Editorial

Keshetti SrisailamDr. Editor-in-Chief, Phcog J

DEAR READERS

It gives me immense pleasure to write to you through this Editorial page for the first time as the Editor-in-Chief of this budding and official journal from the well known group Phcog.Net. Our beloved Executive Editor Dr. Mueen Ahmed has entrusted me the responsibility to serve as Editor-in-Chief of this journal. I hope the journal had a good start with the outgoing Editor-in-Chief and I feel it is our responsibility to continue the endeavor. Dr. Mueen Ahmed and the publishers has set a nice presentation of the journal both online and print editions. It is an Open Access Publication with URL www.phcogj.com. It is published when the articles are ready for publication.

Phcog.Net is bringing out four publications presently viz., Pharmacognosy Magazine, Pharmacognosy Reviews, Pharmacognosy Research and Pharmacognosy Journal. The present publication, Pharmacognosy Journal is exclusively deals with pharmacognostic studies of plants while the remaining publications are dedicated to various aspects and studies on natural products with biological activities. The inception of Pharmacognosy Journal has arised from the scarcity of journals that stress on Pharmacognosy works by various researchers. The Pharmacognosy Journal deals with all aspects of pharmacognostic studies of medicinal plants viz., systematic studies on various medicinally important parts of the plants. The studies will help in standardardization and detection of adulteration of crude drugs and thus pave the way to prepare monographs of medicinal plants and their parts.

A Rapid and swift publication from Pharmacognosy Network Worldwide. Phcog J is not an annual, a quarterly, a monthly, or a weekly. It is being published when the article is ready. Highly indexed & abstracted, Swift editorial decisions, Quality papers, exclusively for Pharmacognostic studies. The journal is indexed and abstracted in DOAJ, Chemical Abstracts, Excerpta Medica / EMBASE, Google Scholar, Index Copernicus, Ulrich's International Periodical Directory, ProQuest, Index Pharmacus, PhcogBase, EBSCOHost's Publishing database, Academic Search Complete, Open J gate.

The present issue deals with a pharmacognostic review on Oroxylum indicum which has potential medicinal importance. The issue also covered pharmacognostical and preliminary phytochemical studies on various parts of few important medicinal plants like Solanum lycocarpum, Bryophyllum pinnatum, Ailanthus excelsa, Clitoria ternatea and Artemisia pallens. A comparison of beneficial hemorheology of decoctions in blood stasis, purification and characterization of antiproliferative and mitogenic plant lectin from Arisaema speciosum, and in vitro antioxidant activity evaluation of some Assamese plants were also included in this issue.

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Pharmacognostical Evaluation of Fruits of *Solanum lycocarpum* A. St.-Hill. (Solanaceae)

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ABSTRACT:

This study describes the chemical and physical-chemical profile of plant drug and ethanolic extract obtained from fruits of *Solanum lycocarpum* A. St.-Hill. (Solanaceae). The physical and chemical analysis involved the granulometry determination, non-compacted apparent density, loss on drying in oven and in infrared scale, pH, ash values and extractive values. The results determined the physical-chemical characteristics of the drug plant. It was also carried out the microbiological control of the plant drug. The preliminary phytochemical screening featured the presence of tannins, flavonoids and saponins in the plant drug and alkaloids and steroids in the ethanolic exctract. The solamargine and solasonine glycoalkaloids were identified through TLC and GC/MS. The levels of total phenols and tannins were quantified in the extract (8.90% and 6,85% respectively). Such studies contribute to the chemical identification and quality control of *S. lycocarpum* fruits.

Keywords: *Solanum lycocarpum,* phytochemical screening, pharmacognostical evaluation, total phenolic compounds, solasonine, solamargine.

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INTRODUCTION

With many representatives in the Brazilian Cerrado, the Solanaceae family is included in the subclass Asteridae, order Solanales, and is one of the largest and most complex among the Angiosperms, with 92 genera and about 2,300 species. It has South America as one of the main centers of diversity and endemism[1]. The genus *Solanum* L. is the largest and most complex of the Solanaceae family[2] and it is known to possess a variety of biological activities, including antifungal, molluscicide, teratogenic and embryotoxic activity[3]. The saponins of basic character, alkaloids belonging to the group of steroidal glycosides, are characteristic of the genus *Solanum*[4]. Besides the alkaloids, flavonoids are also a group of substances in common species of this genus[2]. Some studies with genus *Solanum* species present information about the antioxidant activity, as in *Solanum melongena, Solanum lyratum, Solanum tuberosum*[5,6].

The specie *Solanum lycocarpum* A. St.-Hill., popularly known as lobeira or fruta do lobo, is typical of the Brazilian Cerrado and its fruits are commonly used to reduce glucose and cholesterol levels[7,8,9]. A medicinal preparation obtained from *S. lycocarpum* fruits has been commercialized and it consists in the sediment of green fruit kept in an aqueous solution until it forms a fine grain, known as polvilho de lobeira [7, 10]. Dall'Agnol & Von Poser (2000)[7], through a study of this medicinal preparation related the possible hypoglycemic activity to the polysaccharides content, justifying that they decrease the gastric emptying speed and act on the endocrine system by altering the release of gastrointestinal hormones, thus reducing the glucose blood level, but Oliveira et al. (2003)[8] in the investigation of hypoglycemic effect of this preparation, have not shown evidence of this effect in diabetic and in non-diabetic rats.

In chemical terms, *S. lycocarpum* can be characterized by the presence of two steroidal alkaloids: solasonine and

solamargine[11]. Especially in food science, toxicology and pharmacology related areas, the steroidal glycoalkaloids and their aglycones have received great attention. The solasodine, as well as its triglicosides solasonine and solamargine, is a potential raw material as a prototype for the production of steroid drugs[12]. Researchers also have reported some properties of glycoalkaloids as precursors for the pharmaceuticals synthesis[13], anticancer[14] and antifungal properties[15]. The interest in extracting these compounds and use them for medicinal purposes is shown in the form of several patents or patent applications[16, 17].

Considering its potential as a medicinal plant and its possible economic use, information concerning the *S. lycocarpum* is relevant, since the literature data is still incomplete.

MATERIALS AND METHODS

Collection and identification of plant material

Green fruits of *S. lycocarpum* were collected in Franca – SP - Brazil, in march 2008 (S20° 34′31, 06″, W47° 24′28, 77″). An exsiccate was identified by Prof. Dr. Marcos Antônio Assis and deposited in the Herbarium of the Instituto de Biociências da UNESP de Rio Claro - SP, under number 50125 HRCB. The fresh fruits was used for the physical and chemical analysis. Whereas collected plant materials were drying in the oven with air circulating and powdered a knives mill. This powder was used for the physic-chemical determinations (granulometry, non-compacted apparent density, loss on drying in oven and in infrared scale, pH, ash values and extractive values), microbiological control and preliminary phytochemical investigation was studied as per standard methods.

Extract preparation

The hydroalcoholic extract was prepared by turbo-extraction with absolute ethanol (v/v) using the ratio drug/liquid extractor 10% (w/v). Then the extract was filtered and filtrate was evaporated to dryness, and the extract obtained was stored in sealed amber vial, kept under refrigeration.

Preliminary phytochemical screening

Preliminary phytochemical screening for the detection of secondary metabolites present in the ethanolic extract of *S. lycocarpum* fruits was carried out by using procedures described by Matos (1997)[18] and Baheti et al. (2010)[19], in which chemical tests that result in the development of color and/or precipitate characteristic for each class of substance are described.

Estimation of total phenolic and tannin content by spectrophotometer

The total phenols content was determined by using procedures described Badmanaban et al. (2010)[20], which consisted of a comparison between the UV spectrum absorbance of a plant extract against a curve of tannic acid analysis. For the construction of the curve, solutions of tannic acid at different concentrations (0.1 mg/mL, 0.5 mg/ml, 1mg/ml, 2.5 mg/mL and 3.75 mg/mL) were prepared and solutions of the Folin-Ciocalteau reagent at 10% and Na_2CO_3 at 7.5% were added. After 30 minutes, the analytical curve was generated from the average of triplicate readings in the absorbance patterns of tannic acid at 760 nm. The extract sample to be analyzed (400 mg/mL) was prepared in triplicate by the method described for standard solutions. The readings of the solutions absorbance were determined also at 760 nm, to calculate the total phenols concentration it was used the average from the analytical curve, and results were expressed as percentage of total phenols in the extract.

To determine the tannins content, it was added 1 g of casein powder to a flask containing 6 mL of a solution of the extract (400 mg/mL). After this procedure, the flask was kept under constant agitation at a temperature of 25° C for 3 hours. Then the content was filtered and the volume completed to 25 mL. Three aliquots were taken from this sample, which were prepared and analyzed as described above for total phenols, and the result expressed as percentage of tannins in the extract.

Thin layer chromatography analyses

Thin layer chromatography studies of the ethanolic extract was carried out using Silica gel G as adsorbent, butanol: glacial acetic acid: water (6:3:1) as mobile phase and 10% sulfuric acid as chemical revealing for the detection of solasonine and solamargine glycoalkaloids. In these tests it was used as standard samples authentic of the solasonine and solamargine glycoalkaloids and the R_f values were determined.

Identification of solasonina and solamargina by mass spectrometry

Authentic standards samples of solasonine (1) and solamargine (2) were dissolved in ethanol: water 4:1 (v/v) and analyzed in UltrOTof mass spectrometer (Bruker, Billerica, MA) equipped with electrospray ionization source (ESI-MS) operating in positive mode of analysis. The samples were introduced into the ionization source by direct injection by Rheodyne 7125 injection pump.
The *S. lycocarpum* ethanolic extract was dissolved in

ethanol: water 4:1 (v/v) and analyzed by ESI-MS and ESI-EM/EM under the same conditions as authentic standards of 1 and 2.

RESULTS

The fruits were kept in air-circulating oven for 96 hours, when they reached constant weight. The result of the loss on drying of fruits was 77.25% (Table 1). After drying and grinding of plant material the average particles size was determined. The statistical analysis results are shown in Table 1 and Figure 1, and identified a mean particle diameter of 0,814 mm, featuring a thick dust under United States Pharmacopeia 31 (2008)[21], which says that this average particle size is suitable for turbo-extraction. The physico-chemical characters of powdered drug of fruits of *S. lycocarpum* such as loss on drying in the oven, loss on drying in infrared (IR) scale, non-compacted bulk density, pH, ash value, acid insoluble ash and water soluble extractive are presented in Table 1.

Total and pathogenic counts of microorganisms present in the drug plant were done[21,22]. The results (Table 2) have shown that there was not microbial growth of *Salmonella, E. coli, S. aureus* and *P. aeruginosa*, considered highly pathogenic microorganisms. The growth of yeasts and bacteria was within the limits set for plant extracts

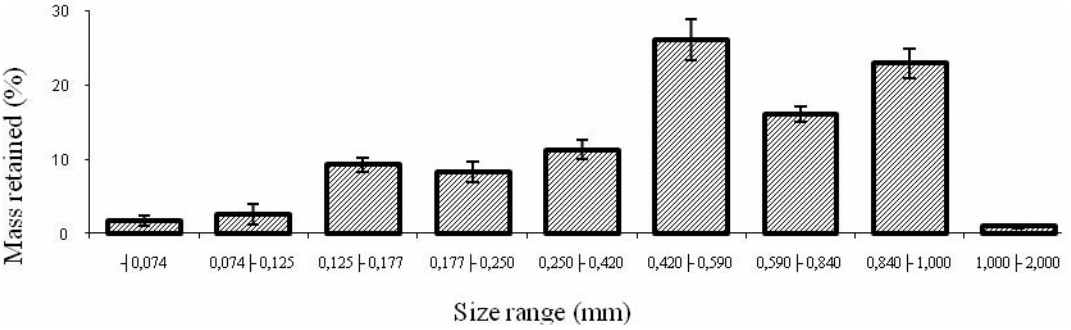

Figure 1 Particle size distribution of powder obtained from *Solanum lycocarpum* fruits

Table 1. Physical-chemical properties of the powder of the *Solanum lycocarpum* fruits

Quantitative parameter	Values obtained (mean ± SD)
Average particles diameter	0, 814 mm[a] ± 0,082
Non-compacted bulk density	0,637 g/cm^3[b] ± 0,025
Loss on drying	77,25 %[a] ± 0,0067
Loss on drying in oven	13,07 %[b] ± 0,061
Loss on drying in IR scale	10,13 %[b] ± 0,473
Total ash	3,18 %[b] ± 0,102
Acid insoluble ash	0,10 %[b] ± 0,033
Water soluble extractive	29,83 %[b] ± 2,081
pH of 1 % solution	4,74[b] ± 0,011

[a] Mean ± SD obtained from 5 determinations,
[b] Mean ± SD obtained from 3 determinations

Table 2. Microbiological analysis of the powder of *Solanum lycocarpum* fruits

Microorganisms	Recommendation[21–23]	Results
Aerobic bacteria	≤ 10^5 CFU/g	75 CFU/g
Fungi and yeasts	≤ 10^3 CFU/g	< 10 CFU/g
Escherichia coli	Absent	Absent
Salmonella sp	Absent	Absent
Staphylococcus aureus	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent

(10^3 CFU/g and 10^5 CFU/g, respectively)[21–23]. The total number of yeast growth was less than 10 CFU/g and the growth of bacteria was 75 CFU/g of plant material.

Data from the preliminary phytochemical analysis carried out with the powder of *S. lycocarpum* fruits showed the presence of tannins, flavonoids and saponins. Phytochemical analysis of the ethanolic extract verified the presence of phenolic compounds, especially tannins and flavonoids, alkaloids, steroids and triterpenes (Table 3).

The glycoalkaloids solamargine and solasonine (Figure 2) were identified through TLC. The results showed dark spots with R_f similar to markers solasonine (R_f = 0.32) and solamargine (R_f = 0.43). The mass spectra of compounds 1 and 2, obtained at high resolution, showed the major ion m/z 868.5052 ([1 + H] +, calculated for C45H74NO15 +: 868.5053) in / z 884.5010 ([2 + H] +, calculated for C45H74O16 +: 884.5002). These ions were selected in the first stage of analysis and activated by collision-induced dissociation (ESI-EM/EM) using nitrogen as collision gas (collision energy: 65 eV). The major ions produced by both compounds showed m/z 722 and 222. The major peaks in the spectrum of high mass resolution of the extract (m/z 868.579 and 884.5043) were selected in the first stage of the analysis by mass spectrometry and then analyzed by MS / MS. The majority of ions produced m/z 868.5079 and 884.5043, which are identical to those produced by solamargine and solasonine, thus confirming the presence of these compounds in the extract.

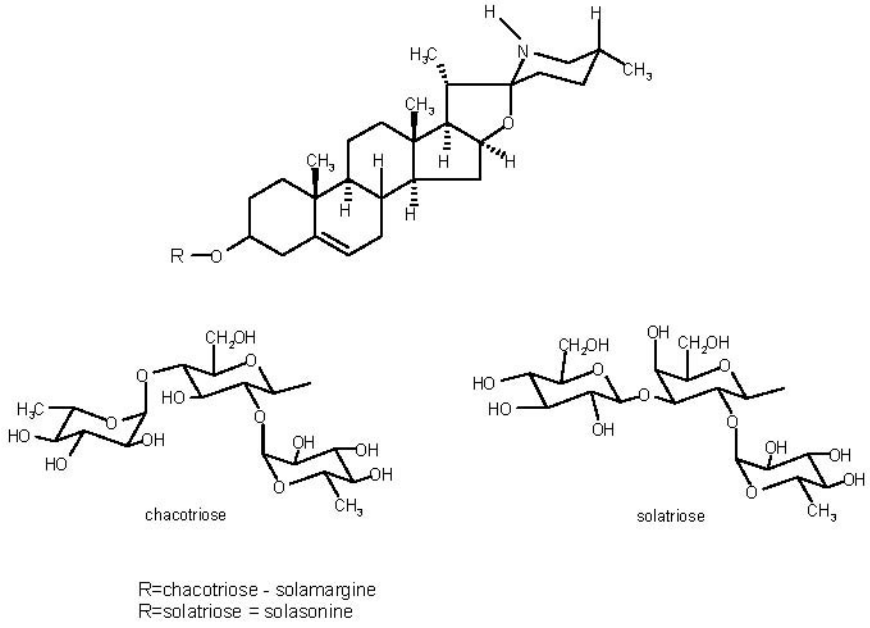

Figure 2 Chemical structure of solasonine and solamargine glycoalkaloids

Table 3. Qualitative analysis of phytochemicals in *Solanum lycocarpum* fruits.

Qualitative parameter	Powdered fruits	Ethanolic extract
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Anthraquinones	–	–
Cardiotonics glycosides	–	–
Alkaloids	–	+
Steroids and triterpenes	+	+
Saponins	–	+
Resins	–	–

+ Present; – Absent

The detection of phenolic compounds in the extract was carried out. The analitical curve for tannic acid showed a linear correlation coefficient (r^2) of 0.9892, and then it used as reference in determining the total phenol and then in tannin. From the straight line equation (y = 0.044 × + 0.0511) and the mean absorbance obtained for the untreated extract with casein (A = 0.055), a concentration of 8.90% of total phenols in the extract was obtained. From the mean absorbance after precipitation of tannins with casein (A = 0.052) it was obtained a concentration of tannin of 6.85% in the extract, showing that most of the phenolic compounds in the extract are tannins.

DISCUSSION

It is evident that more detailed studies of plant species popularly used are needed in order to ensure the quality, an important concept for providing the wished security and reliability for its use. Following the requirements of ANVISA resolution[24] and due to the lack of quality control studies of this kind, the chemical and physical-chemical profiles of the plant drug obtained from *S. lycocarpum* fruits were drown in compliance with the legislative requirements, so that could contribute with the quality parameters for this plant drug[23–25].

The determination of loss on drying of plant material is important because it prevents the material from remaining wet due to inefficient drying or from degradation of compounds by excessive drying[4]. After drying and grinding of plant material, the average particles size was determined. According to the literature[26], the pulverized drug particle size is a direct influence on the efficiency of the extraction process, therefore, for those involved in filtration, very fine powders (below 0.125 mm) may obstruct of the filter.

The determination of loss on drying in oven of the *S. lycocarpum* fruit powder presented 13.07% and the loss on drying in infrared scale presented 10.13% (Table 1), when the stabilization process occurred. This analysis is important since it provides important information about the storage of the plant drug residual. The water found in dried plant drug is directly related to its proper storage, which may result in loss of material by microbial contamination or degradation by enzymatic action on chemical constituents[4]. That values of loss on drying, besides being important information from the technological point of view also serve as a parameter of quality control of the powder of the fruits of *S. lycocarpum*. The maximum recommended moisture to plant drugs is 14%[4] and therefore the analyzed material showed satisfactory result. The pH of the drug plant aqueous solution was 4.74, using water with a pH 6.66, suggesting the presence of acidic substances.

Concerning the determination of total ash content, the content found was 3.18% and the acid insoluble ash was 0.10%. These results are within the parameters set by the reference compendia. In the case of plant drug, the main objective of this test is the verification of non-volatile inorganic impurities that may be present as contaminants[27].

In order to evaluate the amount of extractable substances called extractives content, it was used the aqueous decoction[23]. The yield found was 29.83%. However, it is important to consider the substance one wishes to extract. For example, to the glycoalkaloids solasonine and solamargine showed the use of hydroalcoholic solvent to be the best[28]. In extractions using only water as extractor liquid, the stability of the extract becomes compromised as the risk of microbiological contamination increases significantly[29]. The microbial control is a factor of same importance to ensure the quality of vegetable drugs. It is common to find a large number of fungi and bacteria, either belonging to the natural macrobiotic of the plant or being introduced during the collection and handling. However, this contamination can compromise the quality and integrity of the plant material itself, as well as products arising from its use[29,30,31]. So, total and pathogenic counts of microorganisms present in the drug plant were done.

The detection of phenolic compounds and tannin in the preliminary phytochemical investigation of the drug plant and ethanolic extract led to their quantification. According to the literature, the fruits of *S. lycocarpum* are characterized by the presence of steroidal glycoalkaloids. This information justifies the analysis by TLC and MS for the identification of these substances in the extract. The structural similarity of solasonine and solamargine with many steroid hormones stimulated the creation of many works describing investigations about possible toxic effects related to the reproductive system[10,32].

CONCLUSION

After present investigation it can be concluded that the standardization and preliminary phytochemical investigation study of *S. lycocarpum* fruits yielded a set of 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. The results are within the pre-established in the literature for drugs and plant extracts. This study is substantial in light of the prominent therapeutic potential of this specie, and it requires further studies to evaluate therapeutic efficacy and toxicity of this medicinal plant.

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Microscopical and Preliminary Phytochemical Studies on Aerial Part (Leaves and Stem) of *Bryophyllum Pinnotum Kurz*

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ABSTRACT

The plant *Bryophyllum pinnatum Kurz* (crassulaceae), has potent medicinal values. The plant have been found to possess pharmacological activities as immunomodulator, CNS depressant, analgesic, antimicrobial, anti-inflammatory, anti-allergic, anti-anaphylactic, antileishmanial, antiulcerous, antifungal, antihistamine, antiviral, febrifuge, gastroprotective, insecticidal, muscle relaxant, sedative. The present study deals with pharmacognostic examination of morphological and microscopical characters of *Bryophyllum pinnatum* including leaf constant, ash values, extractive values and preliminary phytochemical screening of the extracts revealed that the plant contains alkaloids, glycoside, carbohydrates, tannins, phenolic compound, steroids, gums, mucilage and lignins. The presence of anisocytic type stomata are the characteristic feature observed in microscopy of leaf. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of monograph of the plant.

Keywords: *Bryophyllum pinnatum Kurz*, pharmacognostic study, phytochemical screening.

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INTRODUCTION

India is one of the richest floristic regions of the world and has been a source of plants and its products, since antiquity, man uses them in different way according to his needs, particularly as food and medicine. Among the entire flora, 35000 to 70000 species have been used for medicinal purpose[1]. The name *Bryophyllum* comes from 'I Sprout' and 'leaf', the plant, classified as a weed is notorious for its growth potential. Shortly after a leaf falls to the grounds, a whole garland of new little plants develops from the notches along the leaf margin. *Bryophyllum Pinnatum Kurz* (syn. *B. Calycium and Kalanchoe pinnata*) commonly known as parnbija, Zakhm-hyat (Hindi), life plant, love plant, air plant (Mexican), Good luck or resurrection plant[2].

It is a glabrous, ornamental, crassulenscent herb, cultivated in houses and gardens. It is of about 1–1.5 m in height, with opposite, decussate, succulent, 10–20 cm long glabrous leaves (with 3–5 deeply crenulated, fleshy leaflet) with obtusely four angled stems. The lower leaves are usually simple, whereas upper ones are usually 3–7 folioate, long-petioled, petioles united by a ridge round the stem, crenatures at the extremities of the lateral nerves furnished with rooting vegetative buds. The flowers are 5cm long, reddish purple, pendent, in large spreading panicles; fruits are membraneous follicles enclosed in the persistent papery calyx and corolla, seeds smooth, ellipsoid. They widely grow in hot and humid areas, around the dwelling places, along road sides and in abandoned farm and fields. They are widely used in folk medicine of its indigenous region (Madagascar, Tropical Africa, India, China, Australia, Hawai and Tropical America)[3,4].

Bryophyllum pinnatum Kurz leaves have great medicinal values in the indigenous system of medicine. It is used for medicinal purpose both, internally as well as externally. The leaves are frequently used for an array of human disorders including hypertension, diabetes mellitus, bruises, wounds, boils, burns, sloughing ulcers, opthalmia, corn, diarrhea, dysentery, vomiting, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches, antifungal,

antibacterial, body pains and acute inflammmation. The leaves are also used for lymphadenitis and ear disease [3]. The main constituents of this the plant are alkaloids, flavonoids, glycosides, steroids, bufadienolide and organic acid are reported[2,5,6]. The pharmacognostical studies of leaf and stem of this plant have not been reported. Therefore, the present investigation was planned to study the pharmacognostical and phytochemical aspects of the plant.

MATERIAL AND METHODS

Collection and Identification

The plant of *Bryophyllum pinnatum Kurz* were collected from Tau Devilal Herbal garden, Churpur and positively identified. The specimen was submitted to the A. R college of pharmacy, Vallabh Vidyanagar, Anand. The collected plant material was made thoroughly free from any foreign organic matter. The aerial parts of the plant were separated, cutted into small pieces, shade dried and powdered with mixer and sieved. Pharmacognostic studies were conducted with fresh leaves and stem.

Pharmacognostical studies

The morphological studies such as type, size, shape, apex, margin, venation, base, petiole, surface, phyllotaxy, color, odour and taste of *Bryophyllum pinnatum Kurz* leaf and stem were carried out[13]. The leaf and stem of *Bryophyllum pinnatum Kurz* were examined microscopically. The sections were stained with Toluidine blue, Saffranin and Fast green, Photograph of different magnifications were taken[7–9].

Physicochemical analysis

Ash values, loss on drying, ethanol soluble and water soluble extractive values were determine as per the procedure given in Indian Pharmacopoeia [10].

Phytochemical analysis

The aerial part of the plant were collected, washed with water, dried in shade and stored properly. The dried material was powdered with mechanical grinder and passed through the sieve no. 60. Coarse powder was used for pharmacognostic work. The dried powder material was extracted by successive extractoion method with Petroleum ether (60–80°C), benzene, chloroform, acetone, ethanol (95%) and water using a Soxhlet apparatus. The extracts were filtered while hot and concentrated under reduced pressure. The extractive values were calculated. The extracts were then subjected to qualitative chemical test for the identification of various active constituents [8–12]. The dried powdered material was subjected to identification tests for detection of various phytoconstituents.

RESULTS AND DISCUSSION

The distinctive macroscopic features of the leaves (Fig. 1, 2) reflecting that the leaves are opposite, decussate, succulent, simple or compound, 8–12 cm and 5–8 cm in size, apex is obtuse, ovate or elliptic in shape, crenate or serrate margin, asymmetric base, reticulate venation, petiole is long, surface is smooth or glabrous, upper surface is dark green in color, lower surface lighter in color, with a characteristic odor and acrid taste.

Fig. 5 demonstrate the microscopic section of leaf. It is broadly shallow on the adaxial side and convex on the abaxial side. It has a thin adaxial epidermal layer of small, less prominent cells. The abaxial epidermis is also very thin and less distinct. The ground tissue of the midrib is parenchymatous and homogenous. The cells are circular or angular and compact. The vascular strand is single, small, collateral and hemispherical in shape. It consists of a thick horizontal band of xylem and fairly wide band of phloem. Xylem elements are narrow, angular, thin walled

Figure 1. Bryophyllum Pinnatum

Figure 2. Leaf

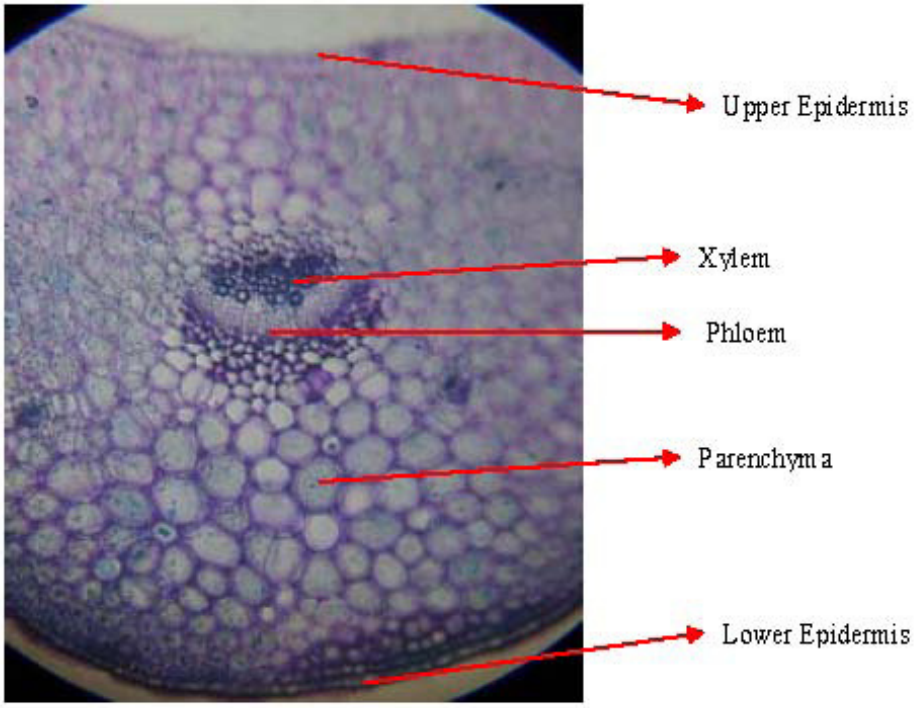

Figure 5. T.S. of Midrib Portion of Leaf

and somewhat diffuse. The lamina is uniformly flat with even surface. The mesophyll tissue is not differentiated into palisade and spongy parenchyma. The epidermal layer appears as flat polygonal cells are variable in shape and size. Stomata are abundant, these are 18–20 stomata per mm^2. The stomata are anisocytic type (Fig. 7, 8).

The cross sectioned outline of petiole shows the shape of circular with shallow adaxial concavity. The epidermal layer consists of layer of rectangular cells; inner epidermis is made up of 2 to 3 layer of collenchyma cells; remaining ground tissue is parenchymatous with large, circular thin walled cells. Vascular system consists of collateral and hemispherical in shape. Xylem elements are narrow, angular, thin walled and diffused. Phloem appears as thick hemispherical arc (Fig. 6).

The distinctive macroscopic features of the stems are highlighted in Fig. 1, 3 reflecting that the stems are of kind herbaceous, direction is upright and spreading,

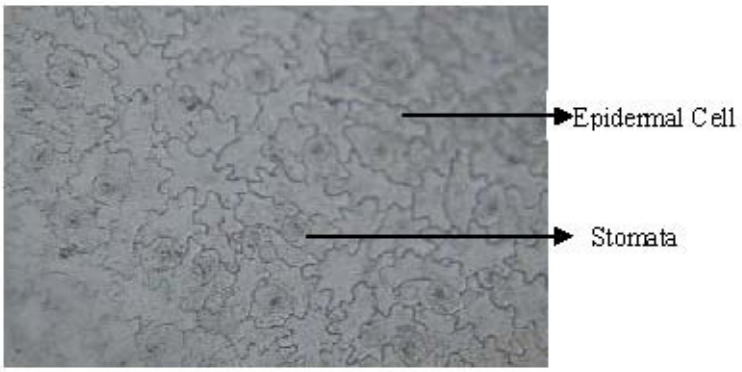

Figure 7. Lower Epidermal Peel

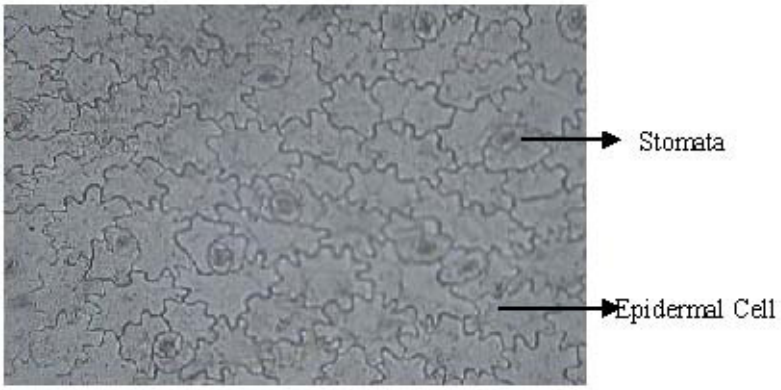

Figure 8. Upper Epidermal Peel

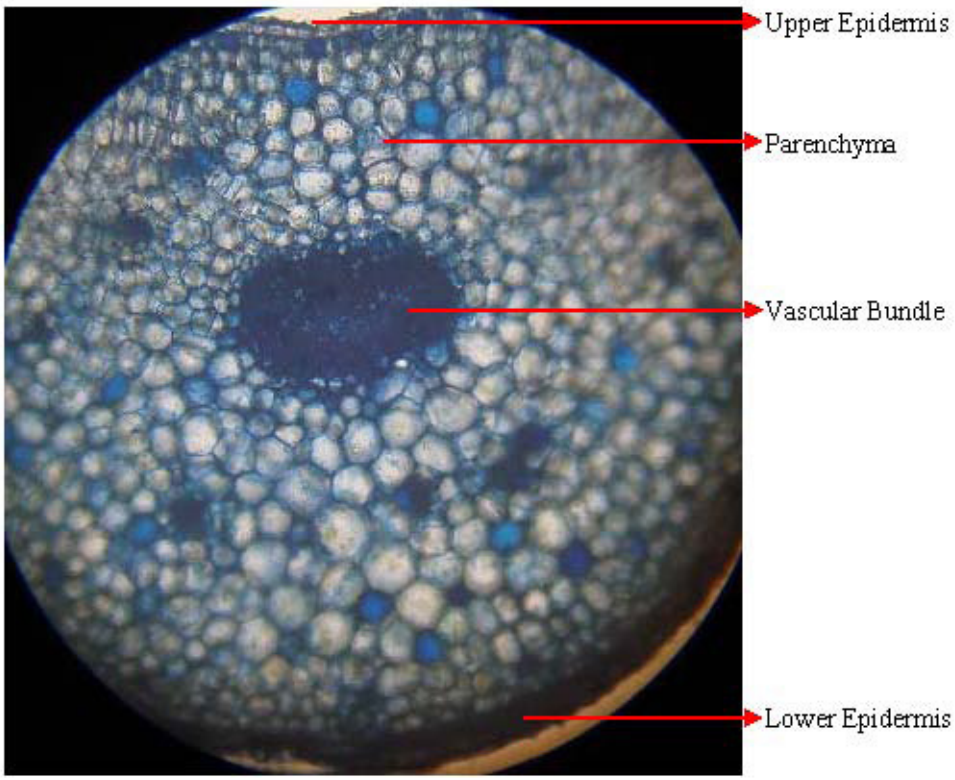

Figure 6. T.S. of Petiole

Figure 3. Stem

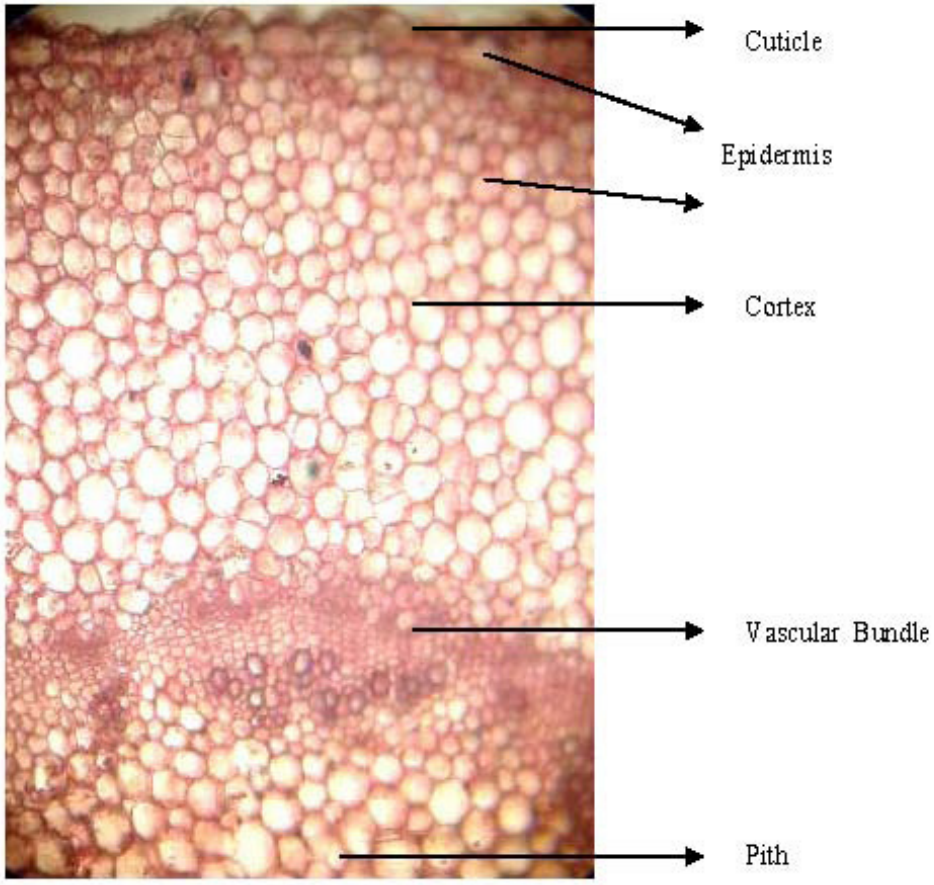

Figure 4. T.S. of Stem

cylindrical or angular in shape, greenish smooth surface when fresh, phyllotaxis is opposite and decussate. The dry stem is pale brownish, shrunken with longitudinal ridges on the surface, hollow in the centre with characteristic odour, and acrid taste.

Figure 4 demonstrate the microscopically diagnostic features of the stem include single layered epidermis consisting of thin walled rectangular cells and covered by moderately thick and striated cuticle. The vascular bundles are 10–20 of open collateral type forming a discontinous ring. Medullary rays showing funnel shaped dilatations. Pith i.e central portion is occupied by large thin walled parenchymatous cells with intercellular spaces.

Physicochemical parameters of aerial part of the plant are like total ash (25.0%), acid insoluble ash (3.00 %),

water soluble ash (23.5 %) and sulphated ash (14.0 %). Loss on drying (4.8%) and % retain was found to be 95.20% w/w.

Alcohol soluble extractive and aqueous extractive values were 8.33 & 38.33% w/w, respectively. The extractive values of aerial parts of the plant in various solvents by successive extraction method using Soxhlet apparatus are 5.04, 1.09, 1.26, 0.43, 3.48, 22.70% w/w in Petroleum ether (60–80°), benzene, chloroform, acetone, ethanol, water respectively and total extractive value was found to be approximately 34.00% w/w.

Phytochemical screening of the extracts revealed that the petroleum ether and chloroform extracts of the powdered leaves and stems of *Bryophyllum pinnatum* showed the presence of steroids and terpenoids. The ethyl acetate extract responded positively to the tests for steroids, terpenoids, phenolics and tannins. Ethanolic extract of the leaves produced positive tests for flavonoids, steroids, terpenoids, phenolics, tannins, alkaloids and glycosides. Aqueous extract showed the presence of carbohydrates, proteins, flavonoids, phenolics, tannins and glycosides. These secondary plant metabolites are known to possess various pharmacological effects and might be responsible for the various actions exerted by *Bryophyllum pinnatum*.

Bryophyllum pinnatum is used in the treatment of various disease conditions. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The results of the present investigations could serve as a basis for proper identification, collection and investigation of the plant. Phytochemical evaluation revealed the presence of various secondary plant metabolites which have been claimed to be responsible for various pharmacological activities.

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Pharmacognostical and Preliminary Phytochemical Evaluation of *Clitoria ternatea* leaves

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ABSTRACT

Clitoria ternatea L. (Family: Fabaceae) a perennial twing herb, steams are terete, more or less pubescent. Root is bitter in taste used to cure sever bronchitis, asthma. In Kokan root juice s given in cold milk to remove the phlegm in chronic bronchitis. *C. ternatea* have reported number of pharmacological activities such as nootropic, anxiolytic, anticonvulsant, sedative, antipyretic, anti-inflammatory and analgesic. In present study pharmacognostic investigation of leaves was carried out by using morphological, microscopic and physicochemical parameters. It was found that leaves are odd pinnately compound, with obovate in shape, entire margin, Emarginate tip and symmetrical base, green in color, bitter in taste, with characteristic odor. Microscopic study reveals that presence of Upper and lower epidermis consists of single layer of cells covered with thick cuticle. Covering trichomes are present on both the surfaces. The upper and lower epidermis is followed collenchymatous cells. The upper epidermis consists of polygonal tabular cells, followed by layer of palisade cells, lignified xylem and paracytic stomata. The powder is green in colour and contains paracytic stomata, covering trichomes, fibres, wavy epidermal cells, covering trichomes and presewnce of starch grain in epidermal cell Physicochemical parameter observed that 13.2 ± 3.49 total ash, 4.8 ± 2.16 acid insoluble ash and 5.3 ± 0.08 water soluble ash. The moisture content found to be 12.5±2.57. Water soluble and alcohol soluble extractive value found to be 25.2 and 18.4 respectively.

Keywords: *Clitoria ternatea,* moisture content, ash value, extractive value.
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INTRODUCTION

Clitoria ternatea L. (Family: Fabaceae) a perennial twing herb, steams are terete, more or less pubescent. There are two varieties of *Clitoria ternatea* white-flower and blue flower varieties. Root bark contain starch, tannin's & resins. Seed contain a fixed oil, a bitter acid resin, tannic acid, glucose. The roots have a sharp bitter taste and cooling, laxative, diuretic, anthelmintic, anti-inflammatory properties; they are useful in severe bronchitis, asthma and hectic fever Stem and flower are recommended for treatment of snake bite. The seed are purgative, Cathartic and laxative in combination with ginger powder.[1,2] The fatty acid content of *Clitoria ternatea* seeds includes palmitic, stearic, oleic, linoleic, and linolenic acids.[3–5] The seeds also contain a water-soluble mucilage, delphinidin 3, 3′, 5′-triglucoside useful as a food dye[6]; beta-sitosterol.[7] *C. ternatea* have number of pharmacological activities such as possessing nootropic, anxiolytic, antidepressant, anticonvulsant,[8] sedative,[9] antipyretic, anti-inflammatory and analgesic activities.[10] Enhance memory, and increase acetylcholine content and acetylcholinesterase activity in rats.[11, 13] Ethanol and benzene extract of *Clitoria ternatea* seeds at doses 75 mg/kg and 100 mg/kg inhibit clonidine induced catalepsy, milk induced eosinophili and leucocytosis in mice.[14,15]

Objective of present study was to perform pharmacognostic investigation and preliminary phytochemical screening on *Clitoria ternatea* leaves.

MATERIAL AND METHODS

Plant material

Leaves of *Clitoria ternatea* were collected from Baramati localities, Pune district (Maharastra), and dried in the

shade at room temperature. Dried leaves were coarsely powdered in grinder and powder material was kept in air tight container for further study. The plant was identified and authenticated by Prof. R. B. Deshmukh Head Dept. of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Baramati.

Morphological and microscopical studies

The morphology of the leaves was studied according to standard methods.[16–18] Hand section of the leaf was taken, stained and mounted [18] and representative photo of section were taken with the help of Digital microscope. The powder characteristics were studied according to standard methods. [18]

Determination of physicochemical parameters

Physico-chemical parameters i.e. percentage of moisture content, percentage of ash values and extractive values were performed according to the official methods. [18,19]

Preliminary phytochemical screening

The shade dried and coarsely powdered leaves were extracted with benzene, ethyl acetate, methanol and distilled water using decoction method. All the extracts were screened for the presence of various groups of phytoconstituents using different chemical tests.[18,20]

RESULTS

Morphological Evaluation

Leaves are green, bitter and characteristic odour (figure 1). These are obviate shape, pinnatified, a Emarginate apex, symmetrical base and 6–11cm in length, 5–7cm in width.

Microscopical Evaluation

Transverse section of *Clitoria ternatea* Linn leaf shows presence of covering trichomes on both surfaces (Figure 2). Upper and lower epidermis consists of single layer of cells covered with thick cuticle. The upper and lower epidermis is followed collenchymatous cells. The upper epidermis consists of polygonal tabular cells, followed by layer of palisade cells. The spongy mesophyll is represented by 3–5 layers cells, which are loosely arranged (figure 3). Calcium oxalate crystals are present in parenchyma cell. Vascular bundle is covered with a single layer of border parenchyma (bundle sheath) contain lignified xylem

Figure 1 Plant and leaves of *Clitoria ternatea*

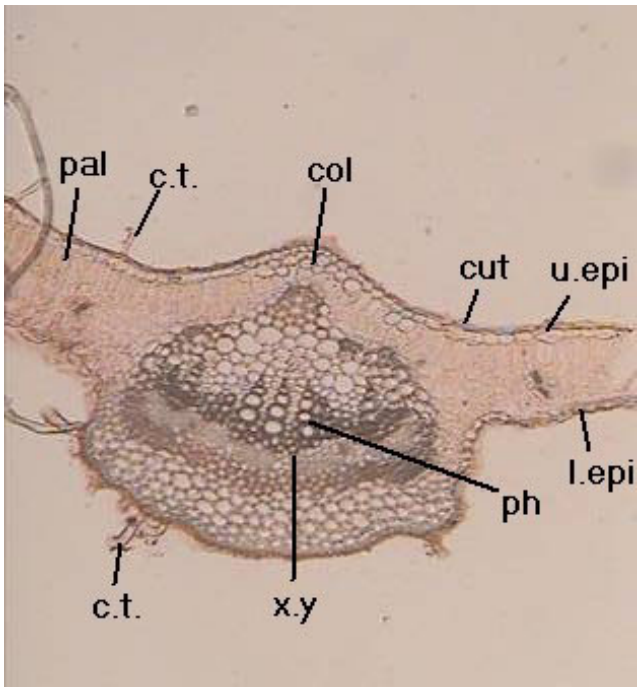

Figure 2 Transverse section of *Clitoria ternatea* leaves

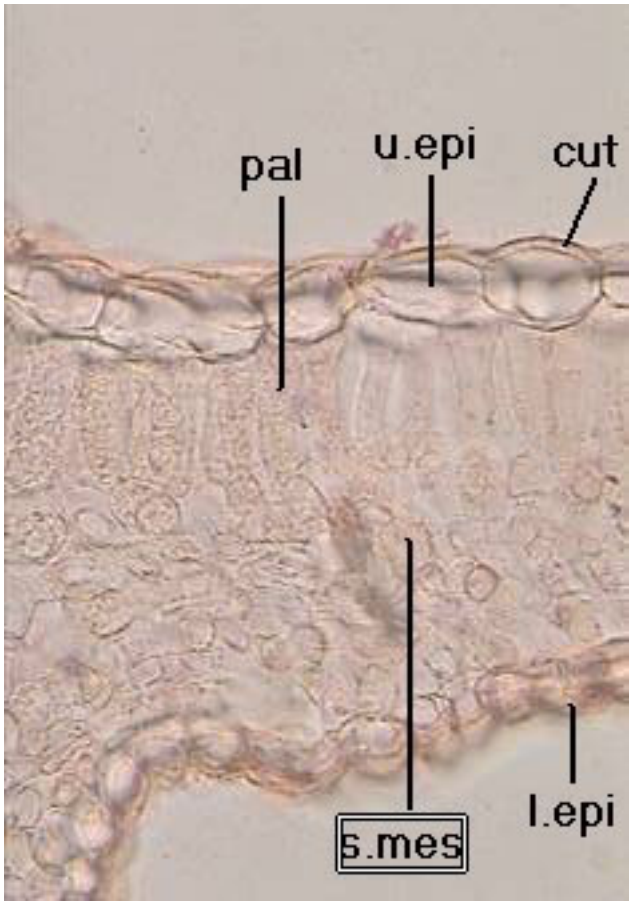

Figure 3. Transverse section of *Clitoria ternatea* leaves though lamina
pal.-Palisade, c.t.- Covering trichome, cut- cuticle,u,epi- upper epidermis, l.epi- lower epidermis, s.mes- spongy mesophyll

vessels (figure 4). Surface preparation showed presence of paracytic stomata, with wavy epidermal walls and presence of cicatrix (figure 5).

Powder characters

The powder is green in colour and contains paracytic stomata, covering trichomes, fibres, wavy epidermal cells, covering trichomes and presewnce of starch grain in epidermal cell (Figure 6).

Physicochemical parameters

Physicochemical parameter observed that 13.2 ± 3.49 total ash, 4.8 ± 2.16 acid insoluble ash and 5.3 ± 0.08 water soluble ash. The moisture content found to be 12.5±2.57. Water soluble and alcohol soluble extractive value found to be 25.2 and 18.4 respectively (figure 7).

Preliminary phytochemical screening

Preliminary phytochemical screening of different extracts revealed the presence of steroids, tannins, carbohydrates, flavonoids, proteins, amino acids, glycosides, saponin, tannins and alkaloids (Table 1).

DISCUSSION

Morphological and microscopic characters are used for identification of crude drug. Leaves of *Clitoria ternatea* are green, bitter and characteristic odour. Microscopical studies showed presence of covering trichomes on both surfaces, epidermal cells are polygonal tabular. Palisade are present below the upper epidermis, followed by spongy mesophylls. Covering trichomes are unicellular,

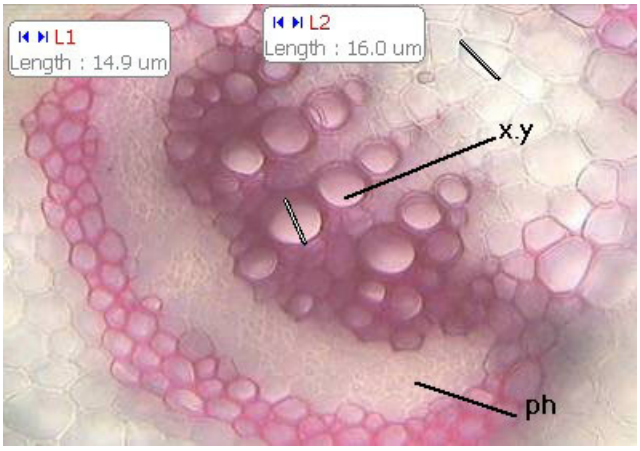

Figure 4. Transverse section of *Clitoria ternatea* leaves showing vascular bundle
x.y.- xylem, ph- phloem cr.- crystal, col- collenchyma, l.epi- lower epidermis

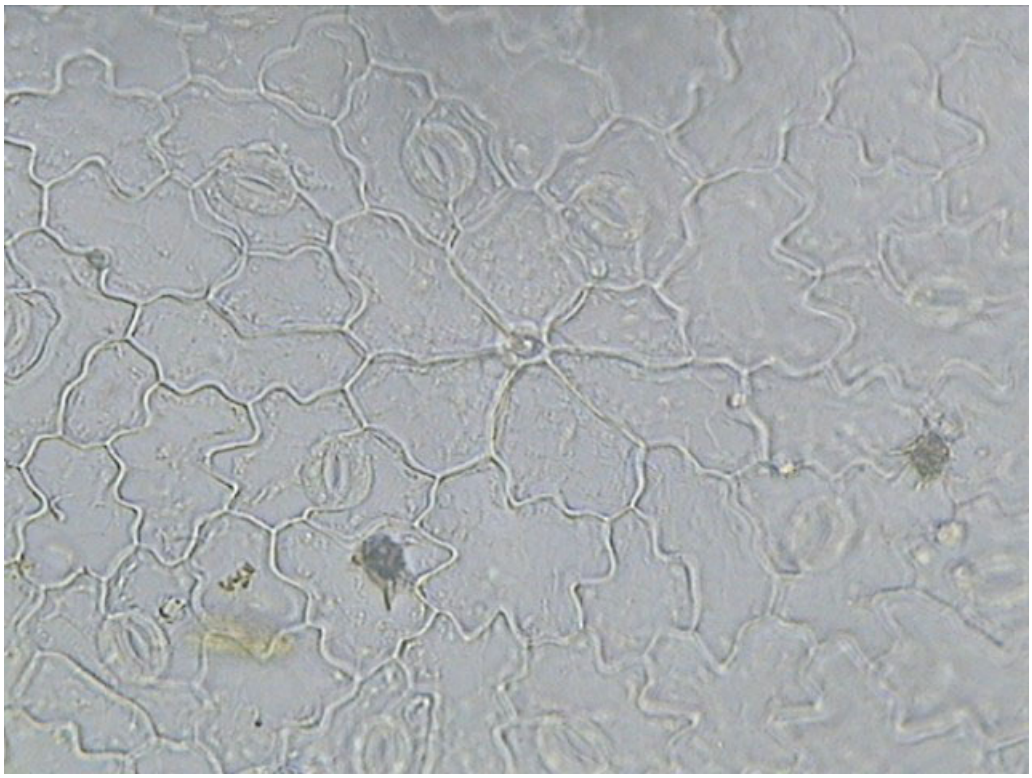

Figure 5. Stomata of *Clitoria ternatea* leaves

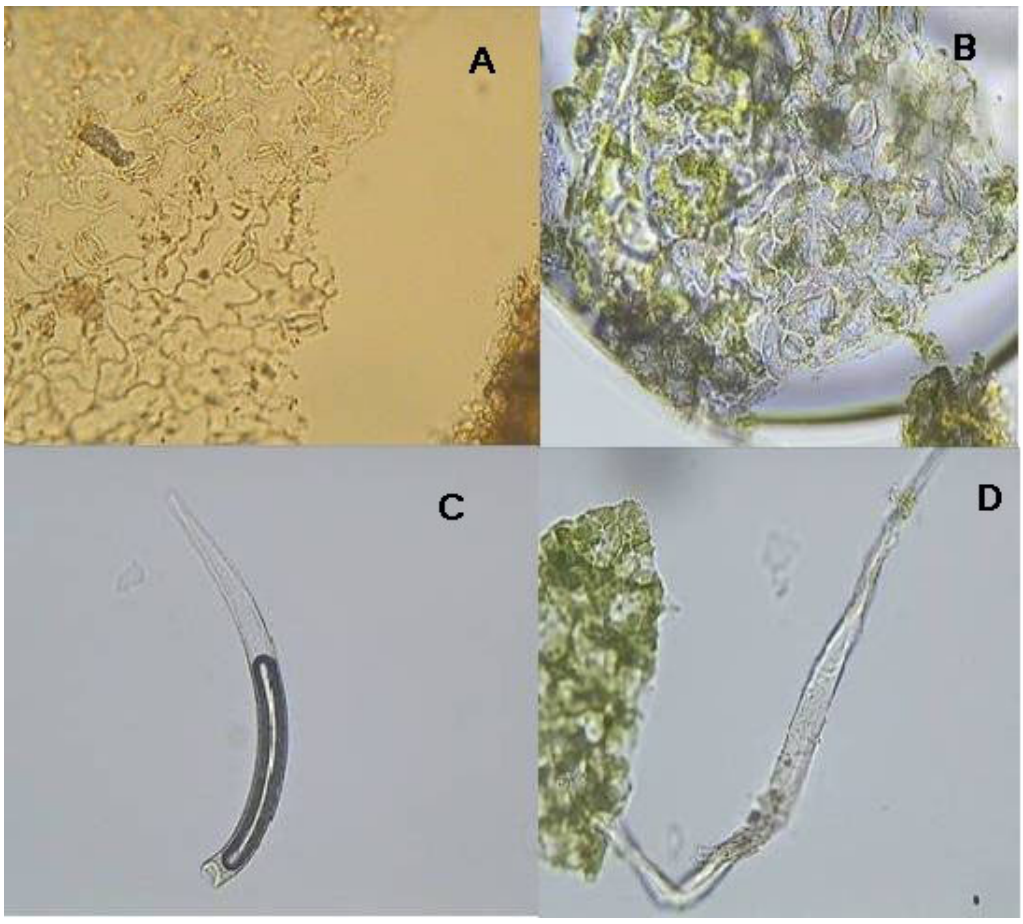

Figure 6. Powder characteristic of *Clitoria ternatea* leaf

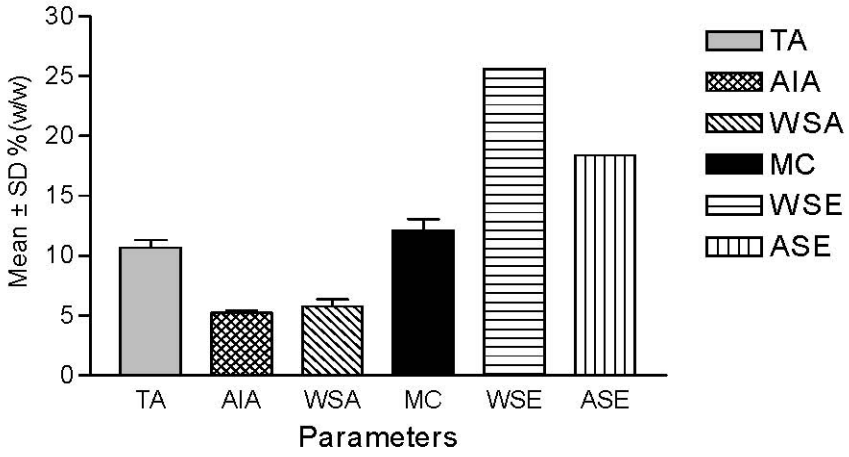

Figure 7. Physicochemical parameters of *Clitoria ternatea* leaves
A-Powder showing stomata, B-Powder showing starch grains
C- Powder showing covering trichome, D-curved trichome

Table No.1- Preliminary Phytochemical Screening of *Clitoria ternatea* leaves

Chemical Tests	Aqueous extract	Methanol extract	Ethyl acetate extract	Benzene extract
Test for carbohydrate	–	–	+	+
Test for reducing sugars	–	–	+	–
Test for Proteins	+	–	–	–
Test for Amino acids	+	–	–	–
Test for Steroids	–	–	+	+
Test for cardiac glycosides	+	+	–	–
Test for Saponins glycoside	+	+	+	+
Test for flavonoids	+	+	+	+
Test for alkaloids				
a) Dragendorff's test	+	+	–	–
b) Mayer's test	+	+	–	–
c) Wagner's test	+	+	+	+
Test for Tannins				
a) 5% Fecl3 solution	+	+	–	–
b)lead acetate solution	–	–	–	–
c)Acetic acid solution	–	–	–	–

+ Indicates presence of constituents. – Indicates absence of constituents

some are strongly waved. Cicatrix which is surrounded by epidermal cells showing characteristic arrangements and stomata are paracytic types. Physicochemical parameter are useful in dermination of purity of drugs, and observed that 13.2 ± 3.49 total ash, 4.8 ± 2.16 acid insoluble ash and 5.3 ± 0.08 water soluble ash. The moisture content found to be 12.5±2.57. Extractive value gives idea about how much quantity of soluble phytoconstituents are present in solvent, water soluble and alcohol soluble extractive value found to be 25.2 and 18.4 respectively. Phytochemical studies revealed presence of Steroids, tannins, carbohydrates, flavonoids, proteins, amino acids, glycosides, saponin, tannins and alkaloids as phytoconstituents.

CONCLUSION

The various morphological, microscopical, physicochemical standards developed in this study will help for botanical identification and standardization of *Clitoria ternatea*. Further, the authentic plant material can be explored for its pharmacological and phytochemical potential.

ACKNOWLEDGMENT

The authors are thankful to the Management S.V.P.M's College of Pharmacy, Malegaon (Bk), Baramati for providing necessary facilities and also to the Prof. R. B. Deshmukh Head Dept. of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar for the authentication of the plant

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Purification and characterization of an Anti-proliferative and mitogenic plant lectin from tubers of *Arisaema speciosum*

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Abstract

A novel lectin having specificity for N-acetyllactosamine was purified from the tubers of *Arisaema speciosum* Martius (family: Araceae) by affinity chromatography on asialofetuin-linked amino activated silica beads. The lectin have four identical subunits of 13.5 kDa each and native molecular mass of 51.2 kDa. ASL agglutinated rabbit, rat, sheep and guinea pig erythrocytes but human ABO blood group erythrocytes were not even after neuraminidase treatment. ASL gave as single band on native PAGE, pH 4.5 and multiple bands in isoelectric focusing and at PAGE 8.3. ASL is a glycoprotein with 0.7% carbohydrate content, stable up to 50°C and at pH 2 to 10. The lectin had no requirement for divalent cations and was stable up to 4M Urea, 3M thiourea and 3M Guanidine-HCl. ASL was rich in acidic amino acids but cysteine was completely absent. Amino acid modification revealed the involvement of tryptophan and tyrosine residues in lectin sugar interaction. The secondary structure estimated in far UV CD spectrum was 38% α-helix, 27% β-sheet and 35% random contributions. ASL showed mitogenic potential towards human peripheral blood mononuclear cell. ASL produced more that 50% growth inhibition of seven human cancer cell lines viz. SW-620, IMR-32, PC-3, SK-N-SH, Colo-205, HCT-15 and HT-29 at 100 µg/ml out of eleven cell lines used.

Keywords: Arisaema speciosum, anti-proliferative, lectin, mitogenic.
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INTRODUCTION

Lectins, the carbohydrate-binding proteins of non-immune origin, have been the subject of intense investigation over the last few decades owing to the variety of interesting biological properties. Most of the lectins which have been purified and characterized from plants have been obtained from dicotyledons. After the discovery of WGA[1], the first lectin from a monocot (Gramineae), more lectins were isolated from this and other monocot families such as the Liliaceae[2] Amaryllidaceae, Alliaceae[3] and Araceae[4]. Various Gramineae lectins from *Triticum aestivum, Secale cereale, Hordeum vulgare, Oryza sativa, Brachypodium sylvaticum* and *Agropyrum repens* have been purified and characterized. Lectins are divided into different groups depending on their carbohydrate specificities as follows: (i) glucose and mannose binding, (ii) mannose binding, (iii) D-galactose binding, (iv) *N*-acetylglucosamine binding, and (v) sialic acid binding[5]. The abundance and the variety of carbohydrate specificities of lectins raised the interest to use these molecules for isolation and analysis of complex carbohydrates, cell separation and studies of cell surface architecture. Lectins display a variety of biological activities including antitumor[6], immunomodulatory[7], antifungal[8], anti-HIV[9], and anti-insect[10] activities.

The first mitogenic agent to be described was PHA, the lectin from red kidney bean (*Phaseolus vulgaris*), by Nowell[11]. The discovery of lectin-mediated mitogenesis led to the detection of many other mitogenic lectins, most notably Concanavalin A[12], WGA[13] and PWM[14]. Some examples include the mitogenic lectins from underground tubers of *Gonatanthus pumilus* and *Sauromatum guttatum*[15]. Carbohydrate epitopes expressed on cell surface undergo certain changes during transformation, for example

synthesis of linear and branched poly-*N*-acetyllactosamine on *N*-glycans, is one of the most important and basic processes leading to the malignant transformation of cells, invasion, and metastasis of carcinoma cells[16]. We report herein purification and characterization of an *N*-acetyllactosamine specific lectin from the tubers of *Arisaema speciosum* Martius belonging to family Araceae having significant mitogenic activity towards human lymphocytes and inhibitory activity towards human cancer cell lines.

MATERIALS AND METHODS

Materials and Chemicals.

Underground tubers of *A. speciosum* were collected from Khajjiar, Dalhousie, India. Fetal calf serum from Sera Lab (GB) and RPMI-1640 from GIBCO-BRL (New York, USA) were procured and stored at 4°C. Carbohydrates, diethylpyrocarbonate (DEP), *N*-acetylimidazole, (NAI), *N*-bromosuccinimide (NBS), 2-hydroxy-5-nitrobenzyl bromide (HNB-Br), bis-dithionitrobenzoic acid, (DTNB), bovine serum albumin, sodium azide, 5-fluorouracil, adriamycin, mitomycin-C and other general chemicals were obtained from Sigma Chemical Co. (St. Louis, USA). Standard molecular weight markers, gel filtration markers and ampholine (pH 3.0-10.0) were procured from Amersham Pharmacia (New Jersey, USA). Amino activated silica beads used were from Clifmar, UK. The Cell-lines viz. MCF-7 (Breast), SK-N-SH (CNS), 502713 (Colon), Colo-205 (Colon), HCT-15 (Colon), HT-29 (Colon), SW-620 (Colon), Hep-2 (Liver), IMR-32 (Neuroblastoma), DU-145 (Prostate) and PC-3 (Prostate) were procured from National Center for Cell Sciences (NCCS), Pune, India. These cell lines were maintained in RPMI 1640 medium with 10% FCS, 10 U/ml penicillin and 100 µg/ml streptomycin at 37°C, in humidified atmosphere (90% air and 10% CO2) in CO2 incubator (Heraeus, Heracell).

Isolation and purification of ASL

Arisaema speciosum tubers peeled, chopped and soaked overnight at 4°C with 10mM phosphate-buffered saline (PBS),1 pH 7.2 (1:5 w/v). After centrifugation at 20,000*g* for 30 min, the supernatant was chromatographed on asialofetuin-linked amino activated silica beads (1000Å; pore size, 100 µ; diameter) as described by Shangary et al[17]. Elution of bound lectin was done with 100mM glycine-HCl buffer, pH 2.5. Eluted fractions were immediately neutralized with 2M Tris-HCl buffer, pH 8.3. Purified protein rich fractions were dialyzed against PBS and stored at 4°C for further analysis.

LECTIN-ACTIVITY ASSAY AND SUGAR INHIBITION OF LECTIN

Lectin-mediated agglutination of human and animal red blood cells was performed in 96-well polystyrene 'U' shaped plates both for crude and purified lectins and after incubation for 1 hr at 37°C, the agglutination was observed visually[18]. The titre was expressed as the reciprocal of the highest dilution of the lectin, showing visible agglutination and this concentration was denoted as one hemagglutination unit (HU). To find the carbohydrate specificity of ASL, sugar inhibition was performed in a manner analogous to the hemagglutination test[19]. Sugars or their derivatives were tested at a concentration of 100mM while polysaccharides and glycoproteins at a concentration of 4 mg/ml. To find the carbohydrate specificity of ASL, sugar inhibition was performed in a manner analogous to the hemagglutination test[19]. For this purpose, a series of 42 sugars/derivatives were used, which included 4 pentoses, 18 hexoses, 10 disaccharides, 3 trisaccharides, 3 polysaccharides and 4 glycoproteins. Lectin was used at twice the lowest concentration causing agglutination of rabbit RBCs as determined through double dilution technique. The minimum concentration of the sugar in the final mixture that completely inhibited the lectin induced hemagglutination was taken as minimal inhibitory sugar concentration (MISC). To ascertain the biological specificity of ASL, the hemagglutination activity was tested against both native as well as neuraminidase treated erythrocytes from rabbit, goat, sheep, guinea pig, rat, and human (ABO) blood[19].

Protein and neutral sugar content analyses

Protein concentration in the crude and purified lectin samples was determined by the method of Lowry et al.[20] using bovine serum albumin as standard while neutral sugar content of the purified lectin preparation was estimated by anthrone method[21] using D-glucose as standard.

Polyacrylamide gel electrophoresis of native and denatured lectin

Native polyacrylamide gel electrophoresis (PAGE) was carried out using 7.5% (w/v) gel at pH 4.5[22] and 10% (w/v) gel at pH 8.3[23]. SDS–PAGE was performed according to Laemmli[24] using 11% (w/v) separating gel. The sample was heated in the presence/absence of 2-mercaptoethanol for 10min in boiling water bath. The gels were stained with Coomassie brilliant blue. The subunit molecular mass of the purified lectin was determined by comparing its electrophoretic mobility with those of molecular weight markers (14.4-94 kDa).

Isoelectric focusing

To ascertain the presence of charged isomers in the purified lectin preparation and to determine the isoelectric point, isoelectric focusing was carried out in 5% polyacrylamide tube gels containing 5% ampholine of pI range 3.0-9.5 according to Robertson et al.[25]. The gels were stained with Coomassie brilliant blue. Isoelectric point was calculated by comparing the mobility of lectin with that of pI markers.

Gel-exclusion chromatography

The molecular mass of the native lectin was determined by gel filtration chromatography on Biogel P-200 column (1.6x62.0 cm) calibrated with molecular weight markers in the range of 29-200 kDa according to the method of Andrews[26]. The column was equilibrated and eluted with 10mM PBS, pH 7.2.

Effect of temperature, pH, metal ions, denaturants, and chelating agents on lectin activity

To determine thermal stability of the affinity purified ASL, 50 µl of lectin sample in PBS was heated for 30min at a defined temperature ranging from 40 to 95°C and cooled to room temperature. As the bound lectin was desorbed from the affinity matrix by employing glycine-HCl buffer, pH 2.5, the effect of such a low pH on lectin-induced hemagglutination was ascertained before standardizing the purification protocol. The lectin sample was incubated with the above-mentioned buffer for time intervals ranging from 15min to 6 h, followed by neutralization with Tris-HCl buffer, pH 8.3. Thereafter, titer of each treated sample was compared with that of controls i.e., lectin sample mixed with glycine-HCl followed by immediate neutralization and lectin sample in PBS alone. The effect of three denaturing agents i.e., urea, thiourea, and guanidine-HCl, at a concentration range of 0.5-8.0M, was tested on lectin activity by incubating 30 µl of each denaturant solution with equal volume of ASL at 37°C for 1h. To examine divalent cation requirement of ASL for hemagglutination, demetallization of purified lectin was carried out by the method of Paulova et al.[27] by using EDTA followed by remetallization of a part of the sample with 0.1M $CaCl_2$ and $MnCl_2$. Following these treatments hemagglutination assay was performed with each sample and titer was compared with that of respective untreated samples.

Amino acid analysis

Affinity purified ASL containing 1 mg/ml protein was dialyzed exhaustively against distilled water lyophilized and then hydrolyzed with concentrated 6 N HCl at 110 °C for 22 h under vacuum. It was analyzed for amino acid composition in Alpha Plus amino acid analyzer (LKB Pharmacia).

Spectroscopic measurements

Fluorescence studies

Fluorescence measurements were performed on a Shimadzu Spectrofluorometer. Samples were excited at 295 nm and emission spectra were recorded between 250 nm to 500 nm. Excitation and emission slit widths were 5 nm. Measurements were made using lectin at a concentration of 0.1 mg/ml protein in deionized water. Base-line corrections were carried out with buffer without protein in all cases. The fluorescence spectra of native and NBS modified enzyme samples were recorded.

Circular dichroism studies

Circular dichroism spectra of ASL were recorded using a Jasco J-715 spectropolarimeter over a wavelength of 200-250 nm at a scan speed of 50 nm/min, under constant N_2 purging according to the manufacturer's instructions (Jasco). The lectin was used at a concentration of 0.15 mg/ml in deionized water, in quartz cuvettes of 0.1 mm path length. The accumulated average of five protein spectra was corrected by subtraction of the spectra measured from a buffer blank. Analysis of CD spectra in terms of secondary structure content was performed using K2D programme.

Chemical modification studies of amino acids

Tryptophan residues of ASL were modified using NBS[28] and HNB-Br[29].Modification of Tyrosine residues was carried out using N-acetylimidazole (NAI)[30]. Histidine, cysteine, serine and arginine residues of ASL were modified as per defined conditions in Table 1. Both modified and unmodified protein samples were dialyzed and residual activity was determined after appropriate dilution. Percentage residual activities were calculated using the native lectin as control possessing 100% activity. For ligand protection ASL, it was pre-incubated for 30 minutes with N-acetyllactosamine and then modification was done.

Biological characterization

Serological Studies

Purified ASL was used to raise antiserum by immunizing healthy rabbits with 1mg/ml of the lectin and 1 mL of Freund's complete adjuvant (Sigma). Three doses were

Table 1: Amino acid modification studies of ASL

Reagent	Solvent	Residue modified	Buffer used	Temperature in ºC	Time (min)	Concentration (mM)
PMSF	Water	Serine	phosphate bufferedsaline, pH 7.2	30	60	10
DTNB	Water	Cysteine	phosphate buffered saline, pH 7.2	30	60	10
Phenylglyoxal	Water	Arginine	phosphate buffered saline, pH 7.2	30	60	10
DEPC	Absolute alcohol	Histidine	phosphate buffered saline, pH 7.2	30	60	10
NBS	Water	Tryptophan	Sodium acetate buffer, pH 6.0	30	60	10
HNB-Br	Dry Acetone	Tryptophan	Sodium acetate buffer, pH 3.0	30	60	10

given at one-week interval each. The blood was collected by puncturing the ear pinna vein of the animal after a weak of the last dose. Antiserum was separated and preserved at –20°C in aliquots containing 0.01% sodium azide. Double immunodiffusion (Ouchterlony) was performed to study serological cross-reactions. The gels stained for 30 minutes with 0.1% amido black and destained with 7% glacial acetic acid to visualize the stained precipitin lines.

Assay of antifungal activity

ASL was tested for antifungal activity against plant pathogenic fungi namely *Fusarium oxysporum, Fusarium graminearum, Trichoderma reesei, Colletotrichum lindemuthianum, Alternaria solani, Rhizoctonia solani and Botrytis cinerea*. Antifungal assay was performed as described by Wang et al.[31].

Mitogenic potential

Mitogenic potential of purified ASL was studied by 3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay[32] towards human peripheral blood mononuclear cells (PBMC) using con A as standard. The PBMC were isolated by the method as described[33].

Anti-proliferative effect on cancer cell lines

Anti-proliferative potential of ASL was tested at 100 µg/ml against eleven human cancer cell lines viz. MCF-7 (Breast), SK-N-SH (CNS), 502713 (Colon), Colo-205 (Colon), HCT-15 (Colon), HT-29 (Colon), SW-620 (Colon), Hep-2 (Liver), IMR-32 (Neuroblastoma), DU-145 (Prostate) and PC-3 (Prostate) procured from National Center for Cell Sciences (Pune, India) using Sulphorhodamine B (SRB) assay[34]. Anti-cancer drugs like 5-flurouracil at a concentration of $1X10^{-5}$ M and mytomycin C and adriamycin at a concentration of $1X10^{-6}$ M were used as positive controls.

Results and Discussion

The present study describes purification characterization of an N-acetyllactosamine specific lectin from the tubers of *A. speciosum* Martius having mitogenic and antiproliferative. Affinity chromatography using asialofetuin ligand linked to amino-activated silica was used to purify the lectin which was one of effective inhibitors of hemagglutination. Lectin isolation results of are summarized in Table 2. Complete adsorption of ASL to the affinity column was because the unbound PBS fractions were devoid of any lectin activity. Elution of lectin from affinity column was carried out using 100mM glycine-HCl buffer, pH 2.5 (Fig. 1). The single step affinity purification of lectin seems to be efficient because 67.03% of total lectin activity was recovered. Interestingly, although ASL constituted only a small proportion of the total weight of tubers, they represented a considerable proportion of the tuber protein suggesting that such high lectin content may fulfill some physiological role in the plant. Similar results in case of other araceous lectins have been reported earlier[35,19]. N-acetyllactosamine (LacNAc), a disaccharide and asialofetuin, a desialylated serum glycoprotein were the only two inhibitors of lectin-induced hemagglutination in sugar inhibition assay. Minimal inhibitory sugar concentration with LacNAc was 25.0mM while asialofetuin was reactive at 250 µg/ml. Preceding reports on araceous lectins established their inhibition by asialofetuin, N-acetyllactosamine[17] and mannose[36]. It is noteworthy to note that monocot lectins from family amaryllidaceae and alliaceae bind mannose with high affinity[3] but in the present study ASL showed no reactivity towards mannose even at very high concentration. It is important to note that poly LacNAc has been reported as very important cancer markers[16]. ASL was inhibited by asialofetuin but not by fetuin which suggest that sialic acid present on terminal position of fetuin hinders the binding of the lectin to the recognition sites present on it. Structure of asialofetuin

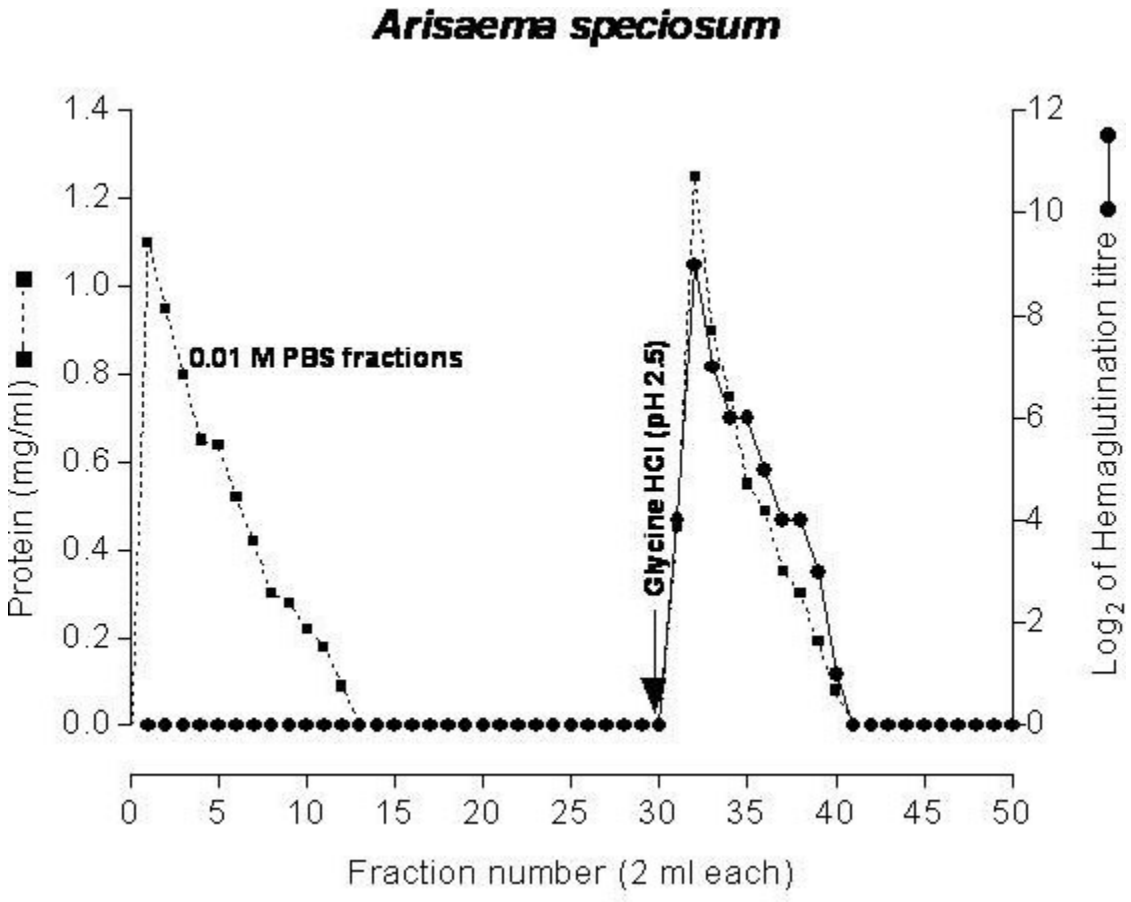

Figure 1. Affinity purification of ASL from tuber extract on asialofetuin-linked amino activated silica beads.

Table 2: Affinity purification of *Arisaema speciosum* lectin.

Lectin/Step	Total Protein (mg)	Total activity (HU)	Specific activity (HU/mg)	Purification fold	Recovery (%)	MEAPC (µg/ml)
Arisaema speciosum						
Crude	959	51200	53.38	1.0	100.0	18.73
PBS fractions	246	--	--	--	--	--
Glycine-HCl fractions	212	34320	161.88	3.00	67.03	6.17

reveals presence of 80% Asn-linked oligosaccharides terminating in LacNAc (Gal-β-1, 4 GlcNAc) and 20% Ser/Thr-linked oligosaccharides having T-Disaccharide (Gal-β-1, 3-GalNAc)[37]. Present findings suggest that ASL binds to LacNAc but not to T-Disaccharide, indicating that the reactivity of araceous lectins towards asialofetuin was due to LacNAc component of the complex molecule.

In hemagglutination assay for biological specificity ASL agglutinated erythrocytes from rabbit, rat, goat, sheep, and guinea pig, human lymphocytes but did not agglutinate human ABO blood group erythrocytes even after neuraminidase treatment (data not shown). However, goat erythrocytes were agglutinated only after neuraminidase treatment. Minimal erythrocyte agglutinating protein concentration (MEAPC) with rabbit and sheep RBCs did not change after neuraminidase treatment but in rat and guinea pig erythrocytes MEAPC lowered by 4 times. In case of human lymphocytes MLAPC reduced to half after neuraminidase treatment. Increase in the reactivity of erythrocytes from rat and guinea pig and human lymphocytes after neuraminidase treatment supports the sialic acid hindrance in binding of lectin to cells.

Affinity purified lectin was tested for homogeneity using various parameters. Under both reducing and non-reducing conditions in SDS-PAGE at pH 8.3, ASL

migrated as single band of 13.5 kDa (Fig. 2A). In native PAGE at pH 4.5 lectin moved as single band (Fig. 2B). In gel filtration chromatography using calibrated Biogel P-200 column, native molecular mass of ASL was 54 kDa (Fig. 3). The results of gel filtration chromatography and SDS-PAGE under reducing and non-reducing conditions suggested homotetrameric nature of the lectin having four identical subunits, not held together by disulphide linkages as reported earlier[3, 38]. In native PAGE at pH 8.3 affinity purified ASL migrated as three bands (Fig. 4A). Above results indicate the existence of charged isomers in the lectin. Similarly, in isoelectric focusing, ASL gave a complex mixture of isolectins, mostly in acidic range (Fig. 4B). Multiple bands in isoelectric focusing indicate charge heterogeneity as reported in most of the lectins[39,40,41]. The microheterogeneity may arise due to variations in oligosaccharide chains[42] or it may stem from few alterations in amino acids of lectins[43]. The carbohydrate content of the lectin was 1.2% indicating the glycoprotein nature of lectin which is being reported earlier in araceous lectin[35, 44]

Thermal stability of the lectin was up to 50°C for 15 minute while above this temperature lectin activity decreased sharply and was completely lost at 85°C (data not shown) indicating high thermal stability of ASL as reported earlier for other araceous lectins[17]. In presence of denaturants ASL was stable up to 4M Urea, 3M thiourea and 3M Guanidine-HCl, declined at higher concentrations but not completely lost even at 8 M (data not shown). Denaturation of ASL by these agents indicates its globular nature, mainly stabilized by hydrophobic interactions[45]. Lectin activity was not affected by EDTA treatment or addition of metal cations suggesting that lectin activity was not dependent on metal cations (data not shown).

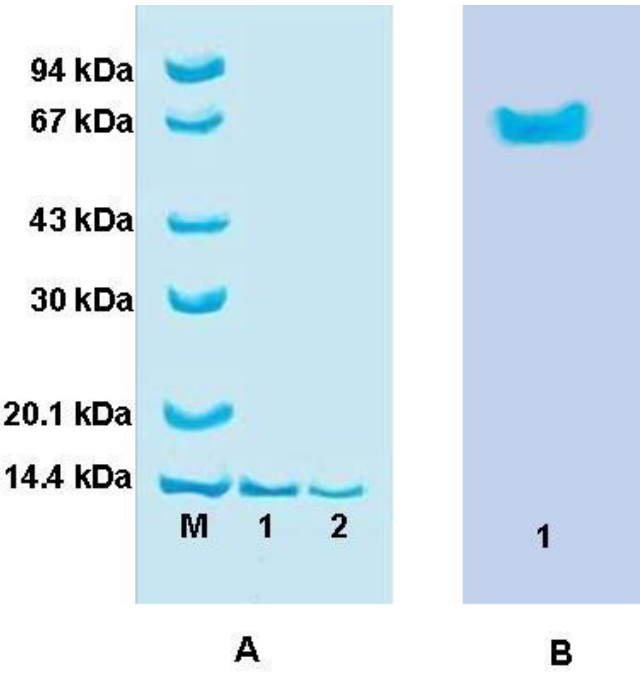

Figure 2. (A) SDS–PAGE, pH 8.3, patterns of purified ASL (lane 1) using 12% gel with (lanes 1) and without (lanes 2) 2% 2-mercaptoethanol. The amount of purified lectin loaded is 20 µg. 40 µg of marker mixture loaded in Lane M, (B) Discontinuous-PAGE, pH 4.5, using 7.5% gel.

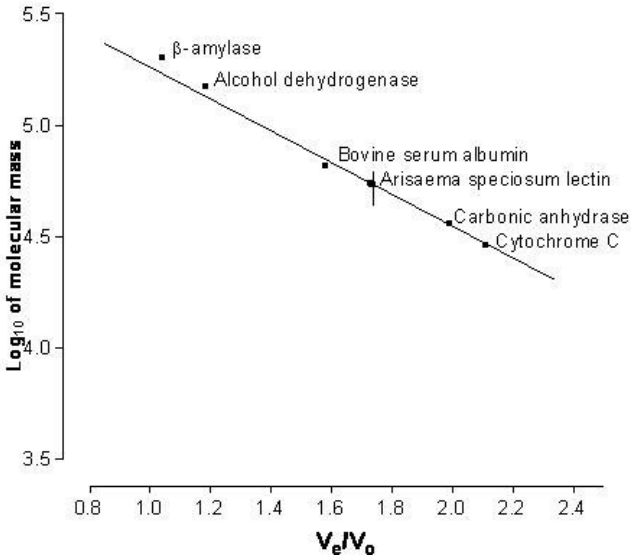

Figure 3. Native molecular mass estimation by standard plot of ASL on Biogel P-200 superfine gel filtration chromatography column.

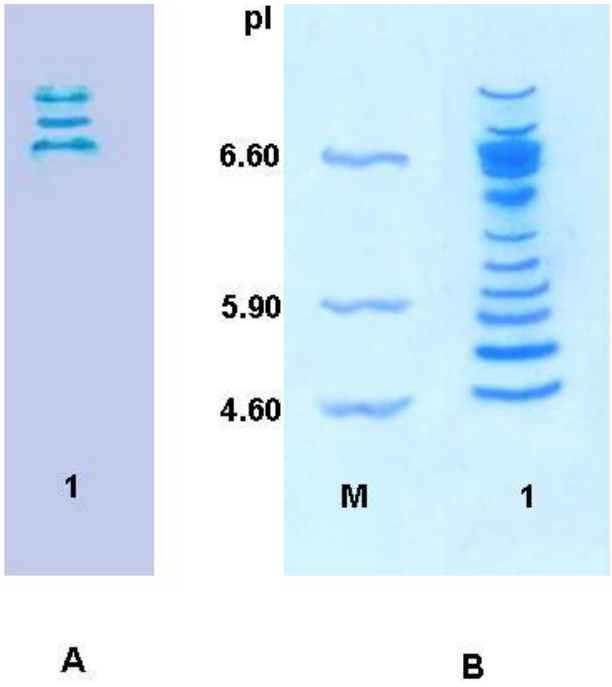

Figure 4. (A) Discontinuous-PAGE, pH 8.3, using 10% gel (running time 6 h at a constant 100 V); amount of protein loaded, 30 µg; lane 1, ASL. (B) Isoelectric focussing of non-denatured ASL lectin on 7.5% polyacrylamide gel using carrier ampholine of pH range 3.5-10.0. protein load, 30 µg; lane M, position of pI marker proteins.

Amino acid analysis revealed high content of aspartic acid, glutamic acid, glycine and proline and very low amount of methionine but complete absence of cysteine (Table 3). Similar reports are available from family amaryllidaceae and alliaceae[3], but gramineae lectins have high content of cysteine[46]. Chemical modification tryptophan residues of ASL with NBS as well as HNB-Br led to complete lost in its activity. Using N-acetyllactosamine in ligand protection assay did not protect lectin activity suggesting that tryptophan may not be present within the sugar binding site but is essential in maintaining the functional three dimensional structure of the lectin. Modification of tyrosine residues with NAI led to 75% loss in ASL activity suggesting importance of tyrosine for lectin-sugar interaction. Ligand protection assay before tyrosine modification completely protected lectin activity which suggests that phenolic hydroxyl groups of tyrosine play important role in the sugar-binding site of the lectin as NAI modified phenolic hydroxyl of tyrosine. Chemical modification of histidine, arginine, serine and cysteine residues with DEP, phenylglyoxal, PMSF and DNB-Br respectively did not affect ASL activity suggesting absence of these amino acids in sugar-binding site. Earlier reports on tryptophan modification in *Glycine max*[47], *Erythrina speciosa*[48] and *Erythrina indica*[49] have shown adverse effects on the lectin activity. Importance of tyrosine residues in carbohydrate binding and hemagglutination activities has been reported in case of *Trichosanthes dioica* lectin using modification studies[50].

Table 3: Amino acid composition of ASL

Amino Acid	Residues/Mol
Aspartic acid and asparagine	56
Threonine	29
Glutamic acid and glutamine	54
Serine	26
Proline	31
Glycine	91
Alanine	24
Valine	28
Methionine	4
Isoleucine	22
Leucine	29
Tyrosine	30
Phenylalanine	28
Lysine	22
Histidine	8
Arginine	14
Cysteine	0
Tryptophan	ND

ND- not determined.

a The nearest integer calculated according to native molecular mass Mr 54 kDa, as determined by gel filtration chromatography.

When excited at 295 nm, native ASL gave fluorescence emission maximum (λmax) at 341 nm. Tryptophan residues when exposed to water, emit 350 and when Trp residues that are exposed to water, fluoresce maximally at a wavelength of 350 nm, while totally buried Trp residues emit at about 330 nm[51]. It shows that tryptophan residue in ASL are located in hydrophobic areas[52]. In modification of Trp residues (mentioned earlier), Trp residues of ASL were required groups and the hydrophobic areas where they were located help in lectin-sugar interaction. The change in fluorescence spectrum on modifying Trp residues of ASL is shown in (Fig. 5). An obvious decline of fluorescence spectrum was caused by modification of Trp.

Far UV CD spectra of ASL were characterized by negative minima at 225 and a positive to negative crossover at around 207 nm (Fig. 6). Secondary structure of ASL has 38% α-helix, 27% β-sheet and 35% random contributions as estimated using K2D programme. In this way the lectin secondary structure is have resemblance with earlier reported ConA[53] and *Arisaema helliborifolium*[54] secondary structures.

Antisera raised against ASL gave precipitin lines with other araceous lectins in Oucterlony's double immunodiffusion, indicating conservation of antigenic determinants in the family (Fig. 7). The closeness in structure and high content of ASL are indicative of an important biological function performed, which is worth investigating. Serological relationship between Amaryllidaceae and Alliaceae has been reported. When antiserum raised against *Narcissus* cv Carlton lectins was tested with purified lectins from *A. moly, A. sativum, A. vineale* and *A. ursinum*, single precipitin lines of identity were observed indicating the evolutionary conservation of these monocot mannose-binding lectins[3]. ASL was found non-inhibitory, when tested for antifungal activity against plant pathogenic fungi.

ASL proved to be a potent mitogen towards human peripheral blood mononuclear cells in MTT assay. Its relative mitogenic stimulation was comparable to Con A at various concentrations (Fig. 8). The optimum proliferation dose of ASL was 20 µg/ml. In mitogenic studies there is inhibition of mitogenesis at higher than optimal lectin concentrations, e.g. Con A has been reported to cause a decrease in mitogenic response at supraoptimal concentrations[55]. Relationship between chromosomal abnormality and human diseases can be studied using mitogenic lectins. As compared to other cells, lymphocytes are usual target cells for mitogenic assays, and lectin-lymphocyte interaction studies may have

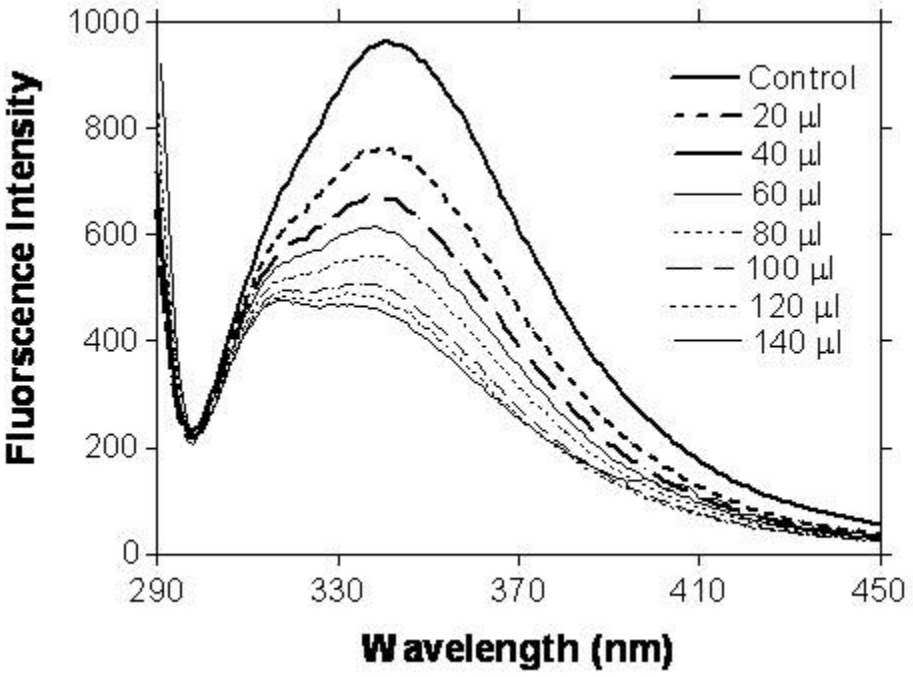

Figure 5. The decline of fluorescence intensity of ASL during tryptophan modification. ASL (100 µg/ml) was excited at 295 nm, and spectra were recorded between 250 and 500 nm.

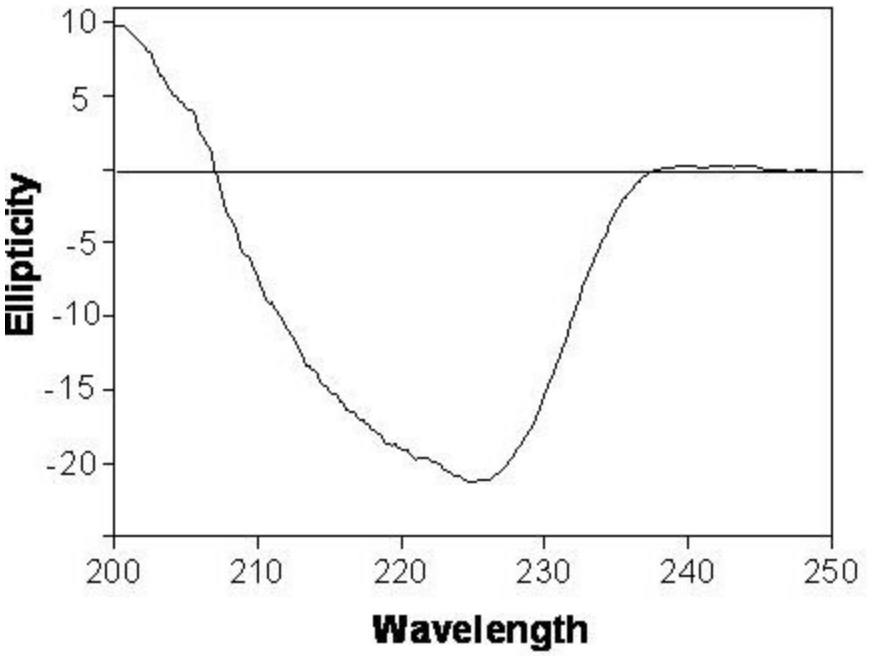

Figure 6. Far-UV CD spectrum of ASL in water.

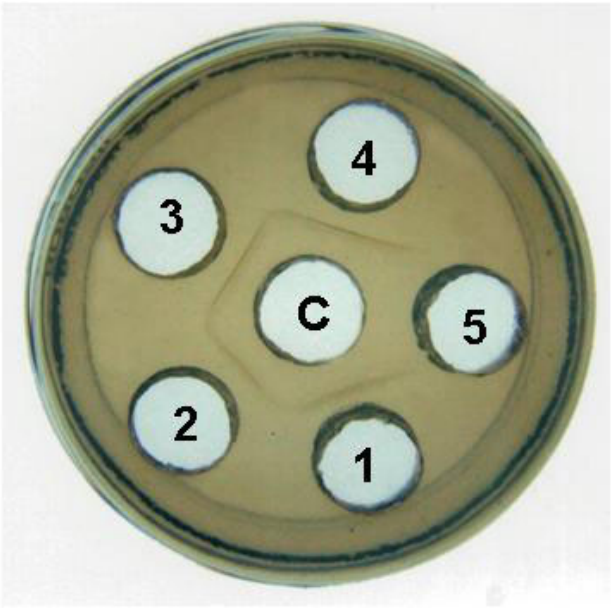

Figure 7. Double immunodiffusion. ASL antiserum was loaded in the central well (A), 20 µg of the purified ASL was loaded in the peripheral (well 1) and the same amount of purified lectin from *Sauromatum guttatum* (well 2), *Gonatanthus pumilus* (well 3) and *Alocasia indica* (well 4). The well 5 was without lectin.

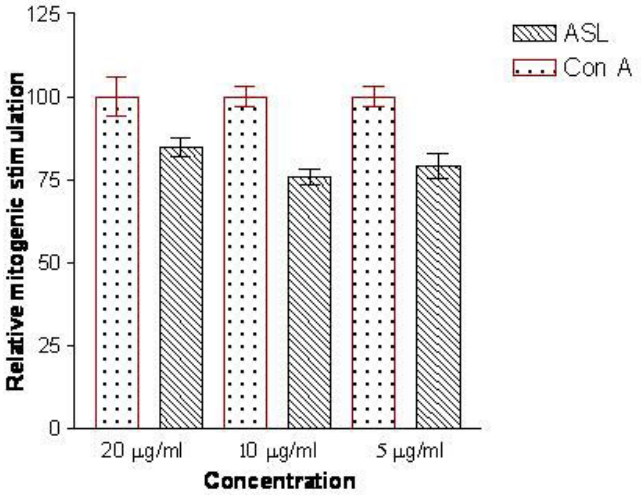

Figure 8. Relative mitogenic index of ASL. Human peripheral blood lymphocytes ($1X10^5$ cells/well) were cultured with ASL at different concentrations. The relative mitogenic index of ASL is its mitogenic index relative to mitogenic index of Con A (taken as 100). (Data represent means ± SD, n = 4.)

enormous contribution in elucidating the mechanism of lymphocyte activation and its control, thereby contributing to our understanding of cell growth and development. In the presence of asialofetuin, an effective inhibitor of ASL, mitogenic response was inhibited in a concentration dependent manner (Fig. 9). Asialofetuin induced inhibition of mitogenicity and hemagglutination suggests that lectin is responsible for these properties by binding to the cell membrane via a site(s) on the lectin, recognized by asialofetuin like structure on the cells.

ASL was tested for its *in vitro* anti-proliferative activity on eleven human cancer cell lines viz. MCF-7 (Breast), SK-N-SH (CNS), 502713 (Colon), Colo-205 (Colon),

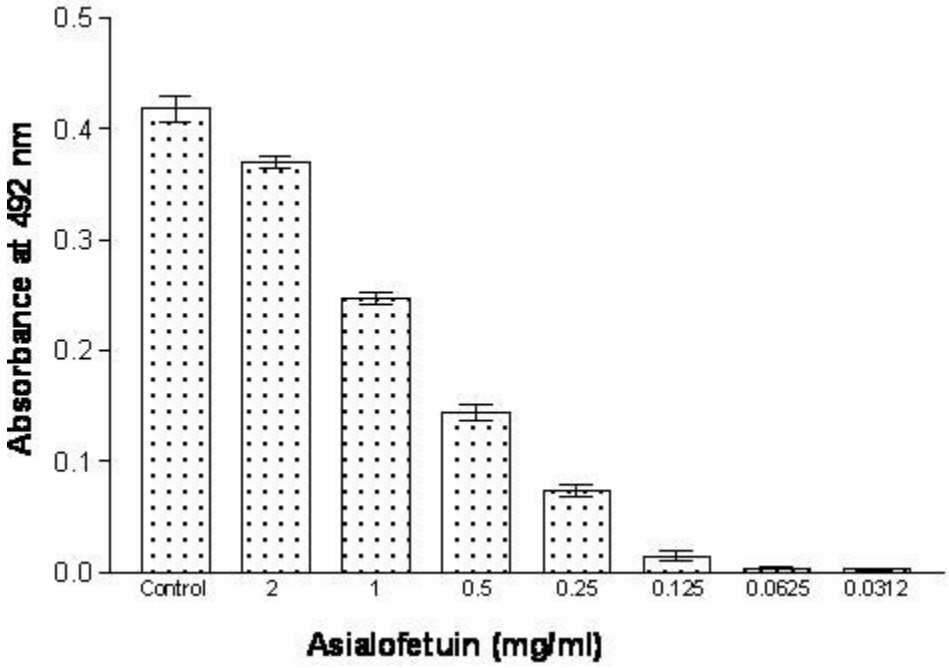

Figure 9. Inhibition of lectin-induced mitogenic stimulation of Human peripheral blood lymphocytes with asialofetuin at a concentration ranging between 2 and 0.03125 mg/ml. Bars represents the percentage inhibition of proliferation. (Data represent means ± SD, n = 4)

HCT-15 (Colon), HT-29 (Colon), SW-620 (Colon), Hep-2 (Liver), IMR-32 (Neuroblastoma), DU-145 (Prostate) and PC-3 (Prostate) (Table 4). More that 50% proliferation inhibition was observed in case of SW-620, IMR-32, PC-3, SK-N-SH, Colo-205, HCT-15 and HT-29 cancer cell lines at 100 µg/ml. Variation in proliferation inhibition of various cell-lines may be due to slight differences in the glycoconjugates expressed on the surface of cancer cells. ASL was found specific for LacNAc in the present study, which is earlier reported as an important cancer marker[16]. Studies have demonstrated that dramatic changes occur in the size and amounts of poly-*N*-acetyllactosamine during differentiation and malignant transformation of embryonic cells[56], hematopoietic cells[57], certain carcinoma cell lines[58, 59, 60], and colon[61,62,] and thyroid carcinomas[63]. In addition, it is suggested that poly-*N*-acetyllactosamine and terminal sugars play some essential roles in carcinoma cell invasion and metastasis[64,65]. As every lectin has unique fine sugar specificity, there is a need to investigate a range of lectins against a number of cancer cell-lines to generate a battery of anti-cancer reagents. A few reports of mitogenic lectins having anti-proliferative activity are available. ASL is similar to the mushrooms lectins having potent mitogenic activity as well as anti-proliferative activity[66,67,68]. There are several hypotheses proposed suggesting, anti-proliferative effect of lectins is associated with their ability to modulate the growth, differentiation, proliferation and apoptosis of premature cells *in vivo* and *in vitro*. The exact molecular mechanism(s) of the anti-proliferative effect of plant lectins is still not clear at present. Anti-proliferative of ASL carried out in the present study appears to be interesting line of investigation and may prove to be a useful tool in cancer research its diagnosis and lectin therapy in modern medicine through further investigations.

Table 4: *In vitro* antiproliferative activity of ASL on human cancer cell lines.

Source of cell lines		Breast	CNS	Colon	Colon	Colon	Liver	Neuroblas-toma	Prostate	Prostate	Colon	Colon
Test Sample	Conc. µg/ml	MCF-7	SK-N-SH	502713	HT-29	SW-620	Hep-2	IMR-32	DU-145	HCT-15	PC-3	Colo-205
ASL	100	37 ± 7.2	54 ± 4.4	42 ± 4.2	63 ± 4.2	53 ± 6.1	44 ± 3.4	53 ± 5.9	40 ± 6.5	49 ± 5.1	52 ± 5.0	52 ± 4.6
5-Fluorouracil	1×10^{-5}M	45 ± 5.4	39 ± 4.3	41 ± 4.5	30 ± 4.2	28 ± 4.9	14 ± 4.3	22 ± 4.2	39 ± 5.4	44 ± 4.6	10 ± 4.2	29 ± 6.4
Adriamycin	1×10^{-6}M	73 ± 6.4	68 ± 6.2	54 ± 5.2	15 ± 4.4	65 ± 6.4	50 ± 5.6	82 ± 5.9	81 ± 5.4	60 ± 5.2	11 ± 5.1	38 ± 5.1
Mitomycin C	1×10^{-6}M	41 ± 5.8	48 ± 4.8	65 ± 4.5	17 ± 3.6	34 ± 5.5	28 ± 3.8	25 ± 4.6	58 ± 5.2	26 ± 5.1	10 ± 4	20 ± 4.5

CONCLUSION

In the present study a lectin having mitogenic as well as anti-proliferative activities has been purified and characterized from *Arisaema speciosum* Schott. N-acetyl-D-lactosamine and asialofetuin inhibited the lectin induced hemagglutination. The lectin was found to have homotetrameric structure with total molecular mass of 54 kDa and four subunits of equal mass i.e 13.5 kDa. The lectin is as mitogenic as con A so it could also be used for mitogenic studies to explore the mechanism of lymphocyte activation as lectin bind to sugars of their specificity. Anti-proliferative property of ASL suggests the binding of lectin to certain receptor on the cell surface which are responsible for cancerous growth. Therefore this lectin may also be detected as histochemical marker

in these types of cancers. The clinical studies using pure lectins has shown promising results therefore additional research, including clinical trials, mechanisms of action at the molecular level, and structure-function relationships, should help researchers continue to examine and elucidate the therapeutic effects of lectins The area of lectin research has great potential for cancer detection and treatment therefore still more study is required in this field.

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Macro and Microscopical evaluation of Trunk bark of *Ailanthus excelsa* Roxb.

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Abstract:

This study presents the detailed Macro and Microscopical study of the Trunk bark of crude drug *Ailanthus excelsa* Roxb. belonging to family simaroubiaceae, an important medicinal plant in Indian system of medicines. The Trunk bark was studied using procedure of light, conofocal microscopy. The morphological and microscopical parameters were studied. The study was help to indentify and establish the authenticity of Ailanthus excelsa Roxb. The parameters will also help to standardize the crude drug and minimize the drug adulteration.

Keywords: *A. excelsa*, Standardization of herbal drugs, Macro and microscopical evaluation.
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INTRODUCTION

There has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance [1]. The use of plant drugs is subject to their correct identification. In general potent drugs are either adulterated or substituted depending upon morphological characters or biological activity. Despite the modern techniques of investigation, identification of plant drugs by pharmacognostical studies is more reliable. *Ailanthus excelsa* Roxb. (Simaroubiaceae) is commonly known as Mahanimb. *Ailanthus excelsa* is a large tree originally from China, is known as the 'tree of heaven'. Different parts of this plant are used widely in traditional medicine for a variety of diseases [2]. However there is none or very minute pharmacognostical report on the Macro and Microscopical standards which is required for the quality control of the Trunk bark of crude drug.

MATERIAL AND METHODS

Plant material

Trunk barks of *Ailanthus excelsa* Roxb. were collected in Aug. 2008 from local area of Pimpri, pune (INDIA) and identified by the Regional Research Institute of Ayurveda Kothrude, Pune (INDIA). A voucher specimen - 899 was authenticated and provided.

Chemical and instruments

The different materials used for the study include basic microscopical instruments like compound microscope, trianocular microscope, glass slides, cover slips, watch glass, and other common glasswares. Microphotographs were taken using Lecia DMLS microscope attached with Letiz MPS 32 camera. Common solvents like ethanol (95%), and reagent like glycerine, Toluidine blue, Iodine solution, Phloroglucinol, hydrochloric acid, chloral hydrate, and sodium hydroxide were procured from Ranbaxy fine Chemical ltd, Mumbai (India).

Macroscopical analysis

The Macroscopy and morphplogy of the plant were studied according to the method of Brain and Turner [3].

Microscopic study of Trunk bark

The paraffin embedded specimens was sectioned with the help of Rotary Microtome [4, 5, 6, 7]. The thickness of the sections was 10 – 12 µm. Dewaxing of the sections

Figure 1 Trunk Barks and Plant of *A. excelsa* Roxb

was done. The sections were stained with Toluidine blue. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with safranin and fast-green and iodine (for starch). The micro powder analysis was done according to the method of Brain and Turner [8, 9].

EXPERIMENTAL

Macroscopical Evaluation

Barks examined were light greyish-brown colour on outer side and yellow or yellowish brown in inner side. It has rough with irregular fissures. Size: 1.2-2.2cm in length, width 2-3 cm and it varies according to size. Bark is approximately 1 cm thick. Taste: aromatic, slightly bitter. Texture: fibrous and rough. Odour: earthy characteristics. Lenticels are vertically elongated with pointed tips, centrally blistered and whitish brown in colour.

Microscopic characteristics of Ailanthus excelsa Roxb. Trunk bark

Microscopic features

The Trunk bark of *Ailanthus excelsa Roxb.* can be differentiated into distinct region i.e. the outer bark and inner bark. The entire bark is 2.8 mm wide.

Outer bark

The outer barks measures about 1.5mm in width. The outer bark is thick and broad, differentiated into several successive bands of periderm and non-peridermous tissue of secondary phloem. This compound structured is termed as rhytidome. The phellem has tangential width of 100 µm and phelloderm is 150 µm wide. (Fig. 2.1) Phellem cells are thick walled, suberised, tabular and

Structure of the bark:

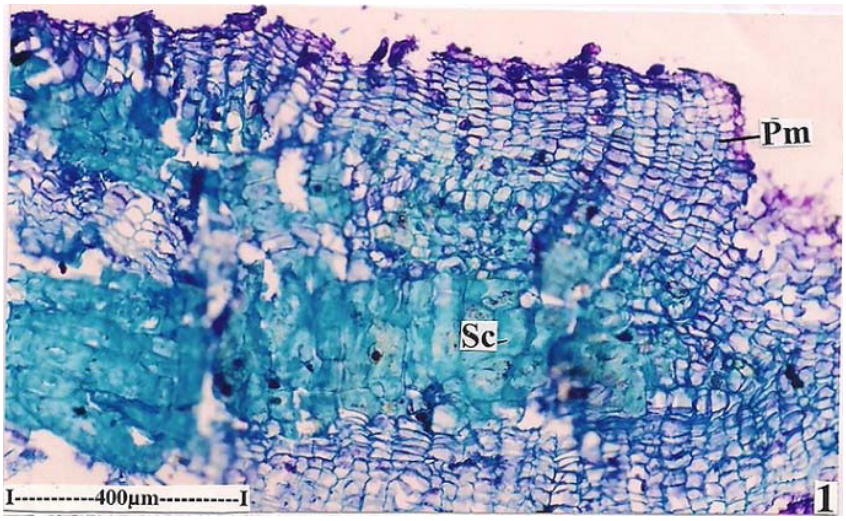


Figure 2.1. T.S of phellem oragnified.
[CZ - Cambial zone; Ncph - Noncollapsed phloem; Pd - Phelloderm; PhR - Phloem ray; Pm - Phellem; Sc - Sclereids]

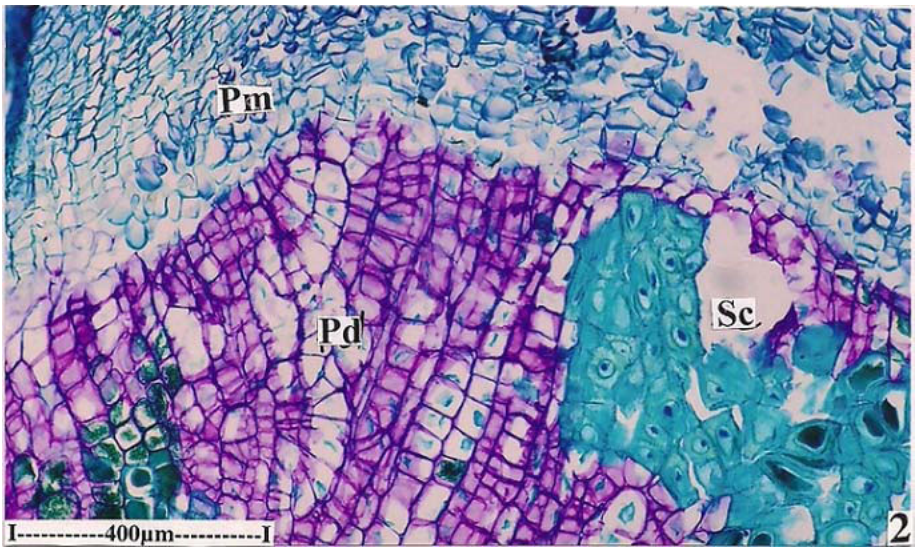


Figure 2.2. Phellem, phelloderm with sclereids enlarged.
[CZ - Cambial zone; Ncph - Noncollapsed phloem; Pd - Phelloderm; PhR - Phloem ray; Pm - Phellem; Sc - Sclereids].

occur in radial series. (Fig. 2.1) Phelloderm cells are thin walled and 5 to 8 layers in thickness cubical or rectangular, arranged in compact radial files (Fig. 2.2). The outer surface Trunk bark consists of many irregular fissures of various shapes. The successive periderm originates from the secondary phloem region, hence the sclerides of the secondary phloem held captive in between the periderm zones (Fig. 2.2).

Inner bark:

The inner bark is broader than the outer bark and it includes all the secondary phloem tissue. Microscopically the inner bark can be distinguished into two distinct regions i.e. (a) Collapsed secondary phloem region and (b) non Collapsed secondary phloem region.

a) Collapsed secondary phloem region: (Fig. 2.2; 2.3)

Collapsed phloem region is the outer to inner part of the bark and middle in position. It is the broadest zone it consists of thick block of phloem sclereids forming tangential cylinders; the cylinders are broken by radially intruding phloem rays. Crushed phloem is seen as dark streaks or lines. The collapsed phloem region measures about 1.8mm in width and extends upto the periderm zone. The dilation of phloem rays towards the periphery gets disturbed and occurs in random due to thick blocks of phloem fibers present in this region.

(b) Non collapsed secondary phloem region: (Fig. 1.3; 2.3)

The non collapsed secondary phloem region is innermost region of the bark and it is the narrow zone lying next to the cambial zone. The region is 600 µm in width. In the non- collapsed phloem regions, the cells are intact and occur in radial files. (Fig. 2.3). Sieve tube members, companion cells, phloem parenchyma are intact (Fig. 3.1; 2.3). The phloem rays are narrow and undilated. In transverse section the sieve tube members are tangentially rectangular or polygonal, the walls are thin. The sieve tube members are 40 µm to 50 µm in tangential plane. Phloem parenchyma cells are prominent and occur along the narrow lateral corners of the sieve element. Sieve plate is simple or compound and prominent. (Fig. 3.2)

In tangential longitudinal section, (TLS) the phloem rays appear non storied and the sieve tube members and axial parenchyma are also non storied (Fig. 4.1). (Fig. 4.1, 2; 5.1, 2) The phloem rays are broad, multiseriate, low in height and heterocellular (Fig. 4.2; 51). The cells are angular and thick walled. The rays are 200-900 µm in height and 40-90 µm in breadth. Occasionally uniseriate rays are also evident. The ray cells are angular and compact without intercellular spaces. In TLS view the sieve tube members appear slightly curved; the sieve plates are compound and oblique (Fig. 5.2). The axial parenchyma cells occur in vertical files of rectangular cells along the sieve tube members (Fig. 5.2).

Cell inclusions:

(Fig. 6.1, 2; 7.1, 2). Calcium oxalate crystals and starch grains are abundant in the outer bark region. They appear bright against dark back ground when seen through the polarized light. The starch grains are small and loosely arranged. Calcium oxalate crystals are also present in the

Anatomy of the bark: -

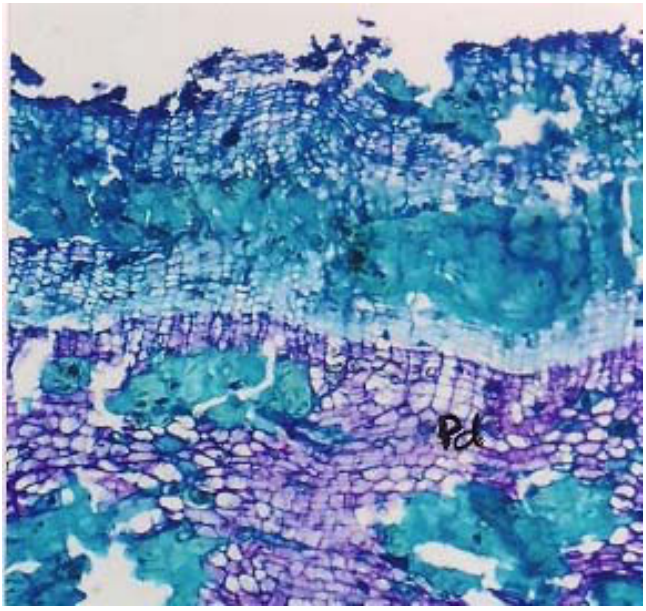


Figure 1.1. T.S of bark- outer periderm region.
[Pe - Periderm; DR - Dilated Rays; Sc - sclereids; Pd - Phelloderm].

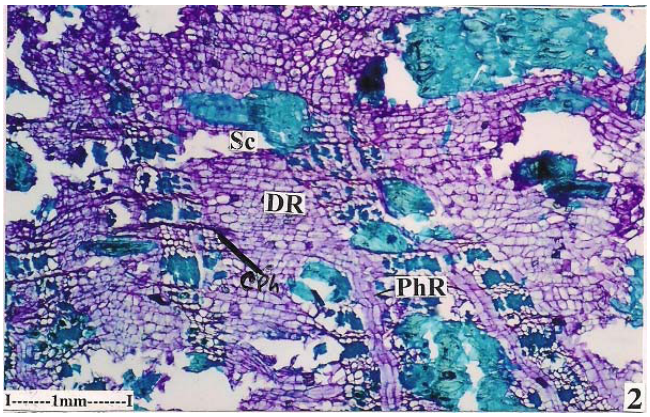


Figure 1.2. T.S bark- middle collapsed phloem.
[Cph - collapsed phloem; DR - Dilated rays; Ncph- Non- collapsed phloem; Pd - Phelloderm; Pe - Periderm; Phr - phloem ray; Sc = Scl- sclereids].

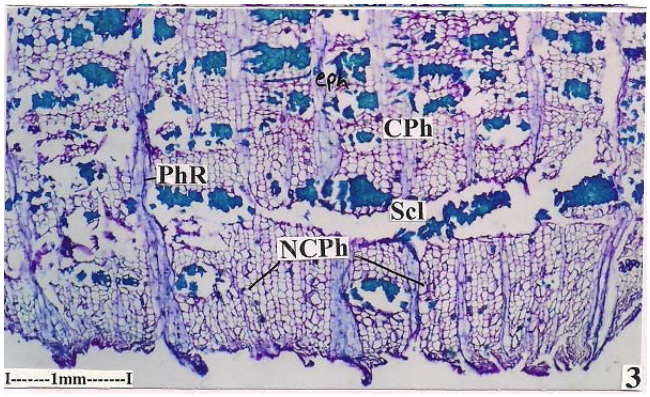


Figure 1.3. T.S of bark- Collapsed and non collapsed phloem.
[Cph - collapsed phloem; DR - Dilated rays; Ncph- Non- collapsed phloem; Pd - Phelloderm; Pe - Periderm; Phr - phloem ray; Sc = Scl- sclereids].

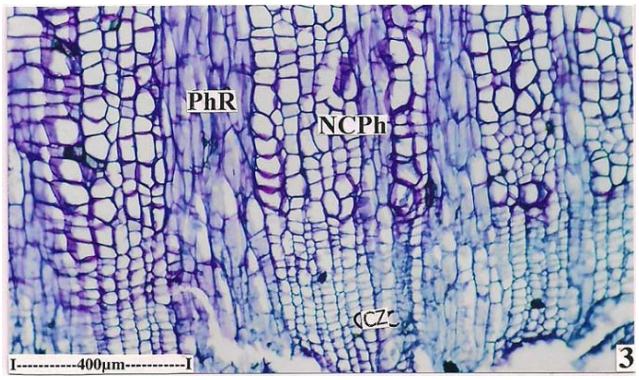


Figure 2.3. Non collapsed phloem cambial zone.
[CZ - Cambial zone; Ncph - Noncollapsed phloem; Pd - Phelloderm; PhR - Phloem ray; Pm - Phellem; Sc - Sclereids].

Structure of the non-collapsed phloem:-

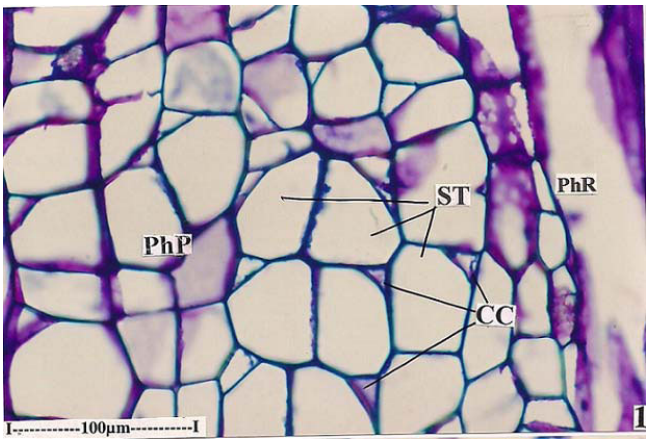


Figure 3.1. T.S of non-collapsed phloem showing companian cells, phloem ray, phloem parenchyma and sieve tube member.
[Php - Phloem parenchyma; PhR - Phloem ray; SPI - sieve plate; CC - companion cells; ST - sieve tube member].

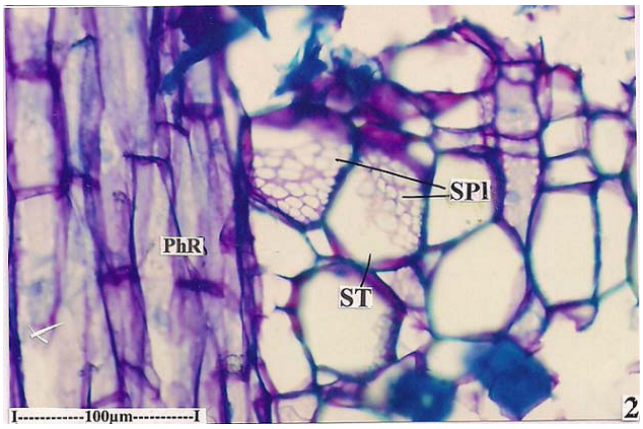


Figure 3.2. Sieve plate with sieve tube members enlarged.
[Php - Phloem parenchyma; PhR - Phloem ray; SPI - sieve plate; CC - companion cells; ST - sieve tube member].

Tangential longitudinal section (TLS) of the phloem:-

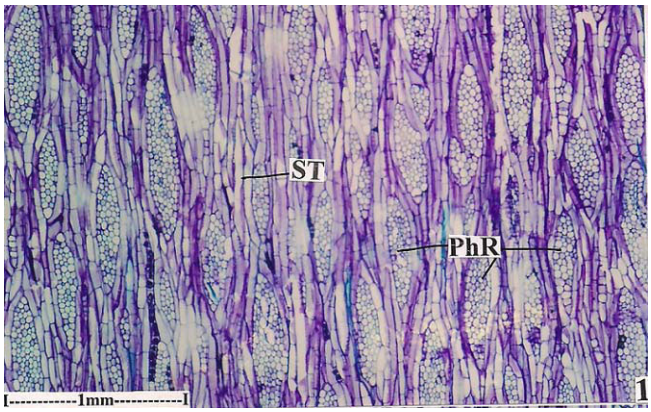


Figure 4.1. TLS of the phloem under low magnification.
[CC - companion cells; CSP - compound sieve plate; Php - Phloem parenchyma; PhR - Phloem ray; ST - sieve tube.]

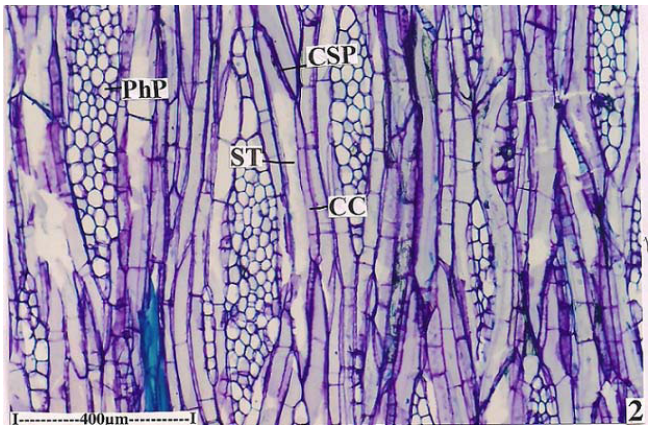


Figure 4.2. Same as above enlarged.
[CC - companion cells; CSP - compound sieve plate; Php - Phloem parenchyma; PhR - Phloem ray; ST - sieve tube.]

Structure of Phloem rays:-

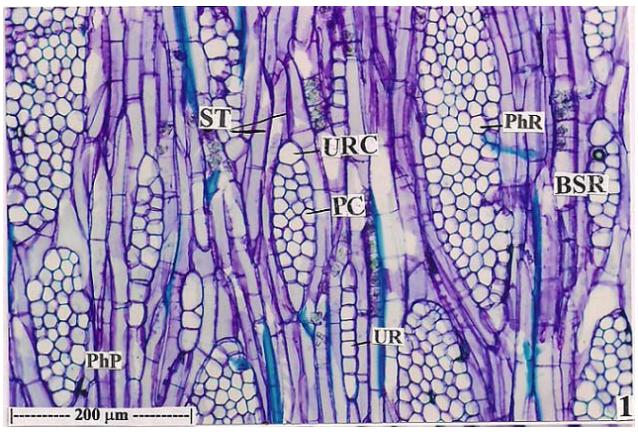


Figure 5.1 TLC of the phloem showing uniseriate biseriate and multiseriate, rays.
[CSP - compound sieve plate; BSR - biseriate ray; Pc - Procumbent cells; Phloem parenchyma; PhR - Phloem ray; ST - sieve tube; URc - upright cell; UR - uniseriate ray.]

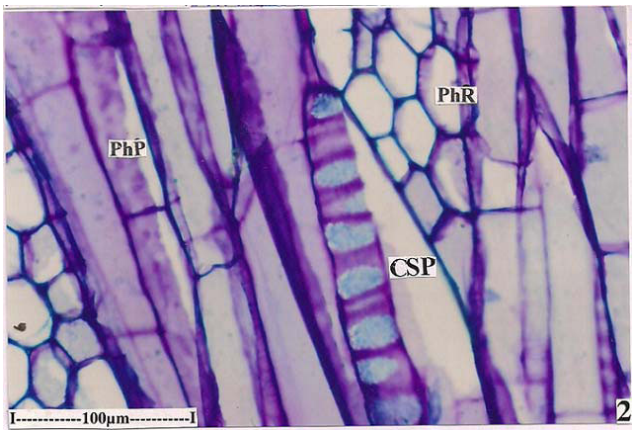


Figure 5.2 A compound sieve plate with phloem parenchyma and phloem rays enlarged.
[CSP - compound sieve plate; BSR - biseriate ray; Pc - Procumbent cells; Phloem parenchyma; PhR - Phloem ray; ST - sieve tube; URc - upright cell; UR - uniseriate ray.]

Crystals distribution in the bark {under polarized light microscope}:-

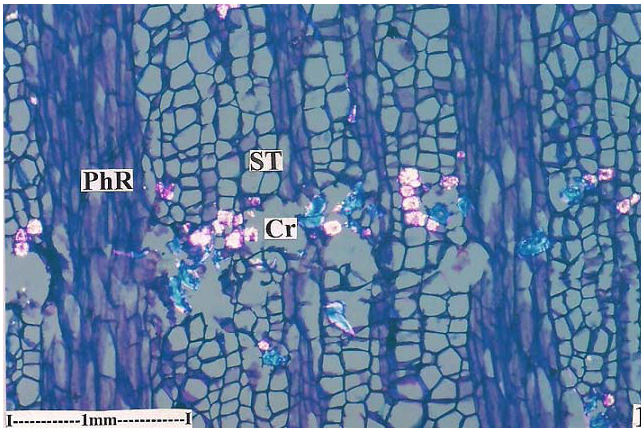


Figure 6.1 Non-collapsed phloem showing druses under low magnification.
[Cr - Crystals; PhR - Phloem ray; ST - sieve tube.]

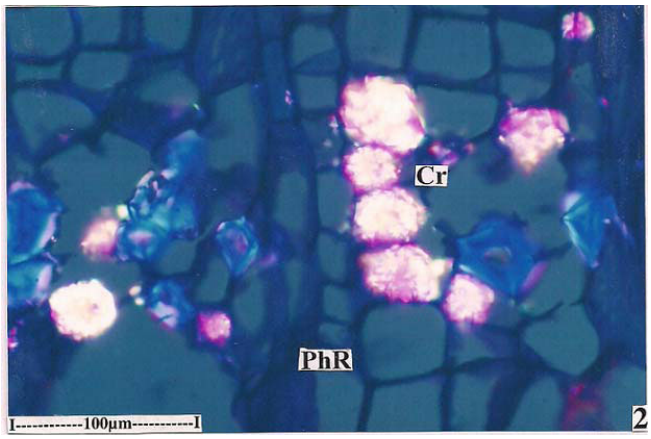


Figure 6.2 Same as above enlarged.
[Cr - Crystals; PhR - Phloem ray; ST - sieve tube.]

Crystals distribution in the non-collapsed phloem {under polarized light microscope}:-

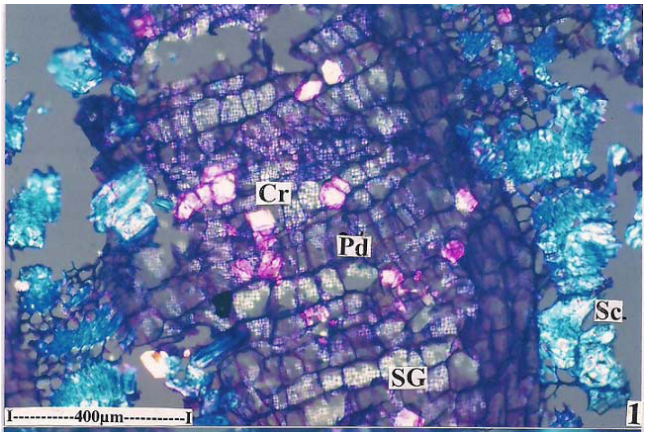


Figure 7.1 Prismatic crystals in the phelloderm.
[Cr - Crystals; Pd - Phelloderm; Sc - sclereids; SG - starch grains.]

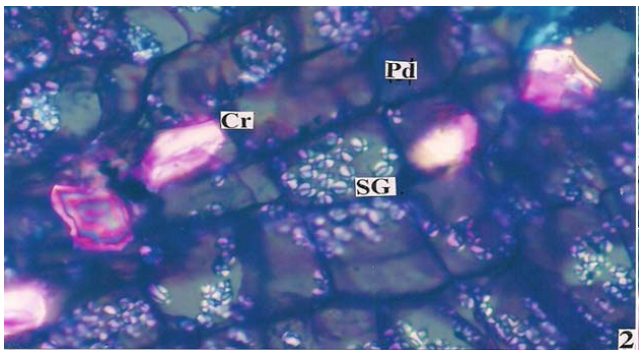


Figure 7.2 Crystals and starch grains enlarged.
[Cr - Crystals; Pd - Phelloderm; Sc - sclereids; SG - starch grains.]

secondary phloem region. They are predominant in the phloem ray. The crystals are typically sphenoid or druses type (Fig. 6.1, 2). Prismatic crystals are seen in the phloem (Fig. 7.1, 2). They are rhomboidal and cuboidal type. The druses are 30µm wide. The prismatic crystals are 20×50 µm in size. The starch grains are simple type; they are ovoidal to elliptical. The hilum is excentric or centic in position (Fig. 7.1, 2).

Powder microscopy:

The bark powder shows druses and prismatic crystal of calcium oxalate. Starch grains are also abundant in the powder. The powder also contains Brachysclereids and fibers. Brachysclereids are isodiametric or elongated (Fig. 8.1, 2). They have thick wall and wide lumen. The fibers are long, narrow cells with pointed ends they have thick lignified walls and reduced lumen. The fibers are 600-800 µm long and 20 µm thick.

Powder microscopy of the bark {under polarized light microscope}:-

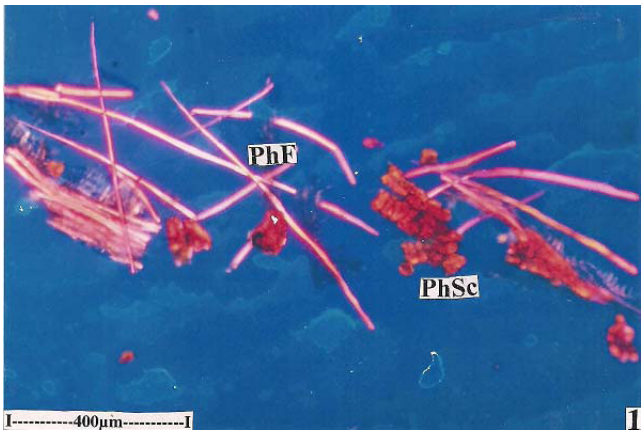
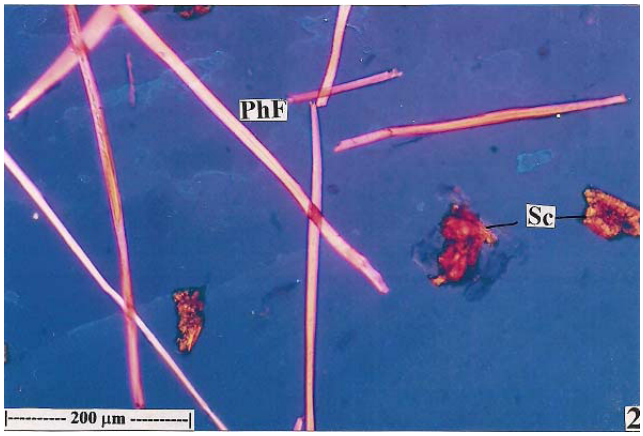


Figure 8.1 Phloem fibres and phloem sclereids under low magnification.



[PhF - Phloem fibers; PhSc - Phloem sclereids; Sc - sclereids]
Figure 8.2 Fibres and sclereids enlarged.
[PhF - Phloem fibers; PhSc - Phloem sclereids; Sc - sclereids]

DISCUSSION

The quality control parameters for the crude drugs as raw materials were established with the help of several official determinations based on morphology, microscopy and physico-chemical studies. These studies were aimed at ensuring standardization of herbal drug under investigation. Morphological examination of drugs refers to evaluation of drugs by colour, odour, taste, size, shape and special features, like touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to impression on organs of senses [10]. All these parameters were recorded for Trunk bark of the plant *Ailanthus excelsa* Roxb. These were helpful in primary identification of *Ailanthus excelsa* Roxb. Microscopical

techniques provide detailed information about the crude drug. Microscopical inspection of crude drugs from plant origin is essential for the identification of the grounded or powdered materials. Rhytidome in outer bark, Collapsed and non Collapsed secondary phloem region in inner bark are the identification characters.

Though microscopy alone cannot provide complete evaluation profile of a herbal drug, still it can provide supporting evidence, which when combined with other analytical parameters can be used to obtain the full evidence for standardization & evaluation of herbal drugs [11]. The above study helps us to standardize the plant or herbal drugs which provide the healthy and maximum potent drug in the market.

AKNOWLEDGEMENT:

We would like to acknowledge that this research was supported by Dr. D.Y. Patil IPSR, Pimpri, Pune-18 (INDIA) by providing the lab facility. We would also like to thank Dr. Rajesh Dabour for authentication the plant sample in R.R. I. Pune (India).

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Pharmacognostic and Phytochemical investigation of aerial parts of *Artemisia pallens* Wall ex.Dc

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Abstract

Artemisia pallens is an perennial herb type plant is a very common medicinal plant used for the various ailments. The aerial parts of *Artemisia pallens* is mainly found in south india (Tamilnadu) and then dried, extracted and calculate the percentage of yield. Phytochemical studies of the Hexane, Chloroform, ethanol and chloroform water extracts showed the presence of carbohydrate, saponins, phytosterol, proteins and amino acid, tannin and phenolic compounds and flavonoids. It was concluded that the all extract contains more important chemical constituents for various pharmacological activities. The present paper deals with the standardization of its aerial parts of plant on the basis of various Pharmacognostical parameters.

Keywords: Artemisia pallens; phytochemical screening; aerial parts.
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INTRODUCTION

Artemisia pallens known as " Davana " in Ayurveda is a versatile medicinal plant used singly or in combination with other medicinal plants for treating a variety of ailments like antidiabetic activity. Artemisia pallens, family composite, mainly found in Mysore city and are a shrub plant. Davana is mostly cultivated in red soil region in south india as Maharashtra, Andhra Pradesh, Karnataka and tamil Nadu[1]. Many Pharmacological activities of Artemisia *pallens* have been reported: perfumenes and as an antifungal and antibacterial agent.[6] It is the most important aromatic plants used in the perfumery and cosmetic industries and india is the major exporters of *Artemisia pallens* oil to the rest of the world. Davana is widely used in Iraqi and Indian folk medicine for the treatment of diabetes mellitus.[2] It is observed that most of the people eat that many parts of the plant. Therefore the objective of this investigation was to evaluate many types of phytoconstituents are present in the aerial parts of davana plant.

Materials and Method

The plant (*Artemisia pallens*) used for this study was collected from south india (tamil Nadu) and identified by Department of Pharmacy, Kamaun University, Nanital, India. A voucher specimen has also been deposited in the herbarium of the institute for future references. Pharmacognostical parameters as per Ayurvedic Pharmacopoeia of India[5] and also primary and secondary plant metabolite. The air dried aerial parts of the plant were cleaned and reduced to powdery form with the help of mechanical grinder. All the reagents used were of the analytical and highest purity grade from standard companies. The stem constants studied and the trichomes morphology observed and size was measured.

Extraction

Powered aerial parts of *Artemisia pallens* (150 gm) were subjected to extraction with different solvent as per the polarity, Hexane, Chloroform, ethanol and chloroform water with the help of sox-let apparatus. Extract were completely air dried and calculate the percentage of yield.

Phytochemical screening

The various extract of the aerial parts of *Artemisia pallens* and subjected to preliminary phytochemical screening was carried out on the their extract using the standard

Table 2: Percentage yield of extracts (Successive extraction) from *Artemisia pallens*.

	Percentage of yield of extracts			
Part of Plant	**Hexane**	**Chloroform**	**Ethanol**	**Chloroform Water**
Aerial parts	17.2	16.2	51.1	27.3

Values are means three reading ± SEM

screening method[3]. The molish's test and fehling's test were carried out for carbohydrate. Foam test for saponins. Salkowiski test and Libermann burchard test for phytosterol. Aq. Sodium hydroxide test, concentrated sulphuric acid test and shinoda's test were carried out for flavonoids. Biuret test, Ninhydrin test and Million's test were carried for proteins and amino acid.

RESULT AND DISCUSSION

Microscopical character

Transverse section of stem is almost circular in outline. The outer most layers are composed of rectangular 15 to 25 layered cork cells, followed by 2 or 3 layered cork

Table 1: Quantitative Physico-Chemical Analysis of aerial parts of *Artemisia pallens*

Paramaters	**Result**
Moisture content (w/w)	18.00%
Total ash (w/w)	12.92%
Acid insoluble ash (w/w)	0.175%
Water soluble ash (w/w)	7.233%
Alcohol soluble extractive (w/w)	5.50%
Water soluble extractive (w/w)	10.125%
Tannin	0.1105%
Sugar	8.57%
Starch	14.54%
Total Phenolic	3.119%
Total Protein	0.954%

Table 3: Summary of phytochemical screening :-

Constituents	**Tests**	**Hexane**	**Chloroform**	**Ethanol**	**Chloroform water**
Carbohydrate	Molish's test	–	+	+	+
	Fehling's test	–	+	+	+
Glycoside	Legal's test	–	+	–	–
	Keller killanis test	–	–	+	–
Fixed oil and fats	Spot test	+	–	–	+
	Saponification test	+	+	–	–
Proteins and amino acids	Million's test	–	+	+	+
	Ninhydrin test	–	+	+	–
	Biuret test	–	+	–	–
Saponins	Foam test	–	–	+	+
Phenolic compunds and tannins	$FeCl_3$ test	–	+	+	+
	Lead acetate test	–	+	+	+
Phytosterol	Salkowiski test	–	+	+	–
	Libermann burchard test	–	+	+	–
Alkaloids	Dragendroff's test	–	–	–	–
	Mayer's test	–	–	–	–
	Hager's test	–	–	–	–
Resin	Resin	+	+	+	–
Flavonoids	Aq. NaOH test	+	+	–	–
	Conc. H_2SO_4 test	+	–	+	–
	Shinoda's test	–	+	+	–

Whereas : (+) Present, (–) Absent

cambium. Just below the cortex, 4 to 12 phloem are present. Cambium is 2 or 3 layered. Vessels in a groups of 2 or 3. The medullary rays thin-walled, radiating, parenchymatous, heterogenous and filled with starch grains.

Extraction

The various extracts obtained were air dried, weighed and calculated the percentage of yields (Table 2), percentage yield of ethanolic extract was found to be higher than other extract of aerial parts of Artemisia pallens. It showed ethanolic extract contain much more chemical constituents other than various extract.

Phytochemical screening

The results of the phytochemical screening were carried out on the various extracts and recorded as shown in table no. 03. Preliminary phytochemical revealed the presence of saponins, phytosterols, carbohydrates, proteins and amino acid, and flavonoids in all extract of aerial parts of *Artemisia pallens*. Phytoconstituents in the various part of the plant vary significantly.

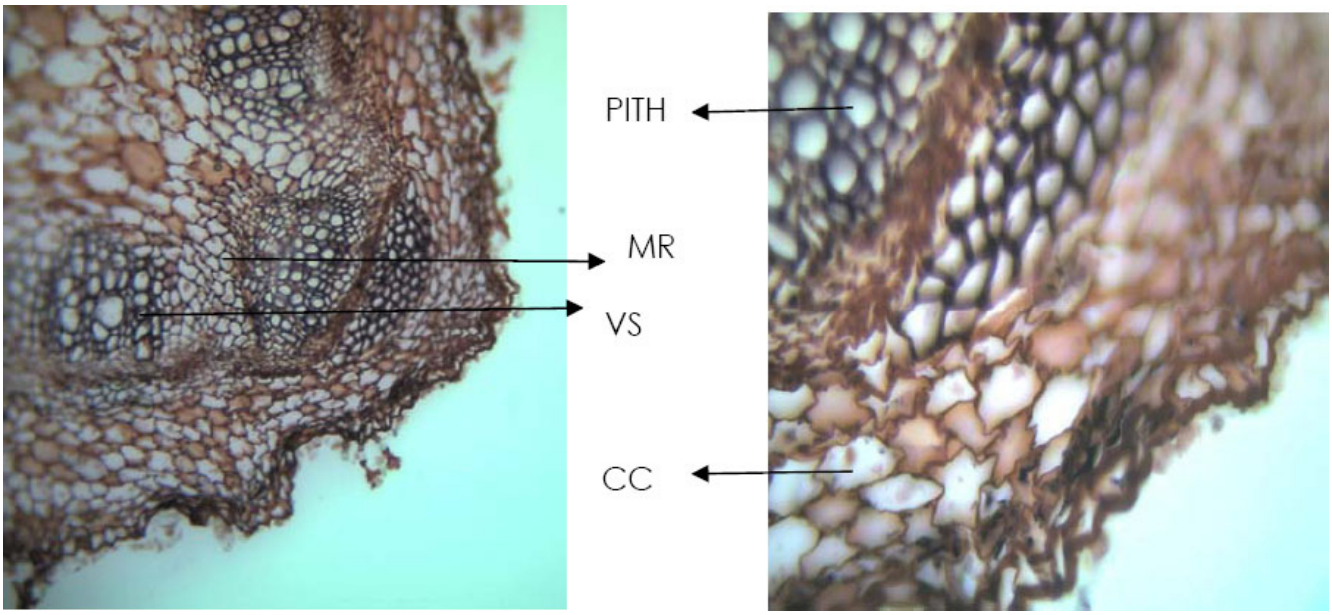

Figure1: T.S. stem of *Artemisia pallens*(× 10X, × 40X)

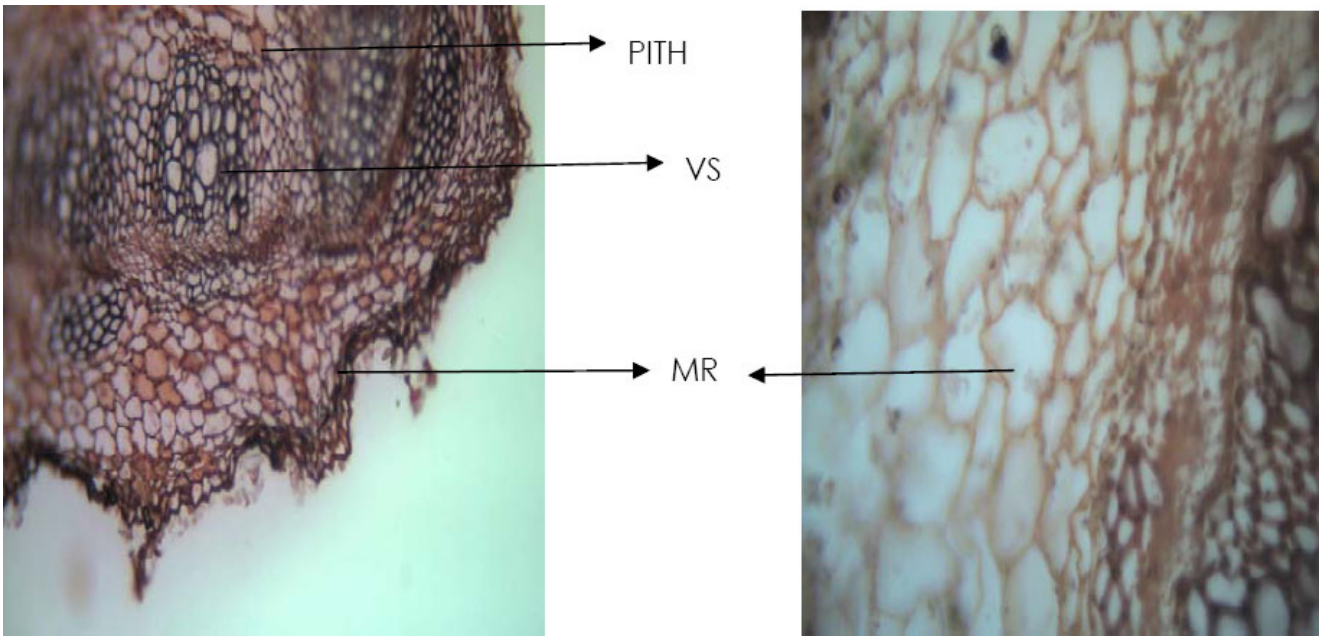

Figure 2: T.S. stem of *Artemisia pallens* (× 10X, × 40X)
Abbreviation:- CC-Cork cambium; CK-Cork cell; MR-Medullary ray; PH-Phloem; Vs-Vessels

The plant contains ascorbic acid and phenolics both which are powerful antioxidants. The presence of saponins protects plant from microbial pathogens. Flavonoidal extracts of aerial parts of *Artemisia pallens* may be shown anti-inflammatory activity.

Flavonoids acts as an anti-inflammatory response in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins[4]. Further studies may reveal the extract mechanisms of action responsible for the analgesic and anti-inflammatory activities of *Artemisia pallens*.

Results reveals that the all extract has large number of phytoconstituents, which may be responsible for many pharmacological activities, further work is required to investigate the all extracts of aerial parts of *Artemisia pallens* for various pharmacological activities.

ACKNOWLEDGEMENTS

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Evaluation of *In vitro* Antioxidant Activity of Some Plants of Cachar District, Assam.

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ABSTRACT

The present study was undertaken to evaluate *in vitro* antioxidant activity of methanolic extract from leaves of four plants viz. *Clerodendron colebrookianum* Walp. (Verbinaceae), *Gnetum gnemon* L. (Gnetaceae), *Sarcochlamys pulcherrima* (Roxb.) Gaud. (Urticaceae), *Garcinia lancifolia* (Don) Roxb. (Cluciaceae), from Cachar district, Assam, India. DPPH (1,1-diphenyl 2-picrylhydrazyl) radical scavenging capacity, reducing power assay (RPA) and photochemiluminescence (PCL) assay were used for evaluating *in vitro* antioxidant activity. Total phenolic content (TPC) was estimated by Folin–Ciocalteu's method. *Sarcochlamys pulcherrima* showed highest antioxidant activity (DPPH EC_{50} 9.70 ±1.51ppm), as compared to *C. colebrookianum* (121.05 ±1.09ppm), *G. gnemon* (255.99 ±0.82ppm), and *G. lancifolia* (344.96 ±0.76ppm). Highest activity of *S. pulcherrima* was also supported by RPA and PCL and highest TPC (0.33 mg gallic acid equivalent/mg of dry extract) amongst the plants, indicated that phenolic compound are mainly responsible for the activity.

Keywords: S. pulcherrima, Antioxidant activity, DPPH, PCL, RPA, TPC.
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INTRODUCTION

Reactive oxygen species (ROS) viz. superoxide radicals, hydroxyl radicals, and hydrogen peroxide are generated as byproducts of biological reactions during normal cell aerobic respiration. Oxidative damage may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations leading to cancer, atherosclerosis, hypertension and other diseases [1].

Natural antioxidants with multifunctional potential are of high interest as alternatives for synthetic antioxidants to prevent oxidation. Northeast India is bestowed with innumerable flora of the world and *Clerodendron colebrookianum, Gnetum gnemon, Sarcochlamys pulcherrima* and *Garcinia lancifolia* are unexplored plants for antioxidant activity. Tender leaves of all plants are used as vegetables by *Khasi* and *Naga* tribe of Machkhal, Binnakandi, Ramnagar, and other tribal pockets of Cachar District, Assam. Tribal people of this area use the water decoction of leaves of *C. colebrookianum* to cure high blood pressure. Leaves of *G. gnemon* are made into paste and applied in athlete's foot. *S. pulcherrima* leaves are claimed to damage tape worm egg present in pork when boiled with it. Leaf juice of *G. lancifolia* is taken in headache. Although, previous study showed presence of different class of phytochemicals in the leaves of *C. colebrookianum* [2–8], and *G. gnemon*[9–10], no phytochemical report was found in the case of *Sarcochlamys pulcherrima* and *Garcinia lancifolia* leaves. Moreover, *in vitro* antioxidant activities of leaves of these plants were not explored yet.

Though these tribal peoples are residing in a tough condition involving much physical labour in every aspect of survival but they are able to bear up. Intake of antioxidant is reported as a remedy for fatigue and tiredness [11–12]. We hypothesized that the antioxidant intake (unknown to them) through the consumption of these medicinal vegetable may be the reason of sound health and physical stamina of these tribal peoples. Therefore, the present investigation was undertaken to study the antioxidant potential of these herbs and to

put forward the evidence of the fact that these plants are having good antioxidant activity.

MATERIAL AND METHODS

Plant material and extraction

Leaves of four plants viz. *Clerodendron colebrookianum* Walp. (Verbinaceae), *Gnetum gnemon* L. (Gnetaceae), *Sarcochlamys pulcherrima* (Roxb.) Gaud. (Urticaceae), *Garcinia lancifolia* (Don) Roxb. (Cluciaceae) were collected from Arun Punjee (Tribal Village) of Machkhal, Cachar District, Assam, India, during the month of April and authenticated by Botanical Survey of India, Shillong, Meghalaya and voucher specimen were kept in the repository of Phytochemistry Division, Defence Research Laboratory, Tezpur for future reference. The plant material was extracted by cold maceration in mixture of methanol and water (80:20). Further, solvent was evaporated to dryness in rotary evaporator under reduced pressure and kept in refrigerator (0°C) for future uses.

Evaluation of Antioxidant Activity

Radical scavenging activity by DPPH method

Radical scavenging activity of the sample extracts were measured by colorimetric assay using DPPH as a source of free radical and according to the method of Blois[13]. Briefly, 1 ml of the crude extract solution at variable concentrations (2.5 –1000 µg/ml in methanol) was added to 1 ml of a DPPH solution at concentration 40µg/ml in methanol, kept for 35 min at room temperature until to produce stable colour and absorbance was measured at 517 nm using a SPECORD-200 UV–vis spectrophotometer (Analytic Jena AG, Jena, Germany). L-Ascorbic acid was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_s/A_0) \times 100]$$

where A_0 is the absorbance of the control, and A_s is the absorbance of extract solution or the standard sample.

Different concentrations of each of the extracts were selected in such a way that percentage of inhibition of colour of DPPH solution is above and below 50%. EC_{50} value was obtained from the linear regression equation.

Reducing power assay

In this assay the ability of the extract to reduce Fe^{3+} to Fe^{2+} was estimated. Reducing power of the extracts was determined by using potassium ferricyanide-ferric chloride system[14]. Briefly, 1ml of extract at different concentration 1000, 500, 250µg/ml was added with 2.5ml of 0.2 M phosphate buffer (pH 6.6), mixed with 2.5ml of potassium ferricyanide (0.1%) and the mixture was incubated at 50°C for 20 min. 2.5ml of Trichloroacetic acid (10%) was added to the reaction mixture and centrifuged at 10000*g* for 10 min and 2.5ml of supernatant was mixed with equal volume of distilled water & 0.5ml of 0.1% ferric chloride was added and the absorbance was measured at 700 nm using a SPECORD-200 UV–Vis spectrophotometer (Analytic Jena AG, Jena, Germany). Increased absorbance of the reaction mixture indicated the increased reducing power.

Total antioxidant activity by Photochemilunescence

Total antioxidant activity was determined by *Photochemilunescence* method using Integral antioxidative capacity of lipid soluble substances (ACL) kit in the antioxidant analyser called Photochem® (Analytic jena AG, Jena, Germany) [15]. In this method, a photosensitizer substance in standardised volumes acts as a source of superoxide anion radical which produces the radicals by optical excitation. Residual radicals remained after partly reacting with the antioxidants present in the sample cause the detector substance luminol to luminesce. The luminescence is then determined.

ESTIMATION OF TOTAL PHENOLIC CONTENT

Total Phenolic content (TPC) were measured by *Folin-Ciocalteu's* method [16]. Briefly, 1 ml of each sample (1000 µg/ml) was added with 25ml distilled water and 5ml of Folin-Ciocalteu's phenol reagent followed by 15 ml of Na_2CO_3 (20%, w/v) and made up to 100ml followed by incubation at room temperature for 2 hrs. The absorbance of all samples was measured at 760 nm using a SPECORD-200 UV–vis spectrophotometer (Analytic Jena AG, Jena, Germany). Results were expressed as milligram of gallic acid equivalent per milligram of dry weight (mg GAE/ mg dw).

RESULTS AND DISCUSSION

Radical scavenging activity by DPPH method

DPPH method allows estimation of hydrogen radical donating ability of the test extract. This model represents the situation in metabolic system where an antioxidant will stabilise a free radical by reacting with hydrogen radical. The results are expressed in EC_{50} (Effective Concentration to reduce initial concentration of DPPH to 50%). Lesser the EC_{50} value for an extract is associated with higher ability to donate hydrogen radical i. e. antioxidant

activity. In the present study EC_{50} values were found to be 121.05, 255.99, 9.70 and 344.96 for *C. colebrookianum, G. gnemon, S. pulcherrima and G. lancifolia* respectively. These data clearly indicate that *S. pulcherrima* is promising radical scavenger.

Reducing power assay

The results are expressed as absorbance X 100 and results are 14.40, 8.37, 161.8 and 7.02 for *C. colebrookianum, G. gnemon, S. pulcherrima and G. lancifolia* respectively. Higher absorbance represents higher reducing ability of an extract. Out of the four plants, highest absorbance was observed in the case of *S. pulcherrima* (161.8), which is in accordance with the result of DPPH assay.

Total antioxidant activity by Photochemilunescence

This automated method allows precise determination of antioxidant activity. Trolox at different concentration (0.5–5 nmol) was run as standard, and sample was diluted so that the results lie within the concentration range of the standard. The results were calculated considering the concentration and dilution factor. *S. pulcherrima* showed highest activity as reflected from the result (6462.63 nmol equivalent of Trolox/mg of dry extract).

Estimation of total phenolic content

Phenolic compound are having wide bioactivity including antioxidant. The antioxidant activity of phenolic compound is due to hydroxyl functional group, however other factors e.g., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. To establish the correlation of antioxidant activity indicated by different assays with phenolic compound, total phenolic content was estimated and observed that *S. pulcherrima* which showed higher antioxidant activity have highest phenolic content i.e., 0.33mg GAE /mg of dry extract.

The result can be explained with previous study as presence of phenolic content is responsible for antioxidant activity [17]. *S. pulcherrima* leaf extract contain highest phenolic content as compared to other plants and showed maximum antioxidant activity. Correlation coefficient between DPPH EC_{50} value and TPC is -0.777 but increases to -0.972, if the data of *C. colebrookianum* is excluded. This is due to the fact that *C. colebrookianum* may contain antioxidant active compound other than phenolics and hence alters the correlation. But in both the cases, there is strong correlation between DPPH EC_{50} value and TPC. Earlier studies show that DPPH and TPC value have a negative correlation i.e. as the TPC value increases, DPPH EC_{50} value decreases in similar fashion[18–20]. Previous studies are in agreement with present one, where *S. pulcherrima* showed lowest DPPH EC_{50}value and highest TPC value. Result of reducing power assay and total antioxidant activity determined by using Photochem®, further supports the highest activity of *S. pulcherrima* and considered as most potent antioxidant plant.

CONCLUSION

Our study provided the clue that leaves of *S. pulcherrima* is having potent *antioxidant* activity and the activity is due to the presence of phenolic compound. The plant is consumed by Khasi and Naga tribe of Cachar District, Assam and the present finding partially validate their traditional knowledge about the goodness of consumption of this medicinal plant as vegetable. Findings of the present work can provide a first hand information in developing antioxidant based antistress products. Further study is going on to isolate phytochemicals and *in vivo* antioxidant study to validate the *in vitro* antioxidant activity.

Table 1: Data of in vitro antioxidant activity of the plants in different bioassays.

Plant	DPPH EC_{50} [$]	PCL[†] (RSD %)	RPA[¥]	TPC[‡]
C. colebrookianum	121.05 ±1.09[a]	375.52 (0.03)[a]	14.40 ±2.24	0.0429 ± 1.92
G. gnemon	255.99 ±0.82	16.59 (2.53)	8.37 ±4.03	0.0452 ± 3.85
S. pulcherrima	9.70 ±1.51 [a]	6462.63 (0.84) [a]	161.8 ±3.10 [a]	0.33 ± 12.25 [a]
G.lancifolia	344.96 ±0.76	192.08 (0.39)	7.02 ±2.17	0.0443 ± 4.14

[a] Results are expressed as mean ±SD (n=6), except PCL data. The significant difference was analysed by one-way Anova followed by Tukeys post hoc test. $p<0.05$ was considered significant. Comparison is made between *G. lancifolia* with other plants.

[$] EC_{50} in µg/ml,

[†] In nmol equivalent of Trolox/mg of dry extract,

[‡] In mg Gallic Acid equivalent/mg of dried extract,

[¥] Absorbance given by 1000ppm solution of extract × 100

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Comparative evaluation of antimicrobial potential of different extracts of *Cuscuta reflexa* growing on *Acacia arabica* and *Zizyphus jujuba*

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Abstract

C.reflexa Roxb. (Cuscutaceae or Convolvulaceae) is a golden yellow leafless plant commonly known as amarbel, akashbel, swaranlata, devils guts, hair weed and love-wine. The methanol extracts of *C. reflexa* is implicated as an antimicrobial. Plant extracts of *C.reflexa* growing on different sources (*Acacia arabica* and *Zizyphus jujube*) were prepared using aqueous and various organic solvents viz. benzene, acetone, ethanol and methanol. Agar well diffusion technique was used to assess the antimicrobial potential of plant from different sources against gram positive bacteria (*Staphylococcus aureus* & *Staphylococcus epidermidis*), gram-negative bacteria (*Escherichia coli* & *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger*). The diameter of zone of inhibition was taken as an indicator of antimicrobial effect. The present study showed a strong inhibitory effect of ethanol and methanol extracts of *C.reflexa* (*jujuba* and *arabica*) on most of the gram positive and gram negative bacteria. The aqueous extract of *C.reflexa* (*arabica*) failed to show any antimicrobial activity while *C.reflexa* (*jujuba*) showed very little effect. Thus *C.reflexa* growing on *Zizyphus jujuba* could be considered as a potential source of natural antimicrobials.

Keywords: C. reflexa, antimicrobial activity, zone of inhibition.

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INTRODUCTION

Over the last few decades, a great interest has developed in searching for antimicrobial drugs from natural plant products. This interest primarily arises from the belief that drugs derived from plants are safe and dependable compared with synthetic drugs that may have adverse effects on host besides their high cost. Natural antimicrobials come from a wide array of sources including plants, animals and microorganisms[1]. Researchers have so far discovered approximately over 10,000 biologically active compounds of microbial origin[2]. Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases[3,4]. Therefore, there is an exigency to discover new and efficient antimicrobials from other sources such as plants[5,6].

Cuscuta reflexa has profound use in Ayurveda and folklore medicine. *C.reflexa* Roxb. (Cuscutaceae or Convolvulaceae) is a golden yellow leafless plant with zig-zag stem, mostly found in all the region[7,8]. It has no underground roots and grows commonly on *Zizyphus jujuba, Clerodendron inerme, Acacia arabica* and other shrubs and trees as a parasitic twiner[9]. Being parasitic plant it earn sustenance from host plant[10]. White crystalline compound cuscutalin and yellow crystalline solid cuscutin ($C_{15}H_{12}O_9$) have been isolated from the hot alcohol extract of *C. reflexa* stem[11]. In the present study, an attempt was made to screen comparative potential of different extracts of *C.reflexa* obtained from two sources *Acacia arabica* and *Zizyphus jujuba* for its antimicrobial action against Gram-positive and Gram-negative bacteria and fungi.

MATERIAL AND METHOD

Cuscuta reflexa was collected from two different host plants i.e. *Acacia arabica* and *Zizyphus jujuba* from wild areas of Hisar and Jind in the month of November and were authenticated by Dr. H.B. Singh Director, Department of Raw Material Herbarium & Museum, National Institute of Sciences Communication and Information Resources,

New Delhi under reference no: NISCAIR/RHMD/Consult/-2008-09/1136/168. The stems of plant (both sources) were dried and then grinded.

MICROORGANISMS

Strains, including fungi and bacteria were obtained from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. Two gram-positive strains, *Staphylococcus aureus* and *Staphylococcus epidermidis*, one gram-negative strains, *Escherichia coli* and one fungus; *Aspergilus niger* were used in the present investigation as test organisms.

CULTURE MEDIA AND CHEMICALS

The culture media, Nutrient Agar (NA), Nutrient Broth (NB) and Czapek Yeast Extract Agar (CYA) were purchased from Hi- Media. All other chemicals, including organic solvents used for the extraction of the plant metabolites, were of analytical grade.

PREPARATION OF PLANT EXTRACTS

130 gm of coarsely ground plant material from both the sources were defatted with petroleum ether (60–80°C) and then the solvent free marcs were extracted with benzene, acetone, methanol and 90% ethanol, using soxhlet. The marcs were extracted with distilled water by maceration. The solvents were removed by *in-vacuo* in rotary evaporator. The dried extracts were stored in a desiccator till further comparative studies.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of all the extracts was determined, using the agar well diffusion assay method[12]. Approximately 20ml of molten and cooled media (NA and CYA) were poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination . The bacterial test organisms (*S. aureus, S. epidermidis* and *E. coli*) were grown in nutrient broth for 24 h. A 100µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. For *A. niger*, a spore suspension of the fungus was prepared in 2 ml sterilized distilled water in a test tube and 100µl of spore suspension was spread on CYA plates. Five agar wells (four on the periphery and one in the center) of 5mm diameter were prepared with the help of a sterilized stainless steel cork borer. The wells were loaded with 100µl of various extracts (for both plants). The central well in each plate were used as a control and loaded with 100µl of solvent or sterilized distilled water. Various antibiotics and fungicides (Table 2) were used as standard control. The plates containing the bacteria and extracts were incubated at 37°C (for *E. coli* and *S. epidermidis*) and at 30°C (for *S. aureus and A. niger*). All the tests were repeated in triplicates.

The antimicrobial activity was assessed on the basis of diameter of zone of inhibition, which was measured at cross-angles after 24 h of incubation (Table 1). Percent inhibition of bacterial/fungal microorganisms was calculated after subtracting the value of control (solvents) from the value of extracts.

STATISTICAL ANALYSIS

All the results were expressed as mean ±S.E.M. Data was analyzed using one way analysis of variance test (ANOVA), p values <0.05 were considered as statistically significant (13).

RESULTS AND DISCUSSION

All the plant extracts tested, exhibited a broad spectrum of antimicrobial activity against the tested microorganisms (Table 1). The aqueous extracts of *Cuscuta reflexa* (*Zizyphus arabica*) did not show any antimicrobial activity, but a significant ($p=0.05$) effect was seen in case of *C.reflexa* (*Zizyphus jujuba*) against *A. niger*. In addition, results show that most of the extracts were significantly effective ($p=0.05$) against the fungus *A. niger*. Moreover, the ethanol and methanol extracts of *C.reflexa* showed significant results ($p=0.05$) against all tested microorganisms with the inhibition zone ranging from 8.2 to 26.8 mm and 13.3 to 25.6mm, respectively, this antimicrobial effect were acceptable with respect to the standard antibiotics (Table 2). The activity of methanol and ethanol extracts is attributed due Dulcitol which is reported in plant as a major component in addition to luteolin and quercitin . Benzene extracts of *Cuscuta reflexa (Zizyphus jujuba*) do not exhibit significant antimicrobial activity against the test microorganisms. The acetone extracts were effective ($p=0.05$) against most test microorganisms. *E. coli* was quite resistant to all the extracts. However, *S. aureus*, the common wound pathogen, was sensitive to most of the extracts. The results support the use of this plant in folklore medicine for treatment of infectious diseases especially for *C.reflexa* (*Zizyphus jujuba*).

CONCLUSION

It is evident from the present study that the extracts of *C.reflexa* (both sources) are active against the tested

Table 1. *In vitro* antimicrobial activity (zone of inhibition in mm) comparatively various extracts of *Cuscuta reflexa* collected from two different sources i.e. *Acacia arabica* and *Zizyphus jujuba*

Extract	Organism	Control	C.*reflexa* (Acacia Arabica)	C.*reflexa* (*Zizyphus jujuba*)
Ethanol	***Staphylococcus aureus***	12.0±0.1	14.3±0.3 (20)	26.8±0.4* (134)
	Staphylococcus epidermidis	12.0±0.1	–	18.8±0.2 (61)
	Escherchia coli	13.0±0.2	8.2±0.2 (–4)	11.8±0.2 (–10)
	Pseudomonas aeruginosa	11.3±0.3	25.1±0.2* (133)	13.8±0.2 (24)
	Aspergillus niger	8.6 ±0.1	13.5±0.1 (64)	21.8±0.1 (22)
Benzene	***Staphylococcus aureus***	7.2±0.2	12.5±0.1 (85)	11.0±0.2 (61)
	Staphylococcus epidermidis	4.5±0.1	15.5±0.2* (314)	12.1±0.2* (217)
	Escherchia coli	20.0±0.3	11.8±0.1 (–43)	–
	Pseudomonas aeruginosa	–	11.0±0.1	–
	Aspergillus niger	11.0±0.4	15.1±0.4 (41)	14.0±0.1 (30)
Acetone	***Staphylococcus aureus***	8.0±0.2	8.3±0.4 (4)	7.2 (–11)
	Staphylococcus epidermidis	8.3±0.1	12.5±0.2 (58)	10.3 (27)
	Escherchia coli	12.0±0.5	17.0±0.4 (45)	–
	Pseudomonas aeruginosa	–	–	–
	Aspergillus niger	13.0 ±0.6	15.2±0.2 (18)	15.5 (21)
Methanol	***Staphylococcus aureus***	12.0±0.3	14.4±0.2 (22)	13.3±0.2 (11)
	Staphylococcus epidermidis	9.8±0.4	15.5±0.3(64)	13.6±0.2 (43)
	Escherchia coli	11.6±0.3	10.4±0.4 (–11)	12.9±0.2 (12)
	Pseudomonas aeruginosa	12.1±0.2	13.6±0.4 (14)	15.6±0.2 (32)
	Aspergillus niger	11.8±0.1	*14.6*±0.2	25.6±0.2* (128)
Aqueous	***Staphylococcus aureus***	–	–	–
	Staphylococcus epidermidis	–	–	–
	Escherchia coli	–	–	–
	Pseudomonas aeruginosa	–	–	13.8
	Aspergillus niger	–	–	17.5

The values in parenthesis show percent increase or decrease (minus values) over the control excluding the diameter of agar wells.
– = No activity

Table 2. Antimicrobial activities of various antibiotics used as positive controls.

Antibiotics	Diameter of zone of inhibition (mm)			
	Escherchia coli	**Staphylococcus aureus**	**S.epidermidis**	***Aspergillus niger***
Streptomycin	15±0.1	16±0.2	12±0.7	N.D
Penicillin	17±0.4	16±0.3	14±0.2	N.D
Ampicilin	18±0.2	17±0.1	15±0.4	N.D
Tetracycline	21±0.1	21±0.2	17 ± 0.3	N.D
Fluconazole	N.D	N.D	N.D	15±0.2
Clotrimazol	N.D	N.D	N.D	17±0.3
Ketoconazole	N.D	N.D	N.D	12±0.2

All values represent ±S.E. of the mean (n=3), * *= significant (p< 0.05) compared to positive control.*

microorganisms. Based on results, *C. reflexa* (jujuba) had dominant antimicrobial activity in comparison to *C.reflexa* (arabica). Further work on isolation of various responsible plant active principles is required to ascertain the antimicrobial potential of *C.reflexa*.

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Pharmacognostic and Phytochemical Studies of Bark of *Oroxylum indicum*

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ABSTRACT

Oroxylum indicum Vent. (Family- Bignoniaceae) syn: Sonapatha & Shyonaka is generally found in sub Himalayan region of India, which yet to be fully explored in terms of medicinal plants. It posses anti-inflammatory, anthelmintic, antihepatotoxic, anticancer, immunomodulator, gastroprotective properties as earlier reported in the literature and hence is an active ingredient of several Ayurvedic formulations like Chyawanprash and Dashmoolarishth etc. It is one of the important 'Rasayana' drugs mentioned in ayurveda.
Preliminary phamacognostic studies were carried out on the bark including morphological, microscopical, physicochemical studies alongwith phytochemical screening. The protocol followed for elucidating physicochemical properties were as per WHO guidelines. The successive extraction of plant bark was undertaken by using various solvents of increasing polarity and the extracts thus obtained were subjected for phytochemical analysis, followed by thin layer chromatographic examination by optimizing the solvent systems. The phytochemical investigation revealed the presence of alkaloids, glycosides, flavonoids, and phenolic compounds mainly. These preliminary data may be helpful in developing the standardization parameters of *Oroxylum indicum* bark.

Keywords: Medicinal plants, Micoroscopy, Physicochemical properties, Standardization, TLC.

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INTRODUCTION

Oroxylum indicum Vent. (Family- Bignoniaceae) syn: Shyonaka, also known as Sonapatha is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years[1]. *Oroxylum indicum* have been used as a single drug or as a component of certain poly-herbal drug preparations in Indian Ayurvedic system of medicine. It is active ingredient of well known Ayurvedic formulations like Chyavanprash, Dashmularista etc[2]. The root bark and stem bark possess antiallergic properties and are used in treating allergic disorders, urticaria, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhoea, dysentery, erythema and measles[3-4]. The normal dose is 8 to 16 g of bark in the form of decoction, extract or powder. The seeds are active in chronic cough and gastralgia at dose of 5 to 10 g daily in the form of a decoction or powder[4-5]. An alcoholic maceration of fresh bark is applied externally for lacquer allergic dermatitis. The fruits of *Oroxylum indicum* are acrid, sweet, stomachic, anthelmintic, and good in diseases of the heart and the throat, piles, bronchitis, used as an expectorant, improves the appetite, useful in leucoderma[2, 6-10].

Oroxylum indicum is native to the Indian subcontinent, in the Himalayan foothills with parts extending to Bhutan and southern China, in Indo-China and the Malysia ecozone. It is visible in the forest biome of Manas National Park in Assam, India. It is also reported from Sri Lanka (Ceylon)[5].

Various studies have been carried out for pharmacognostical evaluation of leaves and roots of *Oroxylum indicum* Vent. But the stem bark of the plant, having a valuable medicinal importance, has not yet been undertaken for the purpose of pharmacognostical evaluation. Therefore the present study was undertaken to evaluate the stem bark of *Oroxylum indicum* Vent. to generate the standardization parameters for the plant.

MATERIAL AND METHODS

PLANT MATERIAL

The stem bark of *Oroxylum indicum* Vent. was collected by self, in the month of November 2009, airdried under shade and were authenticated by Central Instrumentation Facility, National Botanical Research Institute (NBRI), Lucknow (India). A voucher specimen (no. NBRI/CIF/128/2010) was deposited for future reference. Organoleptic evaluation was carried out with intact bark; bark was pulverized in the mechanical grinder to a moderate fine powder to carry out microscopic studies and was stored in a well closed airtight vessel for further analysis

REAGENTS AND CHEMICALS

All reagent and chemicals used for the study were of analytical grade and purchased from S.D. Fine Chemicals Pvt. Ltd, Mumbai, through the authorized dealer Sohan Lal and Sons, Lucknow.

ORGANOLEPTIC AND MICROSCOPICAL EVALUATION

The freshly collected bark of the plant were evenly spread on a clean dry plastic sheet and investigated for different organoleptic features by repeated observations by conventional methods and were recorded. For microscopical evaluation fine sections of the bark were taken with the help of microtome, stained and temporarily mounted following usual microtechniques and representative diagrams were taken with the help of inverted microscope for photodocumentation. The different powder characteristics were studied according to standard methods. Separate slides were prepared for observation of lignified tissues (phloroglucinol + HCl), starch (iodine solution) and non-lignified characters, oil glands etc.[11]

STAINING CHARACTERISTICS OF BARK POWDER

Each time a small amount of powdered bark was treated with different types of chemical reagents as mentioned in Table-2 and the colour characteristics were observed in day light[12].

PHYSICO-CHEMICAL EVALUATIONS

Physico-chemical parameters such as the total ash, acid insoluble ash, acid soluble ash, water insoluble ash, water soluble ash were determined as per reported methods. Considering the diversity of chemical nature and properties of contents of drugs, three different solvents water, methanol and petroleum ether were used for determination of extractive values as per reported methods[12-14]. All determinations were performed thrice and the results were presented as mean ± standard error of mean (SEM).

PHYTOCHEMICAL SCREENING

The dried powdered bark was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents. The dried powdered bark (100 g) was extracted successively with petroleum ether (60–80°C), ethyl acetate, methanol and water in a soxhlet extractor by continuous hot percolation. Each time before extracting with the next solvent of higher polarity the powdered material (marc) was dried in a hot air oven below 50°C for 10 minutes. Each extract was concentrated in vacuum on a rota evaporator (buchi type) and finally dried in hot air oven to calculate the extractive yield with respect to air dried drug. The dried extracts were again dissolved in respective solvents, with which it was extracted, and were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material[15].

THIN LAYER CHROMATOGRAPHIC STUDIES PREPARATION OF SAMPLE

The successive solvent extracts were collected and evaporated on a rota evaporator. The extracts were carefully dried in a hot air oven. 10 mg of each extract was taken and it was dissolved in minimum amount of respective solvent with which it was extracted. This solution was used for the application of spot on the chromatographic plate.

DEVELOPMENT METHOD

One dimensional ascending method by using standard protocol was followed. Methnolic extract was applied 1cm above from the base of the TLC plates .Developmet was done using various solvent systems specific for a class of secondary metabolites.

VISUALIZATION

Development of chromatograms were carried out byusing vanillin-H_2SO_4 reagent as detecting agent, followed by careful gentle heating at 105°C, for 5 minutes till the spots were developed, thereafter observing under UV radiation (366 nm & 254nm)[16-17].

DOCUMENTATION

After visualization as mentioned above different spots were detected. The R_f values of those spots were recorded carefully and the chromatograms were documented by digital photography and scanography.

RESULTS

ORGANOLEPTIC AND MICROSCOPICAL EVALUATION

The organoleptic characters found are described below in table-1.

Table 1: Organoleptic characters of bark of *Oroxylum indicum* Vent.

Characters	Description
Color	Inner surface brown, outer surface light brown
Odour	Characteristic
Taste	Astringent
Size	Approx.10 cm long, 5 cm in width
Texture	Hard
Fracture	Fibrous with snap, easily with longitudinal surface, with sharp snap from transverse surface

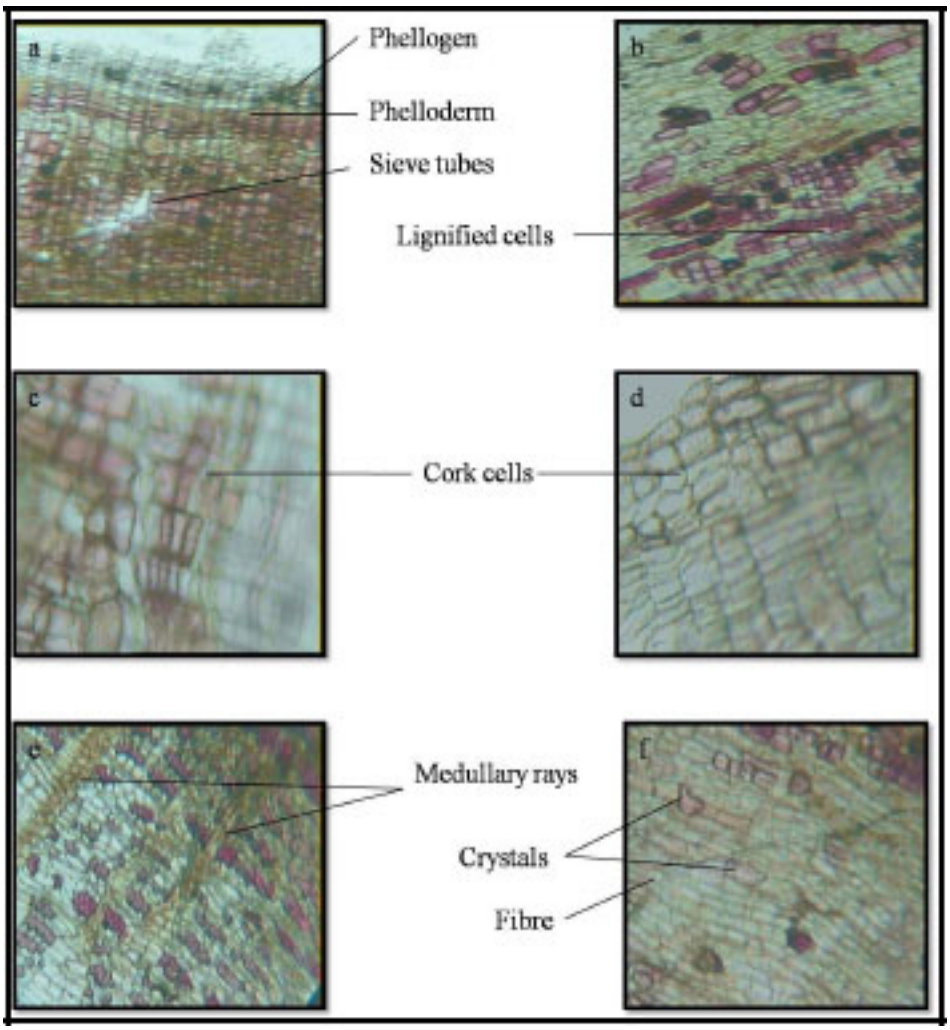


Figure 1: Microscopical characters of t.s. of bark of *Oroxylum indicum* Vent."

a) Phellogen, phelloderm and sieve tubes (stained with safranin at 4x)
b) Lignified cells, sclereids (stained with phloroglucinol at 4x)
c) Cork cells (stained with safranin at 10x)
d) Parenchymatous cells (mounted with glycerine at 4x)
e) Medullary rays (stained with phloroglucinol at 4x)
f) Lignified cells, calcium oxalate crystals and fibre (stained with safranine at 4x)

COLOUR CHARACTERISTICS OF BARK POWDER

The observations of colour reactions are reported in Table-2

Table 2 : Colour reactions of bark with different reagents

Stain	Observation
Powder treated with Iodine	Bluish black starch grains observed.
Powder treated with Sudan red	Orange-pink colored oil observed.
Powder treated with Acetic acid	Ca-oxalate crystals absent.
Powder treated with Phloroglucinol and dil. HCl	Pink colored lignified cells observed.
Powder treated with Ferric chloride	Tannins Present.

PHYSICO-CHEMICAL EVALUATIONS

The physicochemical studies of stem bark of *Oroxylum indicum* Vent. are summarized in table-3.

Table 3: Physicochemical properties of *Oroxylum indicum* Vent.

Quantitative standards	% w/w
Total Ash Value	11.767±0.232%*
Acid insoluble ash	3.270±0.070%*
Water soluble ash	2.10%
Moisture content	13.845±0.737%*
Petroleum ether soluble extractives	0.56±0.046%*
Alcohol soluble extractives	9.44±0.167%*
Water soluble extractives	19.167±0.581%*

* Values are expressed as Mean±SEM, when all the experiments were performed thrice.

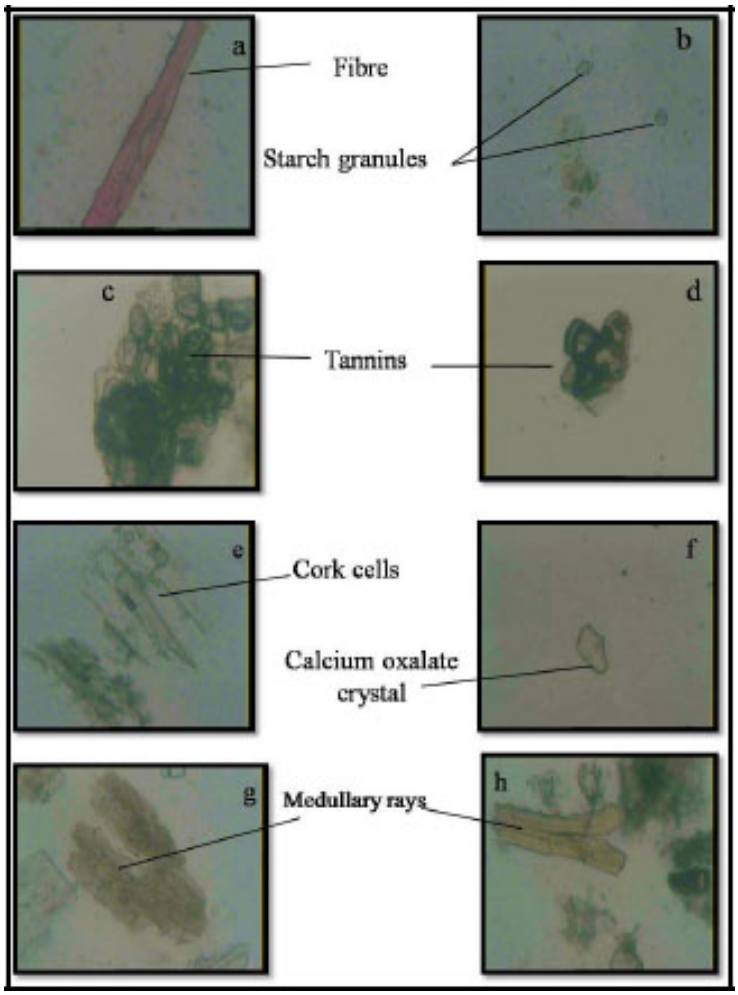


Figure 2: Powder characterstics of bark of *Oroxylum indicum* Vent."
a-fibre, b- starch granules, c & d- tannins, e- cork cells, f- calcium oxalate crystals, g & h- medullary rays and cork cells

The percentage yield in successive solvent extraction is summarized in table-4

Table 4: Percentage yield in successive solvent extraction

Extracts	% Yield
Petroleum Ether Extract	0.58%
Ethyl acetate Extract	2.16%
Methanol Extract	2.87%
Aqueous Extract	18.34%

PHYTOCHEMICAL SCREENING

The results demonstrated presence of carbohydrates, steroids, tannins, alkaloids, glycosides, flavonoids mainly in the stem bark of *Oroxylum indicum*.

The presences of various phytoconstituents in various extracts are summarized in table-5.

THIN LAYER CHROMATOGRAPHIC STUDIES

TLC profile of methanol extract in various optimized solvent systems of *Oroxylum indicum* bark is summarized in table-6.

DISCUSSION & CONCLUSION

Pharmacognosy is primarily the study of natural substances, mainly plants having a role either in prophylaxis or therapeutics of any disease/disorder. Pharmacognosy enfolds the knowledge of history, distribution, cultivation, collection, processing for market and preservation, the study of organoleptic, physical, chemical and the uses of crude drugs. The objective of Pharmacognosy is to contribute towards establishment of rational relationship between the chemical moieties of naturally occurring drugs and their biological and therapeutic effects, which ultimately helps in the standardization of the plant[18].

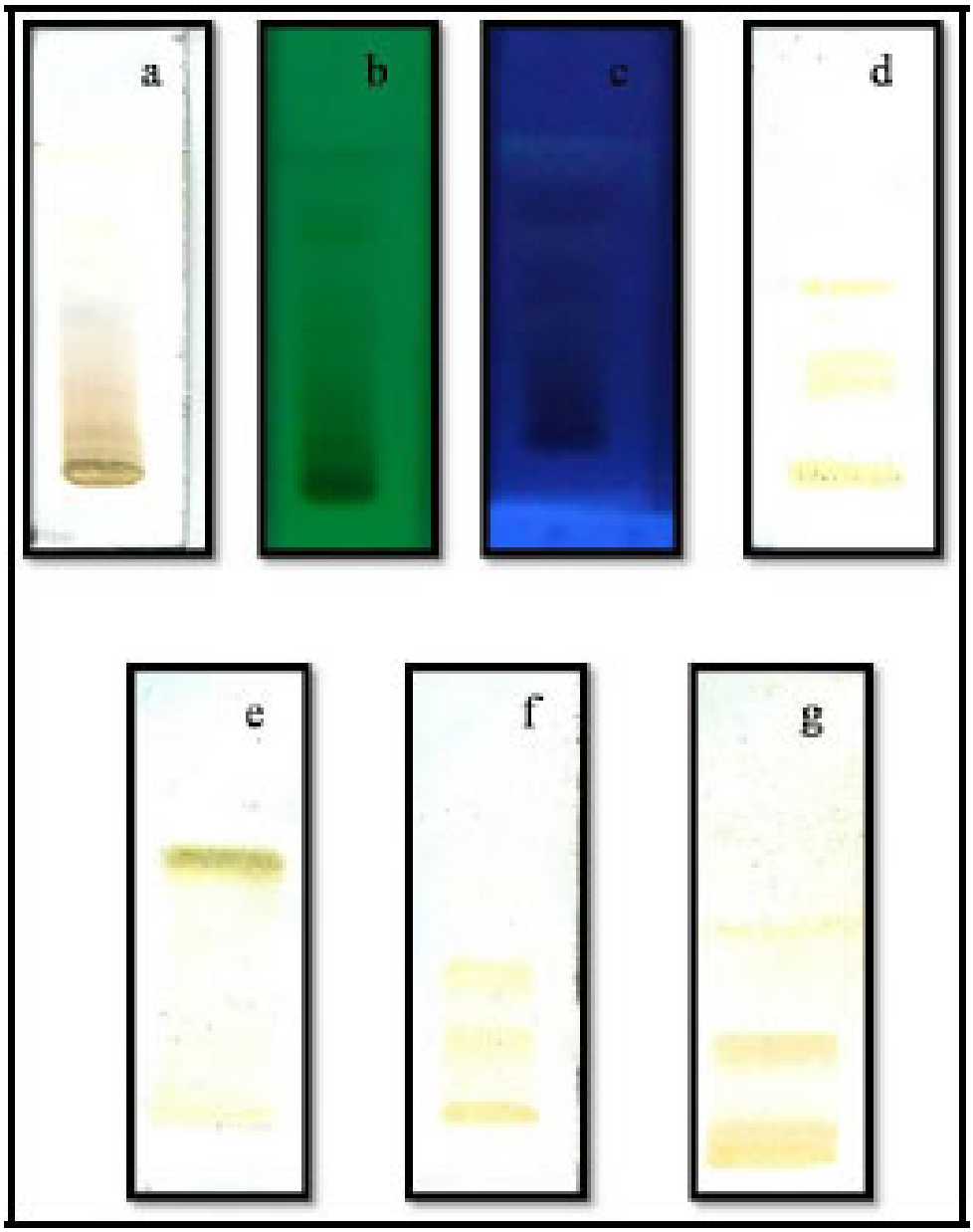
Table 5: Phytoconstituents present in various extracts

Phytoconstituent	Petroleum Ether Extract	Ethyl acetate Extract	Methanol Extract	Aqueous Extract
Carbohydrates	+	-	-	-
Steroids/ Terpenoids	-	+	-	
Tannins and Phenolic compounds	-	+	+	-
Alkaloids	-	-	+	+
Glycoside	-	-	+	+
Saponins	-	-	-	+
Flavonoids	-	+	+	-
Fixed oils & Fats	+	-	-	-

+ Present, - Absent

Table 6: TLC analysis of methanol extract of plant

Solvent System	Visualizing Agent	Rf values
Chloroform: methanol: ammonia: (7:3:1)	Vanillin –sulphuric acid reagent,	0.06, 0.12, 0.47, 0.55, 0.73, 0.76, 0.82
	UV 366 nm	0.06, 0.12, 0.47, 0.55, 0.76, 0.82
	UV 254 nm	0.12, 0.47, 0.55, 0.76, 0.82
Toluene: Pyridine: formic acid (7: 2.5: 1.5)	Vanillin–sulphuric acid reagent	0.29, 0.66
Ethyl acetate: Pyridine: Water: Methanol (8:3:1:1)	Vanillin–sulphuric acid reagent	0.25, 0.87
Chloroform: methanol: water (9:1:1)	Vanillin –sulphuric acid reagent,	0.08, 0.30, 0.58
	UV 254 nm	0.68
Ethyl acetate: benzene (40:60)	Vanillin –sulphuric acid reagent,	0.19, 0.35
	UV 254 nm	0.58, 0.67



"Figure 3: TLC finger printing profile of *Oroxylum indicum* Vent."

a) Chromatograms developed in Chloroform: methanol: ammonia: (7:3:1) after spraying with vanillin-suplhuric acid reagent
b) Chromatograms developed in Chloroform: methanol: ammonia: (7:3:1) under UV 366 nm
c) Chromatograms developed in Chloroform: methanol: ammonia: (7:3:1) under UV 254 nm
d) Chromatograms developed in Toluene: Pyridine: formic acid (7: 2.5: 1.5) after spraying with vanillin-suplhuric acid reagent
e) Chromatograms developed in Ethyl acetate: Pyridine: Water: Methanol (8:3:1:1) after spraying with vanillin-suplhuric acid reagent
f) Chromatograms developed in Chloroform: methanol: water (9:1:1) after spraying with vanillin-suplhuric acid reagent
g) Chromatograms developed in Ethyl acetate: benzene (40:60) after spraying with vanillin-suplhuric acid reagent

Morphological and microscopical characterstics of a plant are of great importance and used for the standardization studies of plant. A detailed study of *Oroxylum indicum* Vent. showed the various morphological as well as microscopical characters. The microscopical study of bark showed the presence of cork cells, phellogen, phelloderm, medullary rays, phloem fibres, sclereids, calcium oxalate crystals and sieve tubes. The cells present in the phallogen were thin walled and 8-10 layered. Just below the phellogen 4 to 5 layered lignified cells was observed followed by medullary rays and sieve tubes. Stone cells was present in groups and smallest group of stone cells consist of 3 to 4 cells but there were also group of upto 100 stone cells was also observed. Powder microscopy showed that the starch grains present in the bark were of subspherical nature and calcium oxalate crystals was of prismatic type. Secondary metabolites like tannins were presents within the cork cells.

The physical evaluation furnished different ash values, extractive values in three different solvents. Total ash, acid insoluble ash and water soluble ash values were also determined. Methanol soluble extractive value was found to be more than pet. ether soluble extractive value however it was less than water soluble extractive value. The phytochemical investigation revealed the presence of alkaloids, glycosides, flavonoids, steroids and phenolic compounds mainly in the bark of *Oroxylum indicum* Vent. Petroleum ether extract was found positive for carbohydrates and fixed oils whereas ethylacetate extract showed the presence of steroids, flavonoids and phenolic compounds mainly.

Alkaloids were present in methanol and water extract whereas glycosides were present only in water extract. Thus a vast array of compounds was found to be present in the plant under present study. The TLC analysis was performed by usual technique using the optimized solvent systems specific for the various classes of compound. Several detecting reagents were used to visualize the chromatograms but in present study most of the chromatograms were detected by vanillin-H_2SO_4 reagent. Thus a variety of standardization parameters viz. morphological, microscopical, physico-chemical, phytochemical and chromatographic parameters were studied and data was generated for the assessment of quality of plant material, and also to check the adulteration and substitution etc., which may be helpful for future reference.

After present investigation it can be concluded that the pharmacognostic study of bark of *Oroxylum indicum*, have furnished a set of qualitative and quantitative parameters that can serve as an important source of information which may substantiate the existing pharmacopoeial data to ascertain the identity and to determine and track the quality and purity of the plant material in future studies. The present results may serve as supplementary chromatographic data and the information thus generated may be explored further as useful tool for diagnostic features in addition to other properties *viz*. reactive pattern of powdered crude drug with different chemical reagents alongwith histological pattern of *Oroxylum indicum* bark and fluorescent characteristics of powdered crude drug on exposure to UV.

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Oroxylum indicum: A Review

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Abstract

Oroxylum indicum (Bignoniaceae), also known as Sonapatha or Shyonaka is commonly used herbal medicine in Ayurvedic system. Roots, leaves and stems of *Oroxylum indicum* have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine for treatment of various disorders as well as used as a tonic and Rasayana drug. It contains flavonoids like chrysine, baicalein and Oroxylin-A. Various studies indicated that sonapatha possesses anticancer, antioxidant, hepatoprotective and immunomodulaory properties mainly. Various other effects like antibacterial, analgesic and gastro-protective properties of sonapatha have also been reported. It is a tree that is found generally in damp region. In the present review an attempt has been made to compile and critically analyse various published reports on *Oroxylum indicum*.

Keywords: Oroxylum indicum, Ayurveda, anticancer

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INTRODUCTION

Oroxylum indicum also known as 'Sonapatha' is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years[1]. *Oroxylum indicum* has been used as a single drug or as a component of certain poly-herbal drug preparations in Indian system of medicine ie. Ayurveda. It is active ingredient of well known Ayurvedic formulations like Chyavanprash, Dashmularistha etc[2]. The root bark and stem bark possess antiallergic properties and are used in treating allergic disease, urticaria, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhoea, dysentery, infantile, erythema and measles[3–4]. The normal dose is reported 8 to 16 g of bark in the form of decoction, extract or powder[4–5]. The seeds are active in chronic cough and gastralgia: 5 to 10 g daily in the form of a decoction or powder and also used as purgative. An alcoholic maceration of fresh bark is applied externally for lacquer allergic dermatitis. The fruits of *Oroxylum indicum* are acrid, sweet, stomachic, anthelmintic, and good in diseases of the heart and the throat, piles, bronchitis, used as an expectorant, improves the appetite, useful in leucoderma[2, 6–10].

BOTANICAL DESCRIPTION

It is a tree which can attain a height of 12 meter (40 feet). The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. The tree is a night-bloomer and flowers are adapted to natural pollination by bats. They form enormous seed pods that hang down from bare branches. Those long fruits curve downward and resemble the wings of a large bird or dangling sickles or swords in the night. The seeds are round with papery wings. Bark is off brown in color. Leaves are 2 to 4 inch long, broad, leaflets are 5 inch long and 3 to 4 inch broad having sharp edges. The flowers stalk is one feet long. The flowers are purple in color. Fruits are 1 to 3 foot long, 2 to 4 inch broad. Seeds are flat and are 3 inch in length and 2 inch in width. The flowers are born in rainy season and fruit appears in December to March[2–3, 5].

GEOGRAPHICAL DISTRIBUTION

Oroxylum indicum is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern China, in Indo-China and the Malaysia

ecozone. It is diversely available in the forest of National Park in Assam, India, reported from Sri Lanka (Ceylon)[5].

TAXONOMICAL CLASSIFICATION[4]

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Bignoniaceae
Genus	:	*Oroxylum*
Species	:	*indicum*

SYNONYMS[9–10]

Sansk	:	Prthsuimba, Katvanga
Hindi	:	Sonapatha, Syonak, Tentoo
Eng	:	Indian trumplet flower
Beng	:	Sonagachh
Guj	:	Tentoo
Punj	:	Tatpaling, Talvarphali
Mar	:	Tentoo
Tamil	:	Peruvaagai

According to Ayurveda it contains[11–13]

Gunna (Properties)	–	laghu (light), tikshan (sharp) and ruksha (dry).
Rasa (Taste)	–	madhur (sweet), tikta (bitter)
Virya (Potency)	–	ushan (hot)

CHEMICAL CONSTITUENTS

The chemical constituents of *Oroxylum indicum* are always of an interest for the researcher. A number of secondary metabolites like flavonoids, glycosides, alkaloids, tannins, terpenoids etc. have been reported from various parts of the plant.

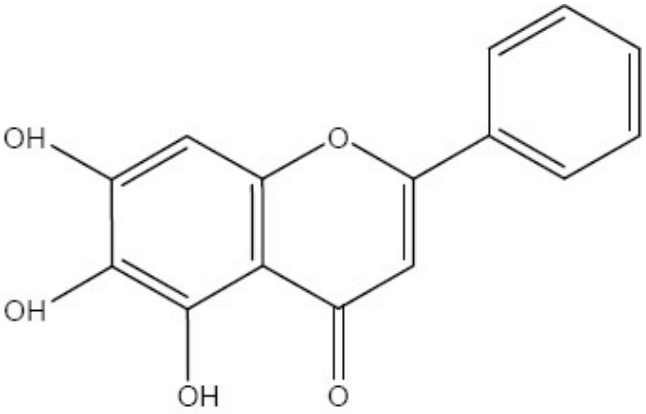
- The leaves have been reported containing flavones and their glycosides baicalein and scutellarein. Leaves also contain an anthraquinone, aloe-emodin[9, 17].
- Bark of the root is reported with chrysin, baicalein and oroxylin-A. Bark also gave dihydrobaicalein. Heartwood yielded beta-sitosterol and an iso-flavone, prunetin. The bark also contains traces of an alkaloid, tannic acid, sitosterol and galactose[14–15].
- Its root and stem contains three flavones named oroxylin A (5, 7-dihydroxy-6-methoxyflavone), baicalein (5, 6, 7- trihydroxyflavone) and chrysin (5, 7- dihydroxyflavone). It also contains pterocarpan and rhodioside with p-hydroxyphenylethanols and cyclohexanols[16–18].
- Four flavonoids, chrysin, baicalein, baicalein-7-*O*-glucoside, baicalein-7-*O*-diglucoside (Oroxylin B)

FIGURE 1: *FLOWERS OF OROXYLUM INDICUM*

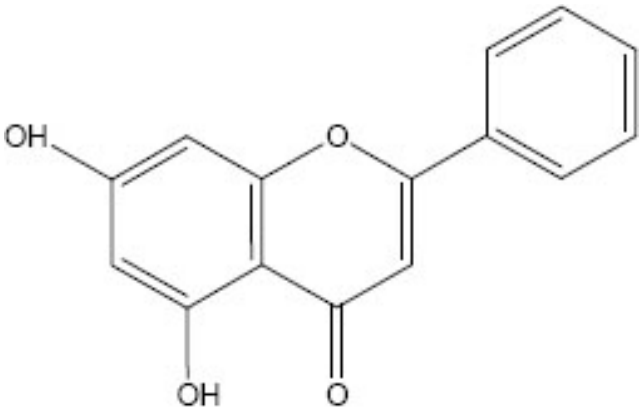
FIGURE 2: *LEAVES OF OROXYLUM INDICUM*

FIGURE 3: *FRUIT OF OROXYLUM INDICUM*

and one unknown flavonoid have also been isolated from the seeds of *Oroxylum indicum*[19]. Seeds also contain shiny oil, the yield of which was 20%[2].



Baicalein



chrysine

ETHNOMEDICINAL USES

- The root bark of plant is acrid, bitter, pungent; astringent to the bowels, cooling, aphrodisiac, tonic, increase appetite, useful in "vata", biliousness, fevers, bronchitis, intestinal worms, vomiting, dysentery, leucoderma, asthma, inflammation, anal troubles. It is used to treat diarrhea, dysentery, diaphoretic, and rheumatism[2–3]. Paste prepared from sesame oil (*Sesamum indicum*) and the powdered bark of the root is given as digestive tonic. The seeds are purgative and taken orally to treat throat infections and hypertension[20].
- The fruits are acrid, sweet; stomachic, anthelmintic; effective in diseases of the throat and heart, piles, bronchitis, used as an expectorant; improves the appetite; useful in leucoderma[6–9].
- In Indian system of medicine the root, bark, stem and leaf are prescribed for snake bite[2].
- Leaves are used externally to treat an enlarged spleen and also to alleviate headaches and ulcers and also reported for its analgesic and antimicrobial activity[20].
- In various tribes of India, bark and seeds of the plant are used in fever, pneumonia and repiratory troubles[21–22]. It is also used to cure various stomach disorders.[23].
- In Nepal a root decoction is used in diarrhoea and dysentery. Seeds are used as a digestive. A seed paste is applied to treat boils and wounds. The root is used as astringent, anti-inflammatory, aphrodisiac, expectorant, anthelmintic and tonic. The bark is diuretic and stomachic and useful in diarrhoea and dysentery. Root bark and seeds are carminative, stomachic, tonic, diaphoretic and astringent. Root bark is also used to treat bile problems, cough, diarrhoea, and dysentery[24]. It is also used in a formulation used for nootropic acitvity[25].

PHARMACOLOGICAL REPORTS

Although a lot of pharmacological and nonpharmacological investigations have been carried out on the plant and its phytoconstituents. A summary of the findings of these studies is presented below.

ANTI-INFLAMMATORY ACTIVITY

The aqueous extract of leaves of *Oroxylum indicum* has been reported to possess significant anti-inflammatory activity. The anti-inflammatory activity has been studied *in vivo* in carageenan induced rat paw edema model and it was reported that aqueous extract of *Oroxylum indicum* leaves exhibited significant anti-inflammatory activity at a dose level of 150mg/kg body weight and 300mg/kg body weight. *Oroxylum indicum* aqueous extract at a dose of 300 mg/kg body weight showed maximum anti-inflammatory activity. However the activity produced by both the dose was less effective than the reference standard diclofenac sodium. Extract at both doses showed significant anti-inflammatory activity at 5 hr. against carrageenan injection suggesting that the extract predominantly inhibit the release of prostaglandin like substances. In conclusion, leaves of *Oroxylum indicum* showed anti-inflammatory activity which may be attributed to the presence of different chemical constituents present within[26]. A number of flavonoidal compounds have also been reported previously as anti-

inflammatory agent and flavonoids present in plant may be responsible for this activity.

Aqueous and alcoholic extracts were tested using three different *in vitro* systems for effects relevant to anti-inflammatory activity of stem bark of *Oroxylum indicum*. The aqueous extracts of *O. indicum* significantly reduced myeloperoxide release. In the rat hind paw edema test, extract also showed significant activity[27]. All these findings suggest, *Oroxylum indicum* may be useful in management of chronic inflammatory conditions like arthiritis.

ANTI-HEPATOTOXIC ACTIVITY

Leaves of *Oroxylum indicum* are widely used as a prophylaxis for liver disorders in Indian system of medicine. Tenpe *et al*. reproted anti-hepatotoxic activity of various extracts of *Oroxylum indicum* Vent. against CCl_4 induced hepatotoxicity. Pet ether, chloroform, ethanol and aqueous extracts were administered to diseased animals (rats) at a dose of 300 mg/kg body weight and serum enzymes levels were observed. All the test groups showed a significant reduction in SGOT, SGPT, ALP, total bilirubin content and a significant increase in the level of total protein was observed in CCl_4 and *Oroxylum indicum* treated rats. Among all the extracts ethanolic extract was found to be more effective[28]. Free redical scavenging activity was also reported and hepatoprotective action of these extracts was likely to be due to its ability to scavenge free radicals and induce microsomal enzymes there by inhibition of the lipid peroxidation induced by CCl_4. The study scientifically proved the folklore use of *Oroxylum indicum* in liver disorders and as an ingredient in various Ayurvedic formulations used in liver disorders.

ANTHELMINTIC ACTIVITY

Jessica *et al*. evaluated the anthelmintic activity of *Oroxylum indicum* against equine strongyle eggs *in vitro* and compared it to ivermectin, one of the most effective deworming agents. At a dose of 2×10^{-5} g/mL and greater, hatching of the strongyle eggs was delayed using *Oroxylum indicum*. 0% hatching was achieved at 2×10^{-1} g/mL *Oroxylum indicum*. At a dose of 2×10^{-4} g/mL and greater, 0% viability of the strongyle eggs and larvae was achieved. The results of the study suggested that *Oroxylum indicum* may be an appropriate anthelmintic against equine strongyles[29].

ANTICANCER ACTIVITY

Various studies have proved anticancer potential of *Oroxylum indicum* using various models. Narisa *et al*. extracted *Oroxylum indicum* with 95% ethanol and tested for cytotoxic effects determing the anti-proliferative effects on Hep2 cell lines. Cell proliferation was measured using a colorimetric method based on the ability of metabolic active cells to cleave the yellow tetrazolium salt XTT to an orange formazan dye and soluble formazan dye was directly quantified using a scanning multiwall spectrophotometer (ELISA plate reader). Ethanol exhibited cytotoxic activity against the Hep2 cell lines at a concentration of 0.05%[30].

Roy *et al*. reported the *in vitro* effects of baicalein on the viability and induction of apoptosis in the HL-60 cell line was investigated. The cell viability after treating with baicalein for 24 h was quantified by counting viable cells using trypan blue staining. The results showed that baicalein caused a 50% inhibition of HL-60 cells at concentrations of 25–30 microM. The inhibition of proliferation of HL-60 cells due to 36–48 h exposure to 10 or 20 microM baicalein was associated with the accumulation of cells at S or G2M phases. However, proliferation inhibition at a higher dose may be associated with induction by apoptosis and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). The results indicate that baicalein has anti-tumor effects on human cancer cells, and *Oroxylum indicum* extract could be used in supplementary cancer therapy[31].

Nakahara *et al*. reported that methanolic extract of *Oroxylum indicum* strongly inhibited the mutagenicity of Trp-P-1 in an Ames test. The major antimutagenic constituent was identified as baicalein with an IC_{50} value of 2.78+/–0.15 microM. The potent antimutagenicity of the extract was correlated with the high content (3.95+/–0.43%, dry weight) of baicalein. Baicalein acted as a desmutagen since it inhibited the N-hydroxylation of Trp-P-2[32].

Tepsuwan *et al*. reported the *in vivo* genotoxic activity and cell proliferative activity in stomach mucosa of male F344 rats by *in vivo* short-term methods after oral administration of a nitrosated *Oroxylum indicum* Vent fraction, which had been found to be mutagenic without S9 mix to Salmonella typhimurium TA98 and TA100. Administration of the nitrosated *Oroxylum indicum* Vent. fraction at doses of 1 and 2 g/kg body weight induced dose-dependent DNA single-strand scission in the stomach pyloric mucosa 2 h after its administration: a dose of 2 g/kg body weight induced an 18-fold increase in the DNA elution rate constant. Administration of the nitrosated *Oroxylum indicum* fraction at doses of 0.7-2.8 g/kg body weight also induced dose-dependent increases, up to 11-fold, in replicative DNA synthesis in the stomach pyloric mucosa 16 h after its administration. Moreover

administration of the nitrosated *Oroxylum indicum* fraction at doses of 0.25-2.0 g/kg body weight induced dose-dependent increases, up to 100-fold, in ornithine decarboxylase activity in the stomach pyloric mucosa with a maximum 4 h after its administration. These results demonstrate that the nitrosated *Oroxylum indicum* fraction has genotoxic and cell proliferative activity in the pyloric mucosa of rat stomach *in vivo*[33].

Leticia *et al*. reported that extract of *Oroxylum indicum* showed the toxicity on tumor cell lines tested, with an IC_{50} value 19.6 µg/ml for CEM, 14.2 µg/ml for HL-60, 17.2 µg/ml for B-16 and 32.5 µg/ml for HCT-8. On the sea urchin eggs, it also inhibit the progression of cell cycle since the frist cleavage (IC_{50} = 13.5 µg/ml). On the basis of all these findings it can be concluded that extracts of *Oroxylum indicum*, could be considered as potential sources of anticancer compounds[34].

IMMUNOSTIMULATING ACTIVITY

The immunomodulatory activity and the mechanism of action of the n-butanol fraction (100 mg/kg body weight, per os, once daily for 22 consecutive days) of the root bark of *Oroxylum indicum*, was reported by Zaveri *et al*. in rats using measures of immune responses to sheep red blood cells (SRBC haemagglutinating antibody [HA] titer) and delayed-type hypersensitivity (DTH) reactions. In response to SRBC, treatment with the n-butanol fraction caused a significant rise in circulating HA titers during secondary antibody responses, indicating a potentiation of certain aspects of the humoral response. The treatment also resulted in a significant rise in paw edema formation, indicating increased host DTH response. Additionally, the antioxidant potential of the drug was exhibited by significant reductions in whole blood malondialdehyde content along with a rise in the activities/levels of superoxide dismutase, catalase and reduced glutathione. Furthermore, histopathologic analysis of lymphoid tissues showed an increase in cellularity, e.g., T-lymphocytes and sinusoids, in the treatment group. In a triple antigen-mediated immunological edema model, the extent of edema raised in drug-treated rats was greater compared to that in control rats, thus confirming enhanced DTH reactions in response to the drug treatment. Based on the all these findings, the reported immunomodulatory activity of an active fraction of *O. indicum* might be attributed to its ability to enhance specific immune responses (both humoral and cell-mediated) as well as its antioxidant potential[35]. This study also justifies the use of plant in various immunomodulatory formulations of Ayurveda like Chyavanprash etc.

ANTIMICROBIAL ACTIVITY

The anti-microbial activity of various extracts of *Oroxylum indicum* has been screened against fourteen pathogenic bacteria (five gram-positive and nine gram-negative) and seven pathogenic fungi by Kawsar *et al*. using disk diffusion method. The crude ethyl acetate extract showed mild to moderate activity against all bacteria and fungi whereas the methanolic extract showed little activity against bacteria but moderate activity against fungi. The minimum inhibitory concentration of two isolated flavonoid compounds from *O. indicum* were determined against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Shigella dysenteriae* and the values were found to be between 64–128µg/ml. A study by Thatoi *et al*. further confirmed the activity by using different strains[36–37]. Ali *et al*. (1998) studied the effect of dichloromethane extract of *Oroxylum indicum* against dermatophytes and wood rot fungi and reported a strong antifungal activity in dichloromethane extract of *Oroxylum indicum*[38].

GASTRO-PROTECTIVE ACTIVITY

Zaveri *et al*. reported the gastroprotective activity of 50% alcoholic extract of root bark of *Oroxylum indicum* and its different fractions viz. petroleum ether, chloroform, ethyl acetate and *n*-butanol fractions in ethanol-induced gastric mucosal damage. *n*-butanol fraction was also studied in Water Immersion Plus Restraint Stress (WIRS)-model. Alcoholic extract (300 mg/kg) and its different fractions (at a dose of 100–300 mg/kg) showed significant reduction in gastric ulceration against ethanol-induced gastric damage. Out of all these fractions, *n*-butanol fraction showed significant maximum inhibition of gastric lesions. In WIRS-model, pretreatment with *n*-butanol fraction showed significant antiulcer and antioxidant activity in gastric mucosal homogenates, where it reversed the increase in ulcer index, lipid peroxidation and decrease in superoxidedismutase, catalase and reduced glutathione levels induced by stress. This study reveals significant gastroprotective effect of *n*-butanol fraction against both ethanol and WIRS-induced gastric ulcers in rats[39]. Flavonoids present in *Oroxylum indicum* Vent. was found to be responsible for its gastro-protective activity[40].

CONCLUSION

Oroxylum indicum is a highly placed drug in the Ayurvedic medicine. It is one of the most versatile plants having a wide spectrum of medicinal activities. This medicinal plant is the unique source of various types of compounds having diverse chemical structure and

nature. Quite less scientific work has been conducted on the possible medicinal applications of these compounds and hence extensive investigation is desirable to exploit their therapeutic utility. Although crude extracts from various parts of *Oroxylum indicum* have been assigned various medicinal applications from time immemorial, the probability of converting these promising activities into modern drugs can be explored further only after extensive investigation of its bioactivity of responsible constituents, mechanism of action, and toxicity and after proper standardization. As this approach would be in line with the global scenario which is now changing towards the use of plant products, that are backed by ethnotraditional medicinal use, which are comparatively nontoxic than currently available marketed drugs of other systems.

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