] and [CASIO, H-21, India].

Preparation of herbal extracts

The ethanolic extracts of herbs were used in the present study due to their acceptability and compatibility with the skin's nature and economy. Plant materials were cleaned to remove the dirt and extra genus material and dried under the shade. The dried barks of *G. glabra, E. officinale* and leaves of *A. indica* were ground using a laboratory mill and their coarse powders [particle size ~0.25mm] were passed through a sieve number 20. Exactly 250 grams of coarse powder of each herb were extracted with a hydro-alcoholic mixture [1000ml, 90:10v/v ethanol:water] at 60–70°C for 24h by a continual hot extraction method[19], until complete exhaustion of the drug using a soxhlet apparatus. Dried seeds of *T. foenum graecum* [250gm] was extracted with a hydro-alcoholic mixture [1000 ml, 90:10v/v ethanol: water] using a cold maceration process according to the Indian Pharmacopoeia process[20] for 8 hrs to make concentrated extracts. The obtained extracts were evaporated under reduced pressure [AU 5 psi] at 50 ± 5°C for 5–15 min and concentrated extracts were dried to obtain actual yields. Fruits of *C. sativus* were chopped, weighed [300gm] and grounded through blender and juice was filtered through proper sieves. Obtained juice was kept at refrigerator. Fresh *A. barbadensis* transparent gel was collected from its fresh leaves after the complete removal of epidermis using a stainless steel knife.

Preparation of natural base

Phase inversion technique[21] was used to prepare natural base [M5]. The internal phase was prepared using ingredients [composition given in Table IV, emulsification was carried out in the mortar pastel. Initially, grated and melted bees wax, natural oil of *T. sativum, C. nucifera, P. amygdalus, O. olivae and S. alba* and other ingredients acacia, soy lecithin, glycerin were mixed using an homgenizer at 200±25rpm at 65°-75°C. After the complete homogenous mixing, a 50ml portion of triple distill water [70±2°C] was added at a rate of 45ml/min at increased speed [250±25rpm]. When the temperature of the internal phase was reduced to 50°C, phase inversion

took place and the solution became viscous; half of the total amount of aloe gel and cucumber juice was added. When the temperature was reduced to 40°C, honey [2% w/w] was added to this mixture.

Formulation of herbal moisturizer

Different concentrations i.e. 0.135–0.9% w/w of extracts, juice and gel were prepared in ethanol and incorporated into the natural base formula as summarized in Table IV. M5 was used as the control product. Commercial herbal moisturizer with synthetic base was coded as M4.

Subjects

Total 20 of volunteers, mean age 30±10yrs, with history of dry and itchy skin, were recruited after signing informed consent for the study of 3weeks. All volunteers having history of dry and itchy skin were selected for the subjective study to determine the effectiveness of formulations with regards to their ability to improve the mechanical and hydration properties.[23]

Study protocol

All volunteers participated were found free from any pathological findings on their arms. All test subjects were informed not to use cleansing or skin care products on the volar forearms[24] for 1week prior to and during the study. Volunteers were equally divided into two groups, each consisting of ten volunteers. One group was tested exclusively using the control [M5], just to observe its initial compliance and safety to the skin. The second group was tested with the M5 control and M1 and M4. Volar forearm of each volunteers of second group were divided into three sites, having a 2cm^2 sample area separated by 0.8cm. Study data were measured six times [n=6]. The measurements were performed in an acclimatized room with a mean relative humidity of 40±3% and a mean room temperature of 23±5°C. They were carried out under standardized conditions as described earlier[25].

Table IV: Composition of ingredients used in formulation of herbal moisturizer

Ingredients	Extracts incorporated in w/w %			
	M5	M1	M2	M3
Acacia	3.0	3.0	3.0	3.0
Bees wax(melted)	2.0	2.0	2.0	2.0
Cocos Nucifera oil	4.55	4.55	4.55	4.55
Glycerin	3.5	3.5	3.5	3.5
Soy lecithin	5.0	5.0	5.0	5.0
Santalum Alba oil	2.0	2.0	2.0	2.0
Prunus Amygdalus oil	3.0	3.0	3.0	3.0
Honey	2.0	2.0	2.0	2.0
Oleum olivae oil	1.0	1.0	1.0	1.0
Triticum sativum oil	0.6	0.6	0.6	0.6
Cucumis sativus	--	0.70	0.6	0.9
Glycerriza glabra	--	0.75	0.5	0.9
Emblica officinale	--	0.210	0.135	0.25
Azadirachta indica	--	0.75	0.6	0.9
Trigonella foenum graecum	--	0.583	0.385	0.75
Aloe barbadensis	--	0.78	0.5	0.9
Triple distill water q.s. (100 ml)	q.s	q.s.	q.s.	q.s.

Physiochemical Evaluation

Several physicochemical parameters were determined for M5 [control] and for prepared formulations [M1-M3], also for commercial moisturizer [M4] according to the Indian Standard Bureau methods.[26–28] These physicochemical parameters provided information regarding formula stability and skin compatibility. The pH, thermal stability (at 20°C, 30°C and 40°C), fatty content and nonvolatile content of the prepared formulations were determined according to Indian standard guideline. Ash examination, saponification values, and acid values were determined according to methods discussed by Lachman *et al*.[19–20] 1992. The viscosity[29] was measured using a Brookfield viscometer at 30rpm. The spreadability and layer thickness was evaluated according to Multimer.[30] Spreadability refers to the % area covered by a fixed amount of cream sample after the uniform spread of sample and layer thickness refers to the thickness of the layer [in microns]. All evaluations were carried out in triplicate presented as mean±standard deviation [SD].

Safety Evaluation

Safety analysis includes determination of microbiological specification and sensitivity profile.

Microbial examination of all herbal sunscreens [1gm/1ml] was tested according to COLIPA guidelines and Indian Standards methods.[26] Total numbers of viable mesophyllic microorganism were recorded by using a colony counter.[31–32] Sensitivity study using a patch test design was conducted on each volunteer of two groups. Formulations were applied on the back of volar forearm with the help of surgical gauze (0.5g/cm^2) and the score was determined using the scale defined in the Indian Standards.[14] Each volunteers was observed for any irritation, erythema score [redness], and edema after 24h to ensure that control [M5] and tested moisturizers [M1-M4] did not cause any side effect.[33]

Efficacy Evaluation

This prospective study was conducted from November to January 2008, as per the ethical guidelines of the Declaration of Helsinki, after prior permission from Institutional Ethical Committee. Efficacy evaluation on human volunteers was carried out for 3weeks. M5 used as the control product and M1 and M4 were used as the test formulations. Twenty subjects were enrolled in the study. Skin viscoelasticity, firmness and hydration parameters were determined by using Cutometer and Multitester respectively.

Skin Viscoelasticity and Firmness

The mechanical properties of the epidermis were determined using a non-invasive, *in vivo* suction skin elasticity meter, Cutometer [MPA 580, Courage and Khazaka, Koln, Germany] equipped with 2mm measuring. The time/strain mode was used with a 5s application of a constant negative pressure of 500mbar, followed by a 5s relaxation period. A typical skin deformation curve is illustrated in Fig. 1. The following parameters were analyzed: *Ue*, immediate distension; *Uv*, delayed distension; [R_0] *Uf*, final distension [skin distensibility]; *Ur* , immediate retraction; *R*, residual deformation at the end of measuring cycle [resilient distension]; [R_2] *Ua*/*Uf*, gross-elasticity of the skin, including viscous deformation; [R_5] *Ur*/*Ue*, neto-elasticity of the skin without viscous deformation; [R_7] *Ur*/*Uf*, biological elasticity, i.e., the ratio of immediate retraction to total distension; [R_6] *Uv*/*Ue*, the ratio of viscoelastic to elastic distension; and *R8*, viscopart, i.e., the area under the suction part of the deformation curve. The average values of two measurements were used in subsequent calculations.

Skin hydration

Hydration of the epidermis was determined with a non-invasive using an electronic device, Multitester [CASIO, H-21, India] that measured resistance based on the commonly known fact that hydrated skin has less resistance to current flow than dehydrated skin. The level of stratum corneum hydration was assessed by measurement of the changes in skin resistance and is referred to as the galvanic skin response or electrical skin resistance. The skin resistance reported in ohms with electrodes [size 1 cm^2] was measured 30min and 6hr after application of the formulation [continuously upto 3weeks] at 1000 khz, 10mA, AC current according to the modification of Nicander *et al*.[34]

Statistical Analysis

Statistical analysis was carried out by using STAT software, obtained values were expressed mean±SD [Standard deviation]. All parameters were statistically analyzed at 99% confidence level in the column. Changes in viscoelasticity, firmness and hydration were expressed in percentage. Analysis of variance ANOVA and Student's

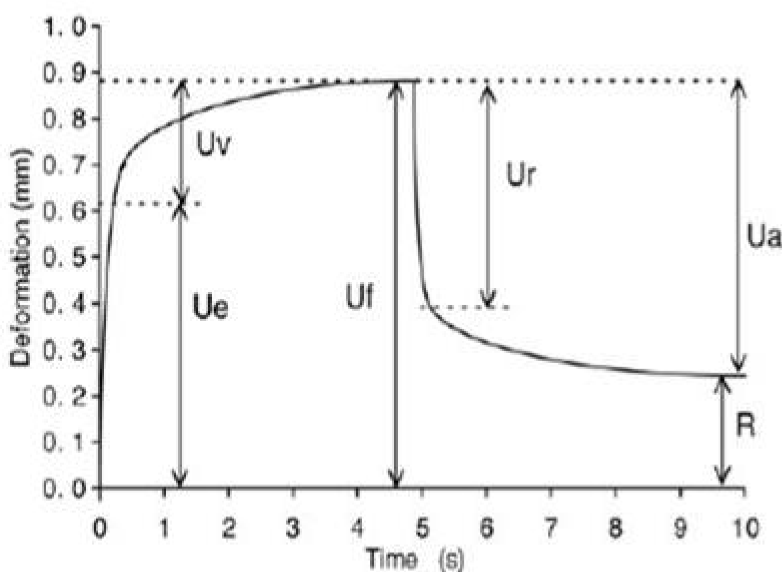

Figure 1: Skin deformation curve obtained with Cutometer

paired *t*-tests were performed. Differences was considered statistically significant if p<0.01.

RESULTS AND DISCUSSION

Natural base M5, formulated moisturizers [M1-M3] containing actives of herbal extracts and commercial moisturizer [M4] was analyzed for their stability at room temperature and physical characteristics.

The pH, erythema score, viscosity and spreadability of M5 was found to be 5.65, 0 erythema score, 5960±30cps, 97±2.0% respectively and found to be stable at 20°C, 30°C and 40°C [Table V]. This formulation showed no signs of phase separation at room temperature which indicated that uniform mixing and the desired consistency remained in the control [M5] cream base formula. The concentration of extracts in the M1-M3 formulations were selected after prior optimization of the individual extracts based on literature and available marketed formulations.[35] Based on the physicochemical parameters shown in Table V, higher acid and saponification values, less thermal stability, more microbial counts and less spreadability resulted in cracking and phase separation of formulations (observed in M3). Key chemical parameters that must be controlled include ash value, acid values [in the range 3.5–6.5], fatty content, nonvolatile content and pH [between 3.5–6.5]. It was observed that formulation M2 and M3 had higher free acid values, which caused more irritation to the skin. This acid value is associated with the free fatty acid and volatile content.

During storage and handling, cosmetic formulation's thermal stability, viscosity and spreadability are the prime parameters which affect the formulation's acceptability. Amongst all of the formulations, the highest fatty content [15.01gm] was found in M3 which accounts for the lowest thermal stability [at 30°C and 40°C, Table V]. Spreadability and layer thickness was found to be in the range of 65–98% and 28.40–30.08μm for formulations M1-M4 [Table V]. The viscosity of all formulations was between 5950–6600cps [Table V].

Microbial examination was conducted for all four herbal formulations [M1-M4] and for the natural control base formula [M5]. The average number of colonies per gm of sample in nutrient medium was calculated. Microbial viable counts were higher in the M3 formulation than in the control cream formula M5 [Table V]. The more microbial counts were observed in M3 indicating these formulations had the highest susceptibility to microbial attack. This microbial growth might be due to the incompatibility of the higher extract content with the base cream.

Table V: Evaluated physicochemical and safety parameters

Formulation Code	pH	Ash Exam				Non Volatile (%)	Acid Value	SV*	FattyCon. (gm)	Thermal Stability			Spreadibility %	LT** (μm)	Viscosity (Cps)	Microbial Count (CFU /gm)	Erythemal Score
		B	CO_3	Cl	S					20°°C	30°°C	40°°C					
M5	5.65±0.2	–	+	–	–	18.0±2.0	6.0±1.5	16.10±1.0	12.50±2.0	P	P	P	97±2.0	29.00±2.5	5960±30	33±2	0
M1	5.55±03	–	–	–	–	21.4±2.0	6.0±0.5	19.30±1.6	12.20±1.0	P	P	P	98±1.5	28.60±1.50	5950±10	31 ±4	0
M2	6.70±0.2	–	+	+	–	25.4±1.8	9.0±1.0	21.17±1.6	14.25±0.9	P	P	N	80±1.9	29.99±1.8	6565±30	43±3	1
M3	6.80±0.5	+	–	–	+	29.2±2.1	9.2±1.3	23.00± 1.4	15.01±0.5	P	N	N	65±2.0	30.00±1.5	6600±15	48±3	1
M4	5.19±0.3	–	+	+	–	21.0±1.6	6.1±1.0	18.10±1.5	12.69±0.6	P	P	P	97±1.0	28.40±1.5	5995±20	31±5	0

*Saponification value, ** Layer Thickness

All the values are represented as mean ± SD (n=3), p<0.01 shows sufficient significant.

(CFU) denotes colony forming units

(+) denotes absence, (+) denotes presence, (P) denotes stability of formulations, (N) denotes unstability of formulations

With respect to safety and the irritant test evaluation, M2 and M3 had shown an erythema score one, indicating the presence of a red spot remaining on the skin. An erythema score of 0 indicates no irritation [no redness] and a score of 1 indicates slight redness of skin. The formulations M1 and M4 were found to be non-irritating, representative by an erythema score of 0. This low erythema score is presumably due to lower pH of these formulations. Based on the results of the physicochemical and stability parameters, formulations M1, which contains the medium amounts of extracts, was the most stable formulation.

M2 and M3 formulations were excluded from *in vivo* efficacy study as they were found to be having erythema score 1 (irritant to skin). Hence, *In-vivo* efficacy studies were carried for M5, M1 and M4. Twenty subjects were enrolled in the study. The panelists communicated noteworthy improvements in the skin surface morphology [skin smoothness and softness] after only a week's application that continued through the duration of the study period. The improvement in the skin's mechanical and hydration properties were compared to the control [M5]. At the end of the 3rd week, the overall performance of the formulated herbal moisturizer M1, compare with commercial moisturizer M4. Statistical analysis was completed according to intent-to-treat principles. All the physiological positive results were found significant when data of M1 and M4 were compared with the data of M5 [$p < 0.01$] [Table VI]. The moisturizing effect of M1 was found highly significant after one-week of twice daily treatment [$p < 0.001$] as compared to the M5 formulation [Table VI]. Regarding the hydration effect, extensibility and firmness, no significant differences between M1 and M4 were observed [$p<0.01$].The results

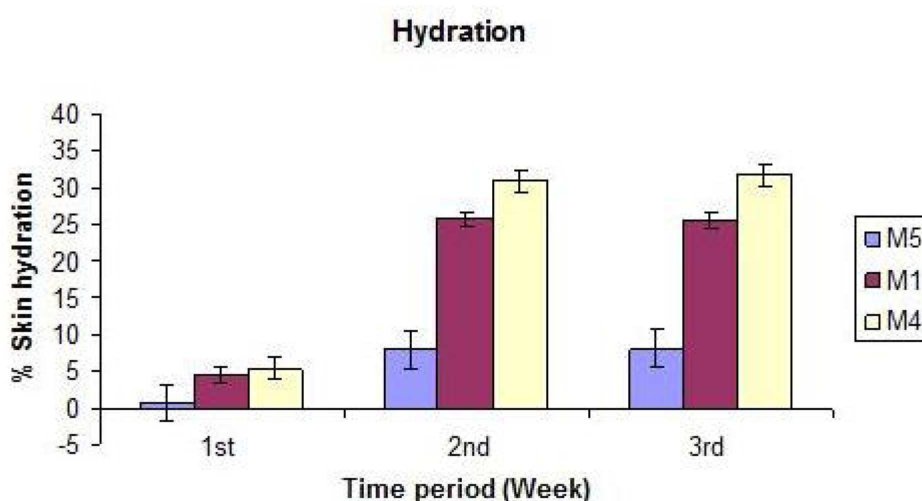

Figure 2: Increase in percentage of skin hydration after 3 week period

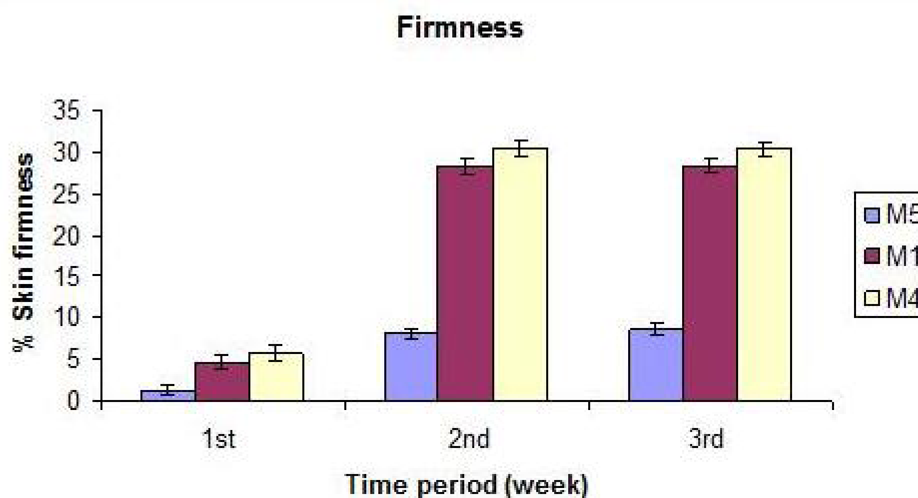

Figure 3: Increase in percentage of skin firmness after 3 week period

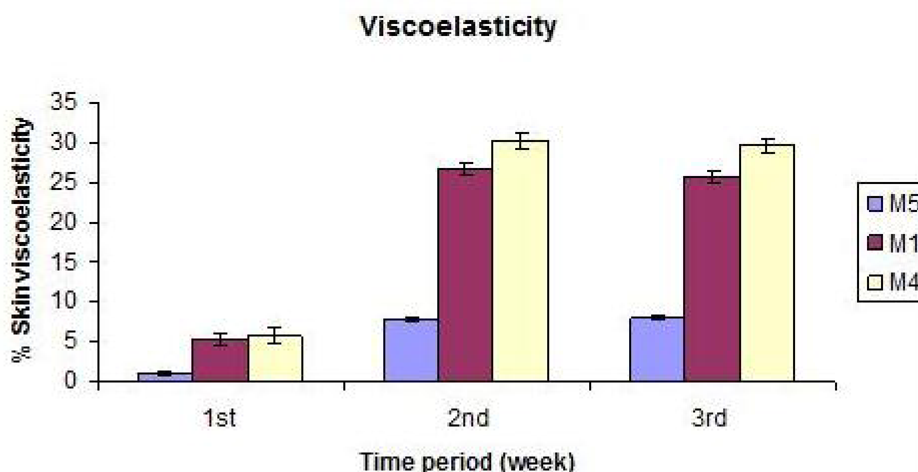

Figure 4: Increase in percentage of skin viscoelasticity after 3 week period

Table VI: Effects of control and tested formulations on the skin physiological properties

Week	% Increase in Skin Physiological Parameters								
	Hydration			Firmness			Viscoelasticity		
	M5	M1	M4	M5	M1	M4	M5	M1	M4
1st	0.69±0.05	7.90±0.51	8.10±1.25	1.27 ± 0.60	8.10 ±0.25	8.63±0.31	0.99±0.28	7.81± 0.55	8.00±0.50
2nd	4.51±2.00	25.85±1.05	25.64± 0.41	4.65 ± 0.30	28.29±0.35	28.36±0.65	5.15± 0.27	26.77± 0.75	25.63±0.92
3rd	5.40±2.51	30.97±0.55	31.77±0.59	5.78 ±0.50	30.46±0.86	30.35±0.91	5.76±0.30	30.27± 0.55	29.69±0.82

All the values are represented as Mean ± SD (n=6), p<0.001; when compared to control (M5)

showed that M1 and M4 had increased skin hydrations levels [30.97±0.55% and 31.77±0.59%] respectively after 3 weeks which were more than the control formulation M5 [5.40±2.51%] [Fig. 2, Table VI]. The improvement in skin firmness was found to increase up to 30.46±0.86% and 30.35±0.91% respectively for M1 and M4 [Fig. 3, Table VI]. The improvement in the skin viscoelasticity was found to be increased for M1, 30.27±0.55%, and M4, 29.69±0.82% as compared to the control product, M5, 5.76±0.30% [Fig 4 and Table VI].

The results revealed that M1 showed remarkable improvements in biomechanical and electrical properties when compare with M4. These improvements may be due to the synergistic effects of active constituents present in the ethanolic extracts of selected herbs. Accurate pathways of these mechanisms are not yet clear, but all these herbal ethanolic extracts possess different kinds of antioxidant, antielastase, photochemoprotective, astringent, face mask toner and anti aging properties due to their chemical constituents [Table III].

Results revealed that it is possible to formulate true herbal cosmetics may be more safe then the synthetic one. Many of the commercial herbal moisturizers contain synthetic ingredients for the base that may be toxic to skin [Table I]. Instead of synthetic adhesive, humectants, emollients, occlusive agents, emulsifiers, perfumes and preservatives we used the complete herbs which impart functional properties also. We utilize the self preserving property of Neem extract, which not only act as antimicrobial agent also imparts firmness to skin. Aesthetic attributes, such as smoothness, softness, firmness and luster found to be comparable to commercial one. The possible putative active constituents in the ethanolic extracts of selected herbs include flavanoids, glucides, polysaccharides, triterpene saponins, polyphenols and santalol etc. Such proven actives may impart a role in the changes in skin properties such as firmness, improved hydration, improved collagen binding and inhibition of indigenous cellular oxidation.

CONCLUSION

Present work attempts to formulate almost complete herbal moisturizer with same functional potentiality

compare to synthetic one. Study concludes that we are blessed with many of the magical herbals it depends upon us to explore then scientifically to treat skin related problems. Formulators must play an active role to replace dangerous and toxic synthetic chemicals from the dermato-cosmetic products so that consumers can get the maximum benefits of our traditional heritage. It is anticipated, this work will kindle more research and faith towards utilization of herbal active ingredients in cosmetics.

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CONFLICT OF INTEREST

None

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Pharmacognostical screening of seeds of Cassia absus

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ABSTRACT

Cassia absus is considered to be useful in many ailments and diseases. It is traditionally used for many medicinal purposes. The seeds have a bitter, bad taste and having diuretic, cathartic and useful in the liver and kidney diseases. It is very helpful in reducing the swelling and is effective in eye ailments. It also prevents hemorrhages. Its alkaline nature helps in scrapping of the extended skin. The seeds are also having astringent properties.[1] In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. Morphological and anatomical aspects as well as differential micro chemical response have been worked out to identify the diagnostic features of the leaf. Physical constant values involving moisture content, ash and extractives as well as qualitative and quantitative estimation of various phyto - chemicals have been studied. The presence of lipid, saponin, tannin, alkaloid, phenol, steroid, flavonoid, and some other chemical constituents are recorded.

Keywords: *Cassia absus* seeds, pharmacognostic and physicochemical standardization

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INTRODUCTION

Cassia absus (Family: Fabaceae) is commonly known as "Jasmeejaz" in English and "Chimed" in "Hindi" also used in India. It grows as a sticky plant in almost all the states of India particularly North-West India[2]. The plant is mainly useful in the skin diseases and eye ailments. The seeds have a bitter, bad taste and having diuretic, cathartic and useful in the liver and kidney diseases. The seeds are used as astringent and cathartic properties. It is very helpful in reducing the swelling and is effective in eye ailments. It also prevents hemorrhages[3]. However, no scientific standards or pharmacognostical parameters are yet available to ascertain the identity and to determine the quality of the crude drug. The pharmacognostical parameters are the major and reliable criteria for the conformation and identity and determination of quality and purity of the crude drug.

The present work therefore attempts to report various necessary pharmacognostic and phytochemical standards of *Cassia absus*.

MATERIALS AND METHODS

Plant Material

The seeds of *Cassia absus Linn*. was procured from a local vendor in Mumbai in the month of January-2010. The seeds were verified by a well known taxonomist and deposited in the Department of pharmaceutical chemistry, SVKM's NMIMS University, Mumbai for further reference. Collected fresh seeds were washed and used for the study of macroscopic and microscopic characteristics. The dried seeds of the plant was powdered and passed through 40 mesh size and stored in an airtight container for further use.

Reagents and Chemicals

All reagents and chemicals used for testing were analytical grade obtained from Fisher Chemicals Ltd., Mumbai and Loba chemie, Mumbai.

Organoleptic Evaluation

The freshly collected seeds were spreaded on a dry plastic sheet and investigated different organoleptic features by repeated observations using magnifying glass and ruler and then recorded.

Macroscopic study

The macroscopic characters of the seeds of *Cassia absus* were studied and reported in the results.

Microscopic study of powdered plant material

1 gm of powder was boiled with Chloral Hydrate solution for about 5 minutes to remove chlorophyll and

any other fatty impurity. Then, powder was placed on the grease free microscopic slide along with the drop of Glycerin: water (1:1).The powder was covered with clean cover slip and observed under the compound microscope at 40X magnification. A camera lucida was attached with the microscope and the powder was suitably traced out.[4]

Physicochemical evaluation

Physicochemical properties such as the percentage of loss on drying (LOD), Total ash, Acid insoluble ash, Water soluble ash, were determined according to the specifications as per Indian Pharmacopeia[5]. Water and alcohol soluble extractive values were estimated by cold maceration according to the method as prescribed by WHO[6].

Phytochemical screening (7, 8)

The powdered seeds were subjected to preliminary Phytochemical screening for qualitative detection of phytoconstituents. The dried and coarsely powder seeds (500 g) were extracted successively with petroleum ether (40° C- 60 ° C), Chloroform (59.5° C-60 ° C) , Ethyl acetate (76.5° C-77.5 ° C) ,Methanol (64.5° C- 65.5° C), Hydro alcoholic mixture (Methanol:water-1:1) and water successively with continuous cold maceration. Each time before extracting with the next solvent of high polarity , the powdered drug (marc) was dried in air oven below 50° C for 10 minutes. Each extract was concentrated by distilling off the solvent., which was recovered subsequently. The concentrated extracts were evaporated to dryness and the extract obtained with each solvent were weighed. Their percentages were calculated in terms of initial air dried plant material. The colors of the extracts were observed. The successive extracts as mentioned above, were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material.

Thin Layer Chromatography study[9]

Accurately weighed 100 mg methanolic extract was diluted with 5 ml of methanol in a clean glass stoppered volumetric flask and used for spotting the chromatographic plates. Silica Gel 60 F Plates were used as a stationary phase. Mobile phase was Butanol: Acetic acid: Water (4:1:1). After development of TLC plates by one dimensional ascending method[9], visualization was performed by spraying the reagent. The R_f values were recorded carefully and the chromatogram was documented graphically[10].

Fluorescence Analysis: (11, 12)

A small quantity of dried powdered seeds was placed on a grease free clean microscope slide and added 1-2 drops of freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 12 minutes. Then, the slide was placed inside the UV chamber and viewed in day light, short (254 nm) and long (366 nm) ultraviolet radiations. The colors obtained by application of different reagents in different radiations were recorded.

RESULTS

Macroscopy

Diameter - Most of the seeds are around 1-1.2 cm in diameter.
Shape - Mostly circular and sometimes uneven in texture

Organoleptic Characters

Color - Externally black and internally yellow
Taste - Bitter
Odour - Odourless

Extra features

Testa is hard smooth and glossy in appearance.

Microscopic study of powdered drug:

Powder of seeds mainly shows wavy sclerenchyma and pigment layer.

Endosperm

Polygonal cells are present with cellulose like material inside the cells.

Epidermis

Polygonal epidermal cells, filled with mucilage are present.

Hypodermis

Rounded collenchymatous cells are present.

Trichomes

Entire or fragments of trichomes are present.

Wavy sclerenchyma

Special rounded wavy sclerenchymatous cells are present.

Pigment Layer

Fragments of pigment layer, square cells with yellow to orange mass are Present.

Table I: Results of phytochemical screenings of successive fresh extracts of *Cassia absus* seeds

No	Test	Extracts Used					
		P. Eth (60–80°)	Ethyl acetate	$CHCl_3$	Methanol	Hydro alcoholic	H20
1.	Alkaloids	–	–	–	–	+	+
2.	Carbohydrates	–	–	–	–	–	–
3.	Phytosterols	+	+	–	–	–	–
4.	Fixed oils and fats	+	+	+	–	–	–
5.	Saponins	–	–	–	–	+	+
6.	Phenolic compounds and tannins	–	+	–	–	–	–
7.	Proteins and amino acids	–	–	–	–	–	–
8.	Gums and mucilages	+	–	–	–	–	–
9.	Volatile oil	–	–	–	–	–	–
10	Flavanoids	–	–	–	–	+	+

+ =Present , – = Present

Table II: Results of Physicochemical properties

Sr No.	Physicochemical properties	Result (% w/w)
1	Ash Values	
	Total ash	4.26
	Acid insoluble ash	0.72
	Water soluble ash	1.02
	Sulphated ash	0.4
2	Foreign Organic Matter	0.025
3	Moisture content (Loss On Drying)	1.71
4	Swelling Index	134.56
5	Mucillage Content	13.9

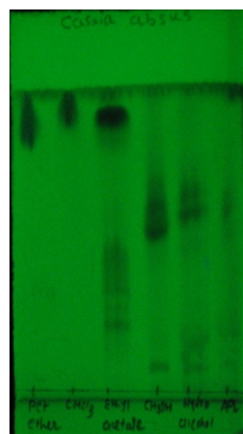

Figure 7: Comparative TLC profiles of all 6 Extracts

Table III: Fluorescence analysis of powdered *Cassia absus Linn.* seeds

Sr No.	Treatment	Day Light	UV (254 nm)	UV (366 nm)
1	Powder as such	Yellow Brown	Dark Brown	Yellow Brown
2	Powder + 1 M NaOH	Yeellowish Brown	Light Brown	Black Brown
3	Powder + Picric acid	Bright Yellow	Greenish Brown	Cream Yellow
4	Powder + 1 M HCl	Cream Brown	Greenish Black	Brown
5	Powder + 1 M Acetic Acid	Cream Brown	Dark Brown	Brown
6	Powder + dilute HNO_3	Orange Brown	Green Brown	Buff Brown
7	Powder + 5% $FeCl_3$	DarkYellow Brown	Black	Black
8	Powder + dilute NH_3	Floroscent Yellow	Black	Green Yellow
9	Powder + Methanol	Yellow Brown	Buff Brown	Black Brown
10	Powder + 50 % HNO_3	Orange Brown	Green Brown	Buff Brown
11	Powder +NH_3 + HNO_3	Yellowish White	Blackish White	Buff Brown
12	Powder + 1 M H_2SO_4	Dark Brown	Greenish Brown	Floroscent White
13	Powder +Dilute I_2	Brownish Red	Blackish Brown	Dark Brown
14	Powder + $K_2Cr_2O_7$	Orange	Greenish Brown	Brownish Orange
15	Powder + Methanol	Brown	Blackish Brown	Dark Brown
16	Powder + Toluene	Dark Brown	Dark Brown	Yellowish Brown
17	Powder + KOH	White Brown	Dark Brown	Brown

Phytochemical Screening

The results are shown in the Table (1). The results demonstrated the presence of phytosterols, Tannins and phenolic compounds and Flavanoids in the dried seeds of Cassia absus.

Physico-chemical Evaluations

The values of all the determinations are summarized in the Table (2). Water soluble ash is somewhat higher than the water insoluble ash. Water and methanolic extracts are having almost same extractive values.

Thin Layer chromatography Results

The results for TLC are shown in the figure (7).

Fluorescence analysis

The results summarized in the Table (3).

Determination of Inorganic elements in ash

Aluminum, Calcium, Chlorides, Phosphates and Potassium are present in ash of the seeds. The results are summarized in the Table (4).

Total phenolic content of *Cassia absus* powder by using Folin-Ciocalteau reagent: 3.39 % w/w.

Total tannin content in *Cassia absus* powder by using Folin-Denis reagent: 0.76% w/w.

DISCUSSION

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years, there has been an emphasis on standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical study is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) , the microscopic and macroscopic determination of the plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

Table IV: Elemental analysis of ash of *Cassia absus* seeds

Elements present in ash	Elements absent in ash
Aluminium	Copper
Chlorides	Carbonates & bicarbonates
Calcium	Iron
Phosphates	Magnesium
Potassium	Nitrates
Sulphates	Zinc
	Sodium

The organoleptic, macroscopic and microscopic studies yielded important characteristics. The present study was focused on the structural features of seeds of *Cassia absus* including micro and macroscopic features and physicochemical properties of grinded seeds. The microscopic characters revealed the presence of wavy sclerenchyma and pigment layer. These characteristics might be useful for distinguishing it from its substitutes and adulterants.

On the basis of qualitative chemical test, it has been observed that chemically therapeutic compounds like Tannins and phenolic compounds, Flavanoids, and saponin glycosides were present in sufficient amount in the seeds of *Cassia absus*. On the basis of elemental analysis, it has been revealed that Aluminum, Calcium, Chlorides, Phosphates and Potassium are present in rich amount. The extracts were subjected to qualitative phytochemicl tests to find out the active constituents. In the fresh seeds extract of *Cassia absus* with chloroform, ethyl acetate , methanol, hydroalcoholic and water sapnin glycosides, Flavanoids and Tannins and Phenolic compounds were present.

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in ash residues consisting of an inorganic material (metallic salts and silica).These parameters were used for the determination of inorganic materials like phosphates, aluminum, calcium, and sodium. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. We can detect the extent of adulterants as well as establish the quality and purity of the drug by using this method. The ash value determines the quality of the drug material. Here, the total ash value obtained was around 4 percent. Acid insoluble ash was 0.72 percent which determines the acid insoluble component present in the ash and water soluble ash was 1.02 percent which was the water soluble fraction of the total ash.

The extraction of any crude drug with particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The extractive value of the crude drug determines the quality as well as purity of the drug material. Methanol and water extractive values were 3.0 and 4.2 percent respectively. The use of a single solvent

can be the means of providing preliminary information on the quality of a particular drug sample. The extractive value of the crude drug determines the quality as well as purity of the drug material. The loss on drying value was found to be 1.71% w/w. It signifies the considerable amount of moisture in seed materials. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence, the moisture content of a drug should be determined and also be controlled to make the solution of definite strength. The moisture content of a drug should be minimised in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination. The objective of drying of fresh material is to fix their constituents i.e. to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug and to reduce their weight and bulk. Not only is the ultimate dryness of the crude drug is important, equally important is the rate at which the moisture is removed and the conditions under which it is removed. If the rate is too slow, much spoilage may occur before the drying process is completed. Therefore, in general, drying should be accomplished as rapidly as is possible with good practices.

Thin layer chromatograms are produced with the aim of identifying the individual substances in a mixture and also for testing for purity or for separation of mixtures. They are particularly useful for checking the mixtures used for synthetic reactions or following the course of reactions. For purposes of identification, it is necessary to relate the Rf values of the investigated substances and those of reference substances. If the Rf value agree, it is probable but not certain, that the two spots correspond to the same substance. Reliable identification is only possible by using spectroscopic investigation alongside with thin layer chromatography.

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. The powder drugs exhibit different fluorescence character in the presence of different chemical reagents under ultra-violet light. The change in the colour of stem powder under UV radiation in reference to day light was observed. The powder drug exhibit different fluorescence character due to presence of different functional groups in drug chemical constituents. The above table is about the fluorescence characteristics of seed powder of *Cassia absus Linn.* in the presence of different chemical reagents and ultra-violet light at 254nm and 365nm respectively.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. [12,16]

Total phenolic content was determined using Folin – Ciocalteu reagent which was found to be 3.39 % w/w and total tannin content was found to be 0.76% w/w using Foiln – Denis reagent.

CONCLUSION:

After present investigation it can be concluded that the pharmacognostical study of *Cassia absus Linn.* seeds yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. As previously mentioned, *Cassia absus Linn.* being a morphologically variable species, these information will also be helpful to differentiate *Cassia absus Linn.* from the closely related other species and varieties of *Cassia.*

Figures: Results for Microscopic study

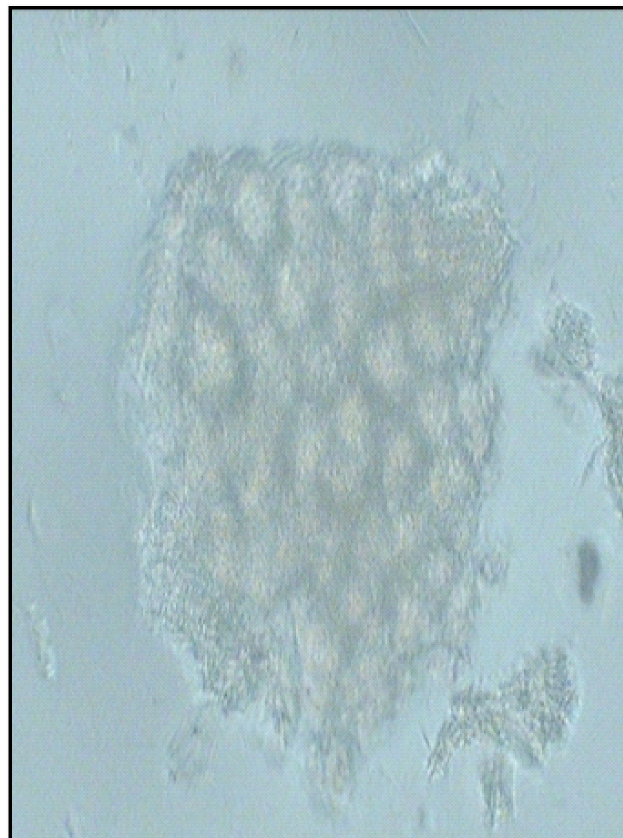

Figure 1: Endosperm

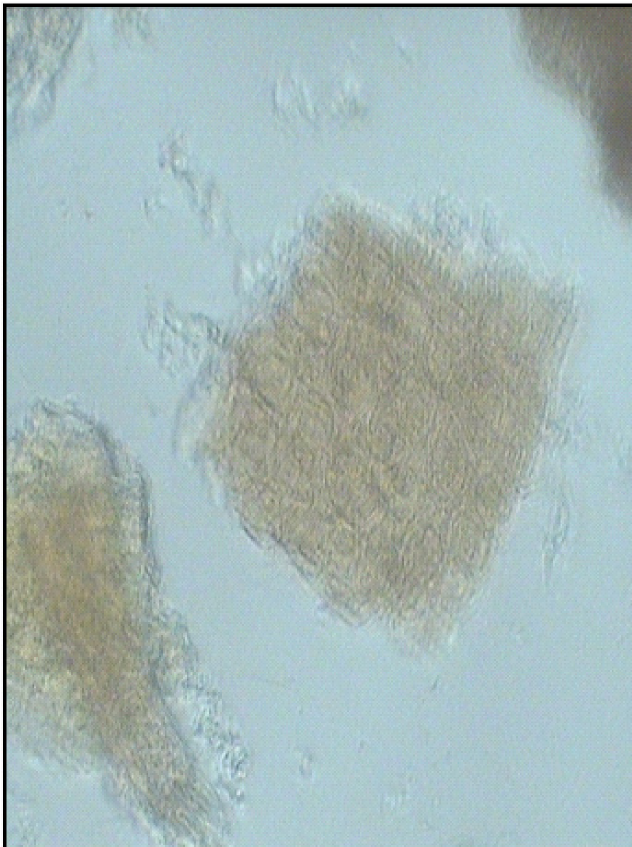

Figure 2: Epidermis

Figure 4: Ca-Oxlate crystals

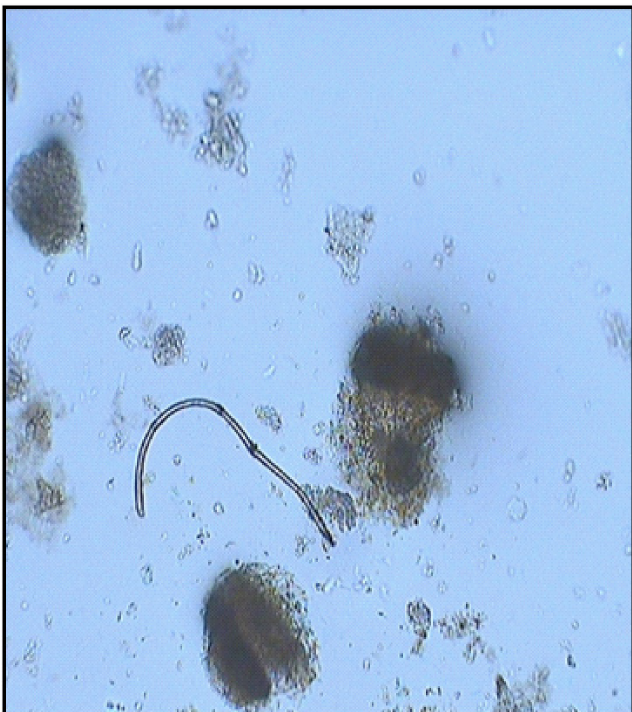

Figure 3: Trichomes

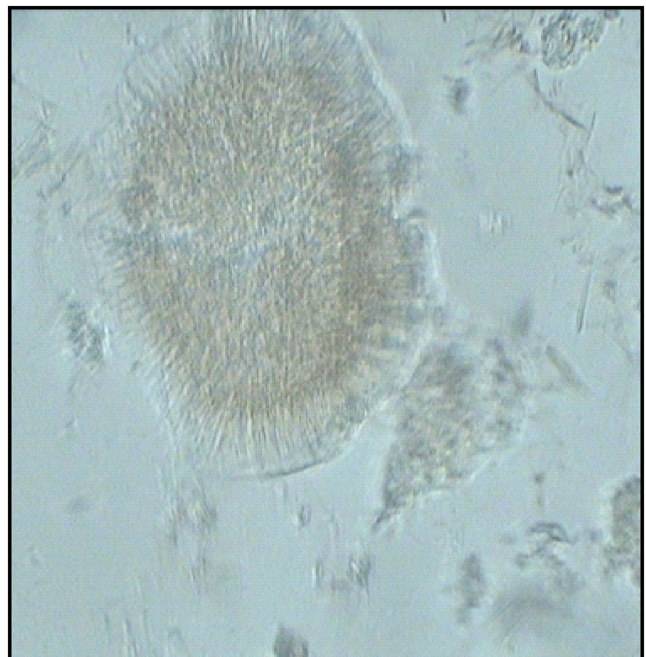

Figure 5: Wavy sclerenchyma

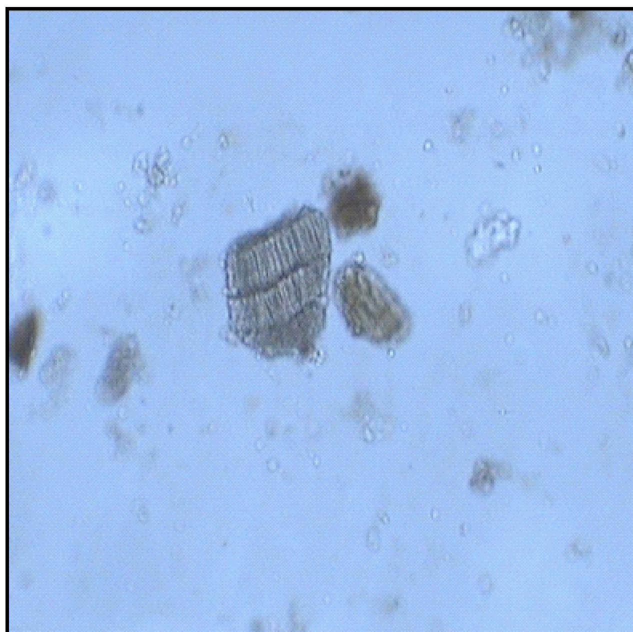

Figure 6: Pericyclic fibres

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Figure 8: Cassia absus plant

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Evaluation of anti-oxidant activities and total phenol and flavonoid content of the hydro- alcoholic extracts of *Rhodiola sp.*

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ABSTRACT

Rhodiola sp. (*R. heterodanta and R. imbricata*), belonging to the stone crop family *Crassulaceae*, is an important medicinal plant and food crop in Trans-Himalayan cold desert. It is a well known medicinal plant in the Amchi system of medicine (Tibetan system of medicine) being used in various ailments like anti-stress, radio-protective, anticancer, anti-inflammatory agent, adaptogen etc. Hydro- alcoholic extracts of *Rhodiola sp.* root and shoot were investigated in the present study to quantify total Phenolic as well as anti-oxidant activity. Total phenolic contents were determined as total flavonoids (TF), and total polyphenols (TPP). *Rhodiola heterodanta* root extract had highest amount of total phenol and flavonoids are found to be 79.21±.26 mg GAE/g and 269.3±.82mg Qc/g respectively. I*n vitro* antioxidant activity was investigated by DPPH radical scavenging activity, reducing power assay. *R. imbricata* hydro-alcoholic root extract showed the highest reducing power and DPPH. radical scavenging. A significant correlation existed between concentrations of the extract and percentage inhibition of free radicals and reducing power. These results clearly indicate that *Rhodiola sp.* are effective against free radical mediated diseases and also helpful to draw special attention for further studies.

Keywords: *Rhodiola imbricata*; *Rhodiola heterodanta*; Total polyphenols; Total flavonoids; Antioxidant activity

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INTRODUCTION

Free radicals can be described as chemical species that have an unpaired electron and play very important role in human health and beneficial in combating against several diseases like cardiovascular disorders, lung damage, inflammation etc. These free radicals are highly unstable and when the amount of these free radicals exceed in the body, it can damage the cells and tissues and may involved in several diseases. Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals.[1–2] The most common reactive oxygen species (ROS) include superoxide (0_2) anion, hydrogen peroxide (H_20_2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO.) and peroxynitrite anion (ONOO).[3] Currently available synthetic antioxidants like BHT, butylated hydroxyl anisole and tertiary butylated hydroquinones have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants.[4] Several studies revealed that phenols, mainly the type of flavonoids, from some medicinal plants, have antioxidant properties and exert anticarcinogenic, antimutagenic, antitumoral, antibacterial, antiviral and anti-inflammatory effects[5] due to their redox properties, acting as reducing agents, hydrogen donors, singlet oxygen quenchers and chelating metals.[6–8] Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability.[9]

Rhodiola sp. (*R. imbricata* and *R. heterodanta*), belonging to the stone crop family *Crassulaceae*, is an important food crop and medicinal plant in Trans-Himalayan cold

desert.[10] Golden root extracts phytochemical are the good source of important commercial preparations widely used throughout Europe, Asia, and more recently in the USA, with biological activities including antiallergenic and anti-inflammatory effects, enhanced mental alertness, and a variety of therapeutic applications.[11–13] The roots of these species are traditionally used as a tonic, adaptogen, antidepressant and anti-inflammatory drugs. The rhizomes and roots of the plants containphenylpropanoides: rosavin, rosin, rosarin and their aglycone: cinnamyl alcohol; phenylethanol derivatives: salidroside, tyrosol; flavonoids (catechins and proanthocyanidins), monoterpenes, triterpenes, phenolic acids (gallic, caffeic and chlorogenic acid), volatile oil, amino acids and minerals. Most of these compounds posses antioxidant activity.[14–15] Considering the importance of this area, present study was focused on *in-vitro* evaluation of antioxidant and antibacterial activity.

MATERIALS AND METHODS

Collection and Identification of Plant material

Plant materials were collected from Trans-Himalayan region in the month of June from Chang-La Top (altitude 17500 ft. Above Mean Sea Level), in India. The collected roots and shoot part were identified and authenticated by a scientist Dr. OP Chaurasia, Medicinal and Aromatic Plant Division, Defence Institute of High Altitude Research (DRDO), C/o 56 APO, India. A Voucher specimen (Specimen no: A – 3, 4) has been deposited at the Herbarium of our division. The plant samples were washed thoroughly to remove clay and dirt from them. The roots were cut into small pieces and shade dried at room temperature for 15 days, finely powdered and used for extraction.

Preparation of plant extract:

Rhodiola sp. roots and shoot powder were successively extracted by 80%ethanol and 20% water with the help of Soxhlet apparatus till the residue remains colourless. The obtained extract was concentrated using rotary evaporator under vacuum and reduced pressure at 40°C and the residue was used for further studies.

Total phenols determination

Total phenols were determined by Folin-Ciocalteu colorimetric method.[16–17] To 0.5 ml of 50% hydro alcoholic solution of each extract was mixed 0.5 ml of the Folin-Ciocalteau reagent and 0.5 ml of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 h incubation at room temperature, with the help of spectrophotometer. The results were given in mg GAE/ g plant extract of gallic acid equivalent. The standard curve was prepared using 0, 50, 100, 150, 200, and 250 mg/L solutions of gallic acid in methanol: water (50:50, v/v).

Determination of Total flavonoids

Total flavonoids were determined by the aluminium chloride colorimetric method[18], where 250 µl of each sample was mixed with 1.25 ml of deionised water and 0.075 ml of 5% sodium nitrite. After 6 min, 0.15 ml of 10% aluminium chloride was added and after another 6 min the product was mixed with 0.5 ml of 1M sodium hydroxide and 2.5 ml of deionised water. Total flavonoids were measured at 510 nm.[19] The results were given in mg CE/ g plant extract of cathequin equivalent.

DPPH radical scavenging assay

The free radical scavenging activity of the sample was measured *in vitro* by DPPH (1, 1 diphenyl 2, picryl hydrazyl) assay.[20] The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH 0.1mM solution of DPPH in methanol was prepared and 3.0 ml of this solution was added to 40.0 µl of extract solution in water at different concentrations (100–1000µg/mL). The mixture was incubated at room temperature for 30 minutes and the absorbance was measured at 515 nm against corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{cont} - A_{sample}) / A_{cont} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of the extract/ standard.

The antioxidant activity of the extract was expressed as IC_{50}, which the concentration (in µg/ml) of extract inhibits formation of DPPH radicals by 50%.

Reducing power assay

Reducing power of ethanol extract of Rhodiola was estimated using the protocol reported by Oyaizu.[21] Different concentrations of ethanol extract of Rhodiola (100–1000µg/ml) were prepared and 1ml of each solution was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.8) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. To this mixture, 2.5 ml

of 10% trichloroacetic acid (TCA) was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and Fecl3 (0.5 ml, 0.1%) was added and the absorbance was measured at 700 nm.

The percentage scavenging was calculated by using the formula $(A_{control} - A_{sample})/A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

RESULTS AND DISCUSSION

Total Phenol determination

The scavenging ability of the phenols is mainly due to the presence of hydroxyl groups. Phenols are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also decrease cardiovascular complications.[22] Comparison of total phenols content (table-1) between *Rhodiola sp.* show significant deference in each extracts. Total phenol content in *Rhodiola heterodanta* root extract is higher than shoot extract. The same behaviour was found in *Rhodiola imbricata* extracts. *Rhodiola heterodanta* root extract with total phenol contents of 79.21±.26 mg GAE/g plant extract had the higher amount among the plant extracts analyzed in this study.

Determination of Total flavonoids

Studying of main group of Phenolic compounds the total flavonoid content was analyzed. Results are exhibited in table-1 and they indicate that *Rhodiola heterodanta* root and shoot extracts obtaining higher total flavonoid content in comparison to *Rhodiola imbricata*. In case of flavonoid concentration in *Rhodiola heterodanta* it was found that root hydro-alcoholic extract presented the highest values (269.3±.82 mg Qc/g plant extract)

DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 515 nm induced by antioxidants. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is widely used to evaluate the free radical scavenging capacity of antioxidants.[23] The DPPH scavenging activity of the hydro-alcoholic extracts from *R. imbricata* and *R. heterodanta* are given in Table 2. The *R. imbricata* hydro-alcoholic root extract showed the highest scavenging activity which was comparable to the reference compounds (ascorbic acid). Free radical scavenging activity was also increased with an increasing concentration. The ability of root extracts to scavenge DPPH radicals suggests that they are electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions.

Reducing power activity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.[24] The antioxidant activity has been reported to be concomitant with the development of reducing power. The reducing power of *Rhodiola* and ascorbic

"Table 1: Total phenols and flavonoids determination results in *Rhodiola sp.*"

Sample	(Hydro-alcoholic extraction)	
	Total phenols (mg GAE/g plant extract)	Total flavonoids (mg Qc/g plant extract)
Rhodiola heterodanta		
Root	79.2±.24	269.3±.82
Shoot	26.16±.42	185.2±1.13
Rhodiola imbricata		
Root	49.8±1.37	167.4±.71
Shoot	21.24±.82	151.6 ±1.31

Values are mean ± S.E.M (n=3)

"Table 2: DPPH radical scavenging activity of Hydro-alcoholic Extracts of *Rhodiola Sp.*"

conc. (µg/ml)	Ascorbic Acid	*R. imbricata* Root	*R. Heterodanta* Root	*R .imbricata* shoot	*R. Heterodanta* shoot
100	36.55±0.61	32.06±0.68	32.34±1.53	11.20±0.65	10.21±0.85
200	39.11±0.80	36.78±1.76	35.46±0.65	12.05±0.88	12.05±0.65
400	41.26±0.83	40.11±1.10	39.43±0.88	13.75±0.88	13.04±0.65
600	43.14±2.82	42.98±0.86	41.84±0.65	14.04±1.12	13.90±0.65
800	46.90±1.01	45.51±0.68	45.39±0.65	16.02±0.88	14.89±0.42
1000	49.19±1.06	48.39±0.71	46.80±0.85	17.16±0.65	16.31±0.65

Values are mean ± S.E.M (n=3)

"Table 3: Reducing power by Hydro-alcoholic Extracts of *Rhodiola Sp.*"

conc. (µg/ml)	Ascorbic Acid	*R. imbricata* Root	*R. Heterodanta* Root	*R .imbricata* shoot	*R. Heterodanta* shoot
100	0.30±0.01	0.16±0.001	0.03±0.002	0.03±0.002	0.09±0.004
200	0.57±0.002	0.35±0.003	0.07±0.005	0.04±0.004	0.12±0.005
400	1.01±0.002	0.88±0.005	0.46±0.003	0.05±0.009	0.13±0.005
600	1.81±0.004	1.71±0.006	0.53±0.003	0.09±0.002	0.15±0.004
800	2.31±0.004	2.09±0.005	0.88±0.006	0.095±0.001	0.20±0.003
1000	2.67±0.004	2.41±0.004	1.04±0.004	0.13±0.004	0.22±0.005

Values are mean ± S.E.M (n=3)

acid is shown in Table 3. The reducing power increased as the extract concentration increased. At different concentrations (100–1000 µg/ml) *R. imbricata* and *R. heterodanta* demonstrated powerful reducing capacity and these differences were statistically significant ($p<0.05$). *R. imbricata* hydro-alcoholic root extract showed the highest reducing power. *Rhodiola sp.* consists of hydrophilic polyphenolic compounds that cause the greater reducing power.

CONCLUSION:

Total Phenolic contents, Antioxidant capacities of *Rhodiola sp.* (*R. imbricata* and *R. heterodanta*) medicinal plant were evaluated. It was found that *R. imbricata* root extract showed the highest antioxidant capacities, which is valuable source of natural antioxidant. A significant relationship between the antioxidant capacities and total phenolic content were found. Antioxidant properties of Rhodiola sp. have recently great interest in the research and food industry. These results can be useful for further application of *Rhodiola sp.* or its constituents in the pharmaceutical preparation after performing clinical in vivo research. With this kind of investigation it would be easier to the treat and prevent the human damages occurring due to free radical and also to replace the synthetic antioxidant in industry.

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Anti-bacterial activity of herbal extracts, EuMiL® and antibiotics against *Helicobacter muridarum*

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ABSTRACT

Aims

The aim of this research was to investigate the anti-bacterial activity of herbal extracts of *Asparagus racemosus* Wild (AR), *Centella asiatica* Linn (CA). *Convolvulus pluricaulis* (CP), *Emblica officinalis* (EO), *Ocimum sanctum* (OS) and *Withania somnifera* Dunal (WS), a polyherbal drug EuMil® and antibiotics -Amoxycillin, Metronidazole, Oxy-tetracycline, Roxithromycin and Tinidazole against *Helicobacter muridarum* a species of helicobactor present in rodents.

Materials and methods

H.muridarum was isolated from gastric mucosa of control and experimental swiss albino mice on selective media and identified by standard methods. Anti-bacterial activity was assayed by Kirby-Bauer Cup-well agar diffusion method at 5 mg/ml concentration.

Conclusions

Treatments of extracts of AR, WS, CP, CA and also EuMil® were found to be anti-bacterial and were inhibitory to *H. muridarum*. *C. pluricaulis* whole plant extract, EuMil® and Oxytetracycline showed the highest inhibitory activity against *H. muradarium*.

Significance and Impact of study

The study signify the importance of these plants as an alternative anti-ulcer and healing agents.

Keywords: Ethanopharmacology, Phytochemicals, Traditional medicine, Helicobacter Sp, peptic ulcer, antiulcer.

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INTRODUCTION

Peptic ulcer disease (PUD) encompassing both gastric and duodenal ulcer, is the most prevalent gastrointestinal disorder[1]. There are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection and second through development of proton pump inhibitors, histamine receptor blockers, and drugs affecting the mucosal barrier and prostaglandin analogs[1,2,3]. But clinical evaluation of these drugs showed development of tolerance and incidence of relapses, thus an indigenous drug possessing fewer side effects is the major thrust area of the present day research, aiming for a better and safer approach for the management of PUD.

Several plant species like *A. racemosus*[4,5], *O. sanctum*[6,7,] , *E. officinalis*[8]; *C. pluricaulis*[9], *C. asiatica*[10] *and W. somnifera*[5], have shown both antibacterial and antiulcer activities. Pepticare[11] and EuMil®[12], a anti-bacterial polyherbal formulation, based on classical Ayurvedic literature comprises of standardized extracts of *W. somnifera, O. sanctum, A. racemosus* and *E. officinalis* has been used commonly by ayurvedic practitioners for Gastric diseases. All these herbs mentioned above

are claimed to increase the resistance of body against infection and other external and internal factors tending to perturb the homeostasis.

This study was thus carried out with the aim to evaluate the anti-ulcer properties with particular emphasis to anti-bacterial and inhibitory action of AR,CA,CP, EO,OS and WS extracts, EuMil® and certain antibiotics against *Helicobacter muridarum*

MATERIALS AND METHODS

All experiments carried out were cleared by institutional ethical committee. CPCSEA (India) Authorization is #: 973/ac/06.

Collection of plant material

The plant materials of CA and CP were collected from Botanical Garden, Univ. College of Science, Udaipur. The methanolic extracts of AR, WS, EO, OS and poly herbal formulation EuMil® was purchased from Envin Bioceutical Pvt. Ltd., Saharanpur (U. P.).

Preparation of plant extracts

Methanolic extract of CA and CP were prepared by reflux method in Soxhlet apparatus. The powder of plant parts was extracted with 100% methanol in 1:7 ratios. The process was repeated till complete extraction took place. Extracted plant material was vacuum dried and placed in hot air drier. Dried extract was stored in air-tight jar and was placed in a refrigerator.

Antibiotics

The antibiotics were purchased from authorized medical shop and stored in refrigerator.

Preparation of stock solutions

Stock solution of extracts as well as EuMil ® was prepared in 1:1 ratio with 50%DMF (N, N di-methyl formamide) and sterile distilled water. The stock solution of antibiotics was prepared in sterile distilled water. Each stock solution contained 100 mg extract or antibiotics per milliliter.

Test bacterium

Helicobacter sp used in the study was isolated from the biopsy samples taken from alcohol induced gastric mucosa of Swiss albino mice. The cultures were identified on the basis of gram staining, culture characters and biochemical tests[13,14]. As per published report[15], the bacteria species was identified as *Helicobacter muridarum* and authenticated by a microbiologist of the university.

Culture medium and inoculum development

Brucella blood agar base (HiMedia) with 10% defibrinated sheep blood was used to culture *H. muridarum*. The inoculum was developed by sub-culturing *H. muridarum* on Brucella blood agar. Inoculated media plates were incubated at 37^{0}C for 48 hours under microaerophilic conditions in a CO_2 incubator. 4–5 colonies of this fresh culture was added in 10 ml sterile distilled water and turbidity was adjusted to 0.5 McFarland opacity standards contained $1.5x10^6$ cells/ml.

Experimental groups

Four experimental groups were - Group 1-six plant extracts (sub groups of AR, WS, CA, CP, OS, EO treatment each); Group 2-EUMIL®; Group 3-Antibiotics (sub groups of amoxycillin, metronidazole, oxy-tetracycline, roxithromycin and tinidazole each) and Group 4-Control group.

Assay of anti-microbial activity

Antimicrobial activity was assayed by Kirby-Bauer cup-well agar diffusion method. 30 ml culture medium was dispensed in respective petri plates and inoculated with 0.1 ml fresh culture[16]. Inoculum of *H. muridarum* was spread onto the surface of Brucella blood agar (BBA) plates using a sterile glass spreader. 8mm wide cup-well was bored in each petri plate and was filled with 50¼l of stock solution to give 5mg/ml final concentration. Each sample was assayed in triplicate. Culture control and DMF control were also maintained along with test samples. All the inoculated media plates were incubated for 48 hours at 37°C under microaerophilic conditions in a CO_2 incubator. The antibacterial activity was interpreted from the zone of inhibition measured to nearest in millimeter (mm).

RESULTS

Zones of inhibition produced by Herbal Drugs, EUMIL® and antibiotics against *Helicobacter muridarum* have been shown in Figure1 (A-H).

Anti-microbial activity of plant extracts.

90mm growth was observed for control. Out of 6 herbal extracts in group1, the methanolic extracts of whole plant of CA and CP produced 25mm and 27mm zones of

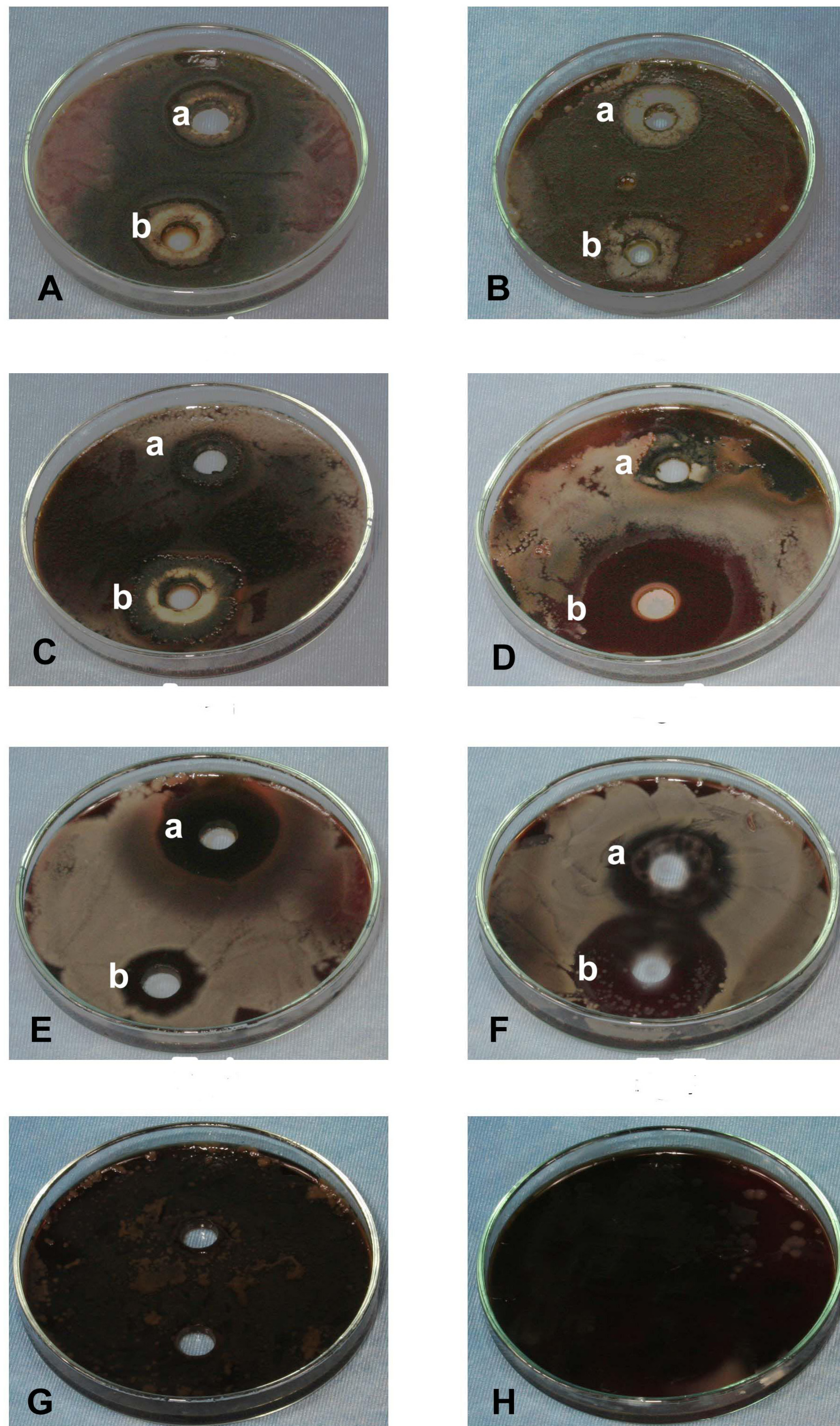

Figure 1. Zones of Inhibition Produced by Herbal Drugs, EUMIL® and Antibiotics against *Helicobacter muridarum*.
A. Inhibition by a-*Asparagus recemosus;* b-*Withania somnifera*
B. Inhibition by a-*Convolvulus pluricaulis;* b-*Centella asiatica*
C. Inhibition by a-*Emblica officinalis,* b-EUMIL
D. Inhibition by a-*Ocimum sanctum;* b-Oxytetracycline
E. Inhibition by a-Metronidazole; b-Amoxycillin
F. Inhibition by a-Roxithromycine; b-Tinidazole
G. Positive control (medium plus test bacterium)
H. Negative control(medium plus DM

inhibition. 25.67 mm zone of inhibition was produced by methanolic extracts of AR as well as WS roots. 12.34 mm zone of inhibition was formed by methanolic extract of EO as well as OS. Maximum zone of inhibition was produced by extract by CP. AR, CA and WS extracts showed similar inhibitory action. Extracts of EO as well as OS showed minimal inhibition as compared to other extracts. Results are summarized in Table-I.

Anti-microbial activity of EuMil® and antibiotics

Poly herbal formulation- EuMil® produced 28 mm zone of inhibition. While various antibiotics amoxycillin, metronidazole, oxy-tetracycline, roxithromycin and tinidazole produced 18.34, 29mm, 38mm, 29.34mm and 34.67mm zone of inhibition respectively. Maximum zone of inhibition was observed with oxy tetracycline followed by tinidazole. Roxithromycin and metronidazole were comparable in antibacterial activity. Amoxycillin showed lowest inhibition as compared to the other antibiotics. Overall highest inhibitory activity was demonstrated by *C. pluricaulis*, EuMil® and oxy tetracycline. 90 mm growth in culture plate was observed in positive control group (Table.I).

DISCUSSION

Helicobacter muridarum is a helical organism harboring gastric region of the rodents. The bacteria are microaerophilic, nutritionally fastidious, and physiologically similar to *Helicobacter pylori* but both could be differentiated by their unique cellular ultra structure[17]. Infection of Helicobacter sp is related with diseases of the digestive system, especially PUD[18]. Comparative study of antibacterial activity of herbal extracts of AR, WS, CA, CP, OS, EO and EuMil® as well as antibiotics have shown interesting results. Methanolic extracts of all the plant extracts showed significant inhibition of bacterial activity. Though maximum inhibition was shown by extract of CP. The inhibition produced by AR, WS and CA extracts was comparable. Extracts of EO as well as OS showed minimal inhibition. EuMil® also produced significant inhibition comparable to that observed for herbal extracts. In case of antibiotics, maximum zone of inhibition was observed with Oxytetracycline followed by Tinidazole. Roxithromycin and Metronidazole produced comparable inhibition. Amoxycillin showed minimal inhibition. Overall highest inhibitory activity was demonstrated by CP, EuMil® and Oxytetracycline

Table I: Antibacterial activity against *Helicobacter muridarum* (at 5mg/ml)

S. no.	Name of agents	Plant part used	Extract	Zone of inhibition (mm ±SD)*
Group 1; Herbal extracts				
1.	*Asparagus racemosus*	Root	Methanolic	25.67±0.58
2.	*Centella asiatica*	Whole plant	,,	25.00±1.0
3.	*Convolvulus pluricaulis*	Whole plant	,,	27.00±1.0
4.	*Emblica officinalis*	Fruits	,,	12.34±0.58
5.	*Ocimum sanctum*	Whole plant	,,	12.34±0.58
6.	*Withania somnifera*	Root	,,	25.67±0.58
Group 2; Herbal Drug				
7.	EuMil®			28.00±1.0
Group 3; Synthetic drugs (Antibiotics)				
8.	Amoxycillin	-	-	18.34±0.58
9.	Metronidazole	-	-	29.00±0.58
10.	Oxy-tetracycline	-	-	38.00±1.0
11.	Roxithromycin	-	-	29.34±1.0
12.	Tinidazole	-	-	34.67±1.0
Group 4; Control group				
13.	Positive control	-	-	+ + + +
14.	Negative control	-	-	– – – –

± = Standard deviation, * = 8mm well diameter included in zone of inhibition, + + + + = 90mm growth all over the culture

Ulcer healing is a complex process that involves combination of wound retraction and re-epithelialization. It also involves other factors, such as, growth factors and angiogenesis. Several drugs such as antibiotics and general inhibitors, channel blockers are used for treatment but each have varying side effects. Drugs of plant origin could be alternative therapy for PUD. As observed in earlier study carried out in this laboratory, root extracts of AR and WS were effective not only in reducing the ulcer and acid secretions but were also effective in increasing the gastric protective secretions[5]. Earlier studies on these drugs have also shown antibacterial activity of AR[4,5], OS[6,7], EO[8], CP[9],. CA[10], WS[5], The exact mechanism of the antibacterial action of these plants species is not known, but a hypopthetical diagram (Figure 2), to demonstrate the possible mechanism of anti-bacterial and anti-ulcer activity of polyphenols, flavonoids etc., present in these plants has been included. Ach release from post ganglionic vagal fibers stimulate gastric acid secretion directly through M3 sub type receptors located on the basolateral membrane of the parietal cells[21]. Polyphenols by inhibiting Ach synthesis might regulate the excess synthesis of acid secretion[22]. Polyphenols also stimulate inflammatory reactions[23]. As Helicobector bacteria is specifically adapted to live deep in the mucus layer close to epithelium and able to survive in acid environment, they may be phgaocytosed by the phagocytic cells stimulated due to inflammatory reaction. Besides, Polyphenols and flavonoids are also known to increase the blood flow and stimulate the secretion of mucus, bicarbonate and NO[24,25]. NO also plays the acid secretion inhibitory role. Polyphenols and flavonoids also reduce ulcer area, increase mucus secretion, and helpful in scavenging free radicals[5].

Antimicrobial activity of the medicinal plants could be attributed to the presence of secondary metabolites such as phenolic compound[26]. In many cases these substances serve as plant defense mechanisms against predatory microorganisms, insects and herbivores. Substance like polyphenols e.g., Catechol and Pyrogallol are toxic to pathogenic microorganisms[27], due to enzyme inhibition by the oxidized compounds, possibly through reaction with sulphydryl groups or through more nonspecific interactions with the proteins. Quinones target the microbial cell surface adhesins; cell wall polypeptides and membrane bound enzymes[28,29]. Flavones, flavonoids and flavonols are known to be synthesized by plants in response to microbial infection. Their antimicrobial nature is probably due to their ability to form complexes with bacterial extra cellular and soluble proteins which then bind to bacterial cell wall. Lipophilic flavonoids may also disrupt microbial membranes Human physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and wide range of anti-infective actions have been assigned to tannins[23,30] Tannins have the ability to inactivate microbial adhesions, enzymes

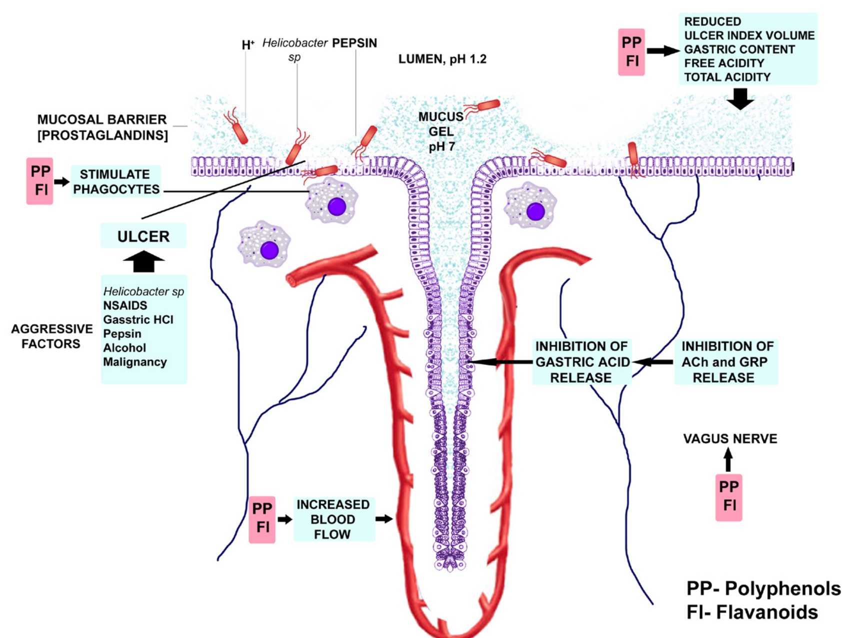

Figure 2: Hypothetical diagram to demonstrate the possible sites of inhibitory action of various constituents of plant extracts on *Helicobactor muradirum* and ulcerative gastrointestinal membrane.

and transport proteins on cell envelope and lipophilic terpenoids and essential oils are also speculated to be involved in membrane disruption[31,32]

In conclusion, study thus demonstrate that *C. pluricaulis* whole plant extract, EuMil® and Oxytetracycline shows the highest inhibitory activity against *H muradarium*. Data presented may serve as background for future studies on *Helicobactor pylori*. Our results fortify importance of these plants as an alternative anti-ulcer and ulcer healing agents.

ACKNOWLEDGEMENT

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Pharmacological Potential of *Atibala* of Ayurveda : A Review

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ABSTRACT

Indigenous drugs used by different ethnic groups of the world for the treatment of diseases have special significance of having been tested on long time scale. They are relatively safe, easily available and affordable to masses of community. Traditional drugs have given the important lead in the search of new drugs. *Balas* in ayurvedic literature are used as *Balya,* or tonic for strengthening the body. *Bala, Brela, Atibala, Mahabala* and *Nagbala* belong to the genus *Sida* of family Malvaceae is in use for medicinal purposes for a long time, in traditional system of medicine, i.e, the ayurveda. *Sida cordata* (Burm.f.) Borssum is *Rajbala* or *Bhumibala; Kharenti Bala is Sida cordifolia* Linn.; and *Sida rhombifolia* Linn. is *Mahabala*. The other *Bala* is *Atibala,* which *is* botanically known as *Abutilon indicum.* Literally meaning, the *Ati* means very and *Bala* means powerful, referring to the properties of this plant as very powerful.

Keywords: Atibala, *Abutilon indicum*, Aphrodisiac and Traditional Medicine.
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INTRODUCTION

The generic name *Abutilon* has been derived from its arabic name *Abutilun. Abutilon indicum* (Linn.) Sweet (Syn. *A. asiaticum* (Linn.) Sweet; *Sida guineenis* Sch.; *S. indica* Linn.), is one of the much used species of genus *Abutilon* in traditional medicines. It is also used in preparation of various ayurvedic formulations of commercial importance. *Abutilon indicum* is known by various names at different locations in different languages[1–6].

These are -

Bengali : Jhampi, Jhumka, Petari, Potari;
Cutch : Balbij;
Goa & Konkani : Petari, Tupkadi;
Gujarati : Dabali, Kansaki;
Hindi : Jhampi, Kandhi, Kanghani, Kanghi, Potari, Tepar;
Kannada : Srimndrigida
Konkani : Voddlipettari;
Malayalam : Jhoukaped, Katturam, Katturan, Pitikkapattu, Uram;
Marathi : Akakai, Chakrabhenda, Kansuli, Karandi, Madmi, Mudra;
Mumbai : Chakrabenda, Etari, Kangoi, Kangori, Madmi;
Punjabi & Sind : Atikhirate, Khapate, Peelee-bootee;
Sanskrit : Atibala, Balika, Balya, Bhuribala, Ghanta, Kankati, Kotibala, Rishiprokta, Shita, Shitapushpa, Vikankata, Vatyapushpika, Vrishyagandha, Vrishyagandhika;
Tamil : Nallatutti, Paniyarattutti, Perundutti, Ponnaithuthi, Tutti;
Telugu : Adavibenda, Botlabenda, Dudi, Kammalaku, Papidlakaya, Thuthurbenda, Tutti;
Uriya : Nakochono;
English : Country Mallow.

Description

This is an herbaceous or shrubby, softly tomentose, perennial plant. Leaves are up to 9 cm long and 5 cm broad, cordate, ovate, acuminate, toothed and rarely sub-

trilobate. Petioles are 3.8-7.5 cm long and stipules 9 mm. long, linear, acute, and deflexed. Pedicel is often 2.5-5 cm long, axillary, solitary and is jointed very near the top. Calyx is 12-8 mm long, divided to the middle; lobes ovate, apiculate. Corolla is 2.5 cm in diameter and is yellow; open in the evening. Staminal tube is hairy at the base; filaments long. Carpels are usually 15-20, longer than the calyx, with a distinct small acute point, hairy, ultimately shining and dark brown. Seeds are brown-black, densely and minutely scrobiculate.[4–9]

The flowering and fruiting in this plant is seen almost throughout the year, but chiefly during the months of August-December. This is very commonly found almost in every region of India and other neighboring countries like Sri Lanka, Pakistan, Nepal, etc. In India, it is met frequently as a weed in the Sub-Himalayan tracts and hills upto 1200 m. Also in the hotter parts of the country.[10–12]

Medicinal Uses

Bala's plants are in use since Vedic times. Theirs' references met in the Athraveda (Paplad Samhita 19/39/1-14). In vedic periods, the roots of the *Bala* plants were used to remove poison, *vata–pitta* diseases, eye diseases, heart problems, bily blood and uterine disorders. In fever its seeds and roots both were used in the form of decoction i.e., powdered plant material dissolve in water or any other solvent.[13]

In modern days, the seeds of the plant are known as aphrodisiac, laxative and expectorant and are used in cough, gonorrhoea, gleet and chronic cystitis. The leaves are known as demulcent and are applied to boils and ulcers. Their fomentation is used for painful parts of the body. They are cooked and eaten in bleeding piles and their decoction is used in toothache and tender gums. These are also given for enema and vaginal infections. Its bark has a sharply bitter taste and is considered febrifuge, anthelmintic and alexteric[3–4], and is used to remove *vata* and *tridosha* and to allay thirst, vomiting and to lesson perspiration[12]. It is also used as astringent and diuretic. The flowers of the shrub are applied to boils and ulcers and their powder in ghee is eaten for blood vomiting and cough. Its roots are nervine tonic and are used in piles and leucoderma[14] and also in strangury, haematuria, bladder-stones and as a wash in the eye diseases.[15] Besides, the powder of the roots is used in cough and leprosy.[16]

In addition, Alcohol and water extracts of its leaves showed significant hypoglycaemic effect[17] and methanolic extract and aqueous extract anti-diarrhoeal activity *in vitro*.[18]

Ethno-Botanical uses

The plant is used in the neurological disorders, epistaxis and heart diseases[19–22], as astringent, diuretic, demulcent[23], in rheumatism[24–25] and post delivery complaints in cattle.[25] Stem and bark of the plant are used as febrifuge, anthelmintic, alexiteric, diuretic[26–27] and in renal colic.[2] Flowers are antipyretic, useful in impotency as fertility enhancer, in piles and gonorrhea.[3] The seeds are used as aphrodisiac[4,5,12], in fever[5], cough[7], bronchitis, piles, gonorrhea[10–11,22–23]. Leaves are used in toothache[12,19], as demulcent[20–21], inflammation and wounds[24], for stomachache, diabetes, diarrhoea, hydrococle[28], jaundice[29], boil[30], piles[31–33], headache[34–35], as a tonic, in rheumatism[36] and as an antipyretic agent[37] and leaf-decoction in gonorrhoea and inflammation of the bladder.[38] Roots of plant are antipyretic[39–44] and used are in uterine haemorrhagic discharge[45], leprosy[46–48], leucorrhoea and menorrhagia[49], toothache[50] and as antiepileptic[51] and on cuts and wounds[52–53]. Besides, the decoction of its stem-bark or that of leaves is used for gargling in mouth ulcers and leaves alone are chewed and kept in mouth to reduce the heat[54].

Chemical Studies

Abutilon indicum contains gum resin and mucilage[50] but is devoid of tannins.[55] The petroleum ether extract of the plant yielded two sesquiterpene lactones identified as alantolactone and isoalantolactone[51] and gallic acid.[52] The aerial parts of the plant on extraction with petroleum ether led the isolation of n-alkane mixture, n-alkanol fraction and β–sitosterol, vanillic, p-coumaric acid, p-hydroxybenzoic, caffeic and fumaric acids, p-β-D-glucosyloxybenzoic acid and gluco-vanilloyl glucose, fructose, galactose, glucose, leucine, histidine, threonine, serine, glutamic acid and aspartic acid. Its mucilage fraction showed the presence of galactose and galacturonic acid.[55]

The essential oil of plant yields βpinene, caryophyllene, caryophyllene oxide, 1,8-cineole, ceraneol, ceranyl acetate, eudesmol and farnesol[56], while α–pinene, borneol, geraniol, geraniol acetate and tetradecane are reported from the oil obtained from flowering tops of the plant[57]. Its flower petals had shown the presence of gossypetin-8-glucoside, gossypetin-7-glucoside and cyanidin-3-rutinoside.[58] Saponins, flavonoids and alkaloids have been isolated from the short flowers of the plant.[59]

Its seeds contain raffinose as the sugar component. Chemical analysis of its seed-oil reveals the presence of lenolenic, linoleic, oleic, palmitic, stearic[60–61], malvalic, sterculic and 12,13 epoxyoleic acids.[62] Crude protein, pentosan and water soluble mucilage contents have also been isolated from its seeds.[63] Three HBr-reactive fatty acids viz. cis-12,13 epoxyoleic (vernolic acid), 9,10-methylene-octadec-9-enoic (sterculic acid) and 8,9-methylene-heptadec-8-enoic (malvalic acid) are also identified in its seed-oil. Amino acid profile of the seed proteins (31.0%) indicated to contain aspargine, threonine, serine, glutamine, proline, glycine, alanine, cysteine, methionine, isoleucine, valine, leucine, tyrosine, phenylalanine, histidine lysine and arginine.[64] Its fruits are reported to contain flavonoids and alkaloids.[59]

β-sitostirol and tocopherols have been reported from its leaves.[65] In addition, the roots of *A. indicum* contain flavonoids, steroids, sterols, terpenes and terpenoids.[66–67] Besides, the preliminary chemical studies revealed the presence of cyanogenetic glycosides, saponins, cardiac glycosides, tannins, phenolic compounds and alkaloids in leaves, stem, and roots of the plant.[68]

Uses in Commercial Formulations

Along with its uses in ethnobotanical aspects, the plant is being used in making various ayurvedic preparations of commercial importance. The important ones include: Bala taila, Atibalaghrit, Mahanarayan taila and Mahavishgarbh taila.[5,12] It also forms one of the ingredients of *Chayvanprash Linctus*, used as a general tonic for restoring health and vigour in each and everyone's house in India.

CONCLUSION

Various plants have been widely used as curative agents for variety of ailments since the time immortal. Some of them are used to strengthen the body. The extensive survey of literature revealed that *Abutilon indicum* is an important plant of ayurveda. It is being used since a long time in making ayurvedic drugs. Medicinally coumarins have diuretic activity, saponins hypoglycemic and antifungal activities. Linoleic acid, oleic acid, palmitic acid, lauric and stearic acids and other fatty acids found in the plant claim analgesic activities. β-sitosterol is reported to possess antipyretic reactions and flavonoids hypoglycemic activities[69]. Besides, the gum and resin obtained from the plant are used in rheumatism and show antiplasmitary reaction, which support the use of this plant for the various purposes since ancient times. Thus, the chemicals found therein possess various utilities and they can be used to yield the better results in making commercial drugs in allopathic system of medicine. So the importance of this plant should not be neglected.

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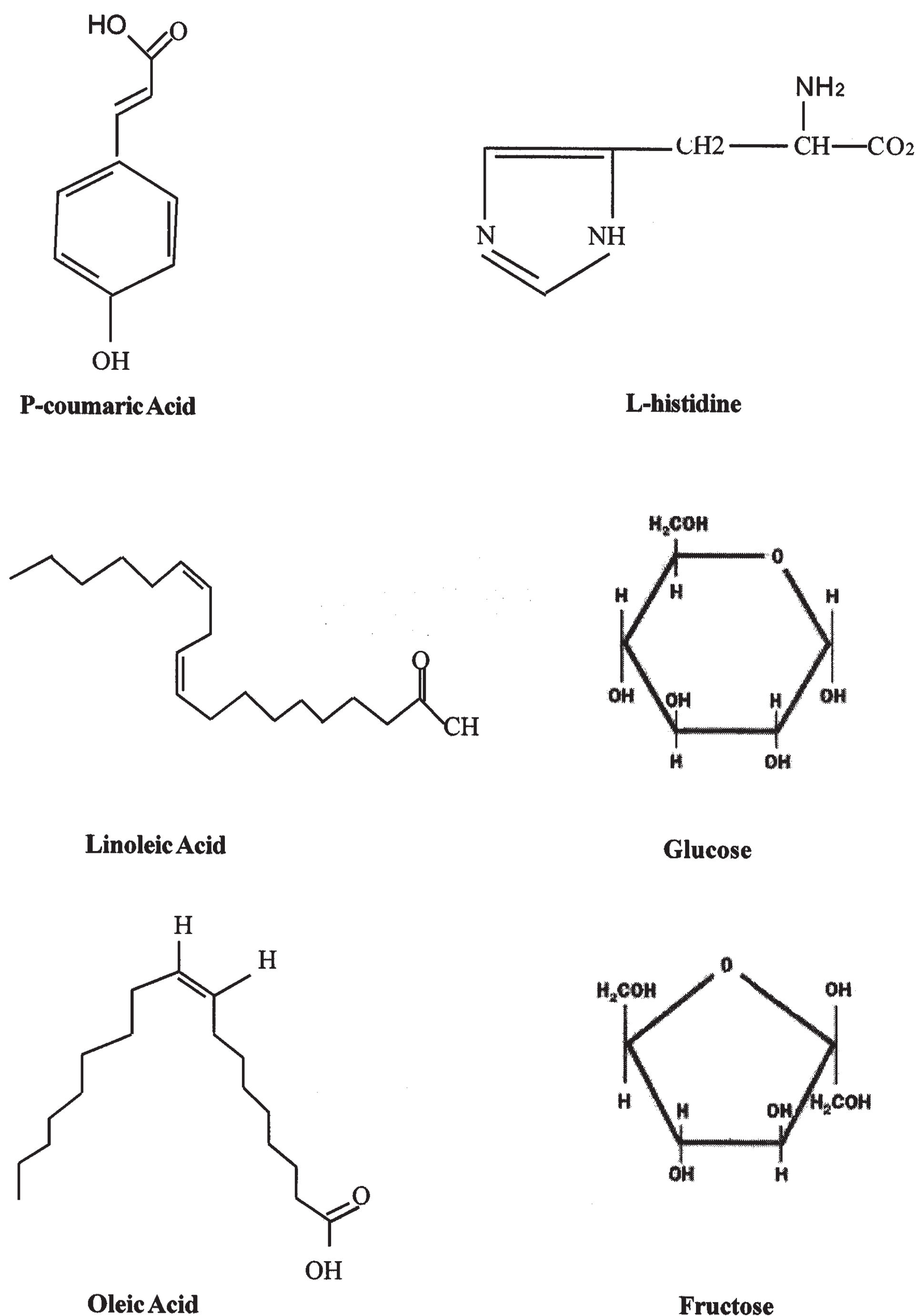

Figure 1 : Important Chemical Constituents

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