A Review on Phytochemistry of *Cuminum cyminum* seeds and its Standards from Field to Market

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ABSTRACT

The small boat shaped seeds of Cumin (*Cuminum cyminum*) has been used for many medicinal and culinary purposes from the ancient time in the various countries. Cumin is a popular spice in the world from Latin America to Northern Africa and all over the Asia and also used as a flavoring agent in many products such as cheese, pickle, soup, bean dishes or liqueurs. Essential oils of the seeds are also used as a flavor or in aromatherapy. Many pharmacological effects have been reported from this spicy plant as, anti-diabetic, Immunologic, anti-epileptic, anti-tumor and antimicrobial activities. Cumin used in the medicinal preparations is supposed to be produced with high quality encompasses all the properties of the final product which makes it optimal suitable for use. Reproducible quality is a goal, which achieved by the process of standardization. The focus here is rather on harvesting and processing of the cultivated species, because the quality of plant material and processing technology lead to the high quality of the final product. The quality of Cumin seeds and its essential oil can only be assessed with analytical methods, which include physical, microscopic and chemical analyzing assays. In this paper, the phytochemistry, medicinal properties and the standards from the field cultivation, harvesting and storage until marketing are reviewed.

Key words: *Cuminum cyminum,* phytochemistry, adulterants, standards.

INTRODUCTION

Cuminum cyminum L., belonging to the family Apaiaceae, is one of the old cultivated medicinal food herbs in Asia, Africa and Europe. This plant is well-known as Cumin and named Zireh-Sabz or Cravieh in Persian language. Its seeds have been commonly used for culinary and flavoring purposes and folklore therapy since antiquity in various countries.[1-3] There are two species of *Cuminum* which growing wildly in Iran, *C. cyminum* L (Zireh-Sabz means green Cumin) and *C. setifolium* Boisskos. Pol (Zireh-Sefid means white Cumin). Some literature reported that *C. setifolium* is the sub-species of *C. cyminum*.[1-4]

C. cyminum is an annual herbaceous plant which grows up to 15-50 cm height somewhat angular and tends to droop under its own weight. It has a long, white root. The leaves are 5-10 cm long, pinnate or bi pinnate, with thread-like leaflets and blue green in color and are finely divided, generally turned back at the ends. The leaves are highly dissected. Whitish-red flowers are on a compound umbel (arrangement of flowers looks like an umbrella). The fruit is an elongated, oval shaped schizocarp (an aggregate fruiting body which doesn't break open naturally and has two single seeded units called mericarps). The fruits are similar to fennel seeds, when chewed has bitter and pungent taste. The fruit are thicker in the middle, compressed laterally about 5 inch long, containing a single seed.[5-6]

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MEDICINAL PLANT MATERIAL

Dried ripe seeds of *C. cyminum* are usually used for medicinal or culinary purposes. In Iranian traditional medicine, Cumin seeds were used for their therapeutic effects on gastrointestinal, gynecological and respiratory disorders, and also for the treatment of toothache, diarrhea and epilepsy. The seeds were also documented as stimulant, carminative and astringent.[2] Johri has been recently reported that medicinal usage of Cumin seeds has also been widespread in diverse ethnomedical systems from Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia and North America, where these have remained as an integral part of their folk medicines.[7]

PHYTOCHEMISTRY AND MEDICINAL PROPETIES

Antimicrobial activity has been reported from the volatile oils and aqueous extract of Cumin. Cumin seed oil and alcoholic extract inhibited the growth of *Klebsiella pneumoniae* and its clinical isolates by improvement of cell morphology, capsule expression and decreasing urease activity. Cuminaldehyde (**1**) is the main active compound of Cumin for this property.[8-9] Limonene (**2**), eugenol (**3**), α- and β-pinenes(**4, 5**) and some other minor constituents have been found in cumin oil and suggested as the active antimicrobial agents.[7,10]

The Cumin oil is reported as a high antioxidant mainly due to the presence of monoterpene alcohols.[11] The presence of phytoestrogens in Cumin has been reported which related to its anti-osteoporotic effects. Methanol extract of Cumin showed a significant reduction in urinary calcium excretion and augmentation of calcium content and mechanical strength of bones in animals.[12] Furthermore, the aqueous extract of Cumin seeds indicated the protective effect against gentamycin-induced nephrotoxicity, which decreased the gentamycin-induced elevated levels of serum urea and enhanced the clearance of the drug.[13]

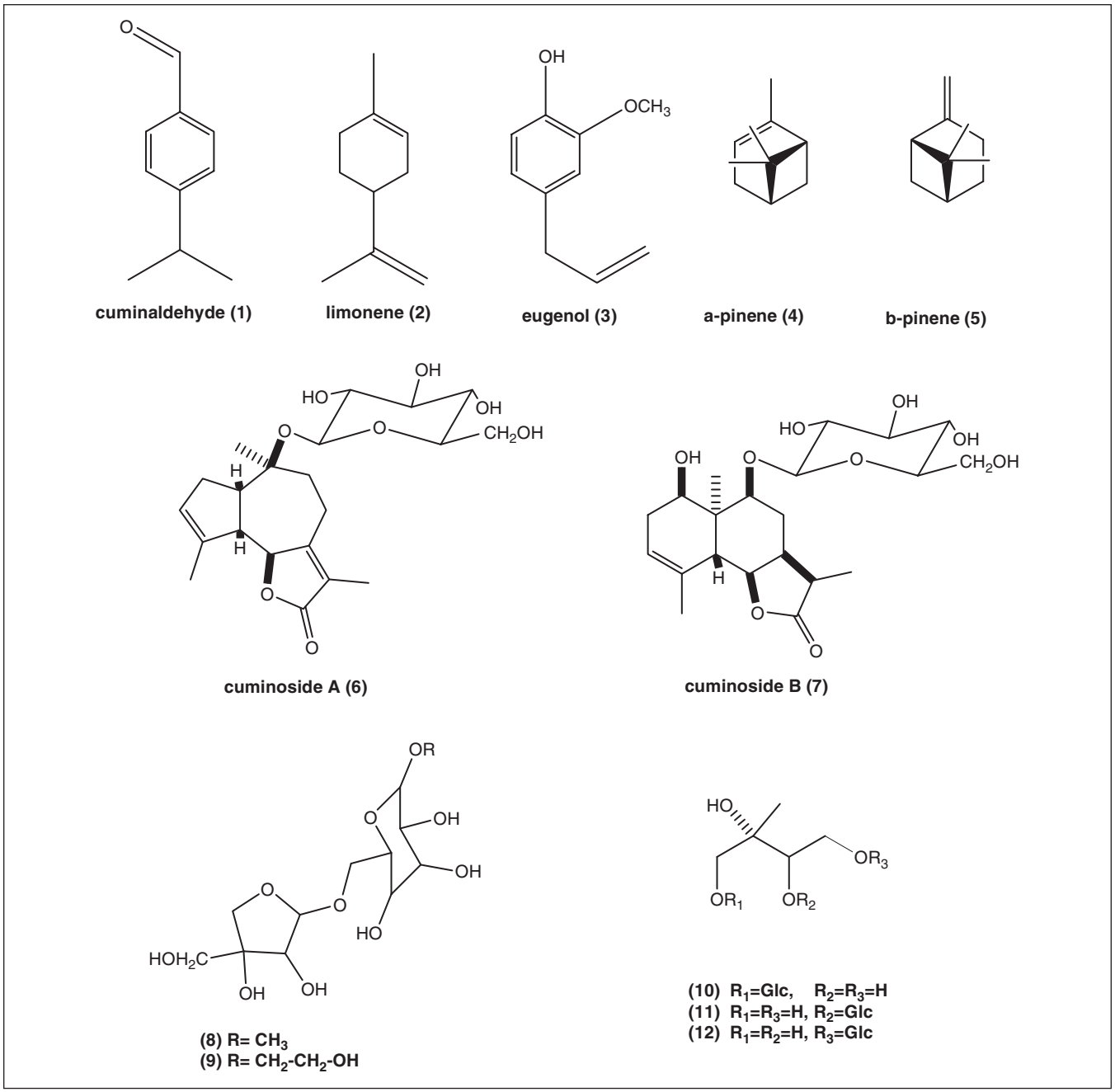

Figure 1: Chemical structures of the isolated compounds from Cumin.

Anti-epileptic activity of cumin oil was also reported, which decreased the frequency of spontaneous activity induced by pentylene tetrazol (PTZ).[14] Recently, Cumin oil has been found to act as a significant analgesic by formalin test in rats and suppress the development and expression of morphine tolerance and also reverse the morphine dependence.[15-17]

Other important reports consider that dietary Cumin can inhibit benzopyrene-induced for stomach tumorigenesis, 3-methylcholanthrene induced uterine cervix tumorigenesis, and 3-methyl-4-dimethyaminoazobenzene induced hepatomas in mice, which was attributed to the ability of Cumin in modulating carcinogen metabolism *via* carcinogen-xenobiotic metabolizing phase I and phase II enzymes.[18]

Literature review on phytochemistry of the Cumin seeds revealed the presence of various bioactive compounds, the important secondary metabolites of which are discussed as followed.[19-20]

Two sesquiterpenoid glucosides, cuminoside A (**6**) and B (**7**), and two alkyl glycosides (**8, 9**) were isolated (Figure 1) together with some known compounds from the methanol extract of Cumin seeds. Their structures were established as (1*S*,5*S*,6*S*,10*S*)-10-hydroxyguaia-3,7(11)-dien-12,6-olide β-D-glucopyranoside (**6**), (1*R*,5*R*,6*S*,7*S*,9*S*,10*R*,11*R*)-1, 9-dihydroxyeudesm-3-en-12, 6-olide 9-*O*-β-D-glucopyranoside (**7**), methyl β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**8**) and ethane-1,2-diol 1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**9**).[19] In another report, three glycosides (Figure 1), 1-*O*-β-D-glucopyranoside (**10**), 3-*O*-β-D-glucopyranoside (**11**) and 4-*O*-β-D-glucopyranoside (**12**) have been isolated and structural elucidated from the seeds (fruits) of Cumin.[20]

NATURAL HABITAT AND THE LAND UNDER CULTIVATION

Cumin is the native species growing in Egypt (North Africa) and Asia. It was originally cultivated in Iran and the Mediterranean region. In Iran, Cumin is wildly growing in Khorasan. The species, *C. setifolium* is also found in desert aria such as Damghan, Sabzevar, Tabas and Borazjan. Cumin (*C. cyminum*) is mainly cultivated in Khorasan followed by East Azerbaijan, Yazd, Semnan, Esfahan and some parts of Golestanand Kerman provinces. Ninety percent of the whole products for the export are cultivated in Khorasan. Iran provides 20-40% of the world production and export of Cumin. The Cumin seeds are valuable, because the prices of one kilogram seeds are equal to 10 kilogram wheat. Cultivation of cumin requires a long, hot summer of 3-4 months, with daytime temperatures around 30 °C. This herb is resistant to drought, and is mostly grown in Mediterranean climates. It is grown from seed, sown in spring, and needs fertile, well-drained soil. The plant blooms in June and July. The seeds are normally ripe four months after planting. The plants are threshed when the fruit is ripe and the seeds are dried.[21-22]

STANDARDS CRITERIA FOR HARVESTING, DRYING AND STORAGE

Appropriate Season

The Cumin seeds are usually ready to harvest during 100-120 days after cultivation. Seed harvesting season is different from June to July on the basis of the weather conditions, because the flowering season is influenced by day long and temperature.[23] Literature showed that Cumin is better to be in rotation with summer crops such as soybean, millet and sesame. In order to produce satisfied yield in Iran, application of 30 kg N, 60 kg P and 30 kg K per hectare has been recommended. Cumin crop water requirement is 335 mm/ha. Average yield of Cumin is reported 1000 kg/ha with percentage of 2.1 to 3.5 for seed volatile oils.[24]

Appropriate Harvesting Methods

The seeds are harvested about 4 months after planting when the plant begins to wither and the seeds change to brown-yellow color. In traditional method, the whole plants were removed from the soil and collected as sheaves. The sheaves were set up in the fields and sifting and cleaning by winnower. The isolated seeds were then further dried to 10% (moisture content), wither by placing on mats or trays in the sun or using a drier in the humid conditions (Like Pakistan). The dried seeds are winnowed using a traditional winnowing basket to remove the dirt, dust, leaves and twigs. Nowadays, the modern and high capacity combines are used for harvesting, sifting and cleaning of the plants.[23]

Main Physicochemical Characteristics

The seeds are elongated, oval shaped schizocarp and similar to fennel seeds, when chewed have bitter and pungent taste. The seeds (fruits) are thicker in the middle, compressed laterally about 5 inch long. Five out-standing lines are observed in each parts of mericarp. The seeds are too flavored and covered by hairs (sometimes without hair). The fruit pericarp contains high amount of tannins which change color in presence of Iron contained compounds. The seeds must contain at least 7% of oil, 13 & resin and 2.5-4% essential oil. The maximum total crude ashes are 9.5% and the maximum acid insoluble ashes are 2% with no more than 9% humidity.[22]

Qualification and Quantification Parameters of Essential oil

Different factors may impact on the physicochemical properties of the essential oil of Cumin seeds, of which plant

variety, cultivation area and conditions, date of harvesting and extraction methods are important. Quantification of the total essential oil of seeds is conducted by hydro-distillation method. In this method, about 20 g of the grind fruits disperse in 500 ml of distilled water (in a 1000 ml flask) and hydro-distilled for 4 h with 3-4 ml/min distillation rate. The oil volume is measured by using xylol.[25-26] Physical Properties of the essential oil obtained by steam distillation from Cumin seeds are summarized in Table 1.[22,27]

Packaging and Storage

Cumin seeds are sensitive to crashing and mechanical damages hence protection of seeds during sifting and cleaning or winnower is too important. Sometimes the products lose some parts of humidity and become drier than standards. The quality of seeds has been decrease during the prolonged storage. Today, Cumin is packed in gunny bags and cleaned by machines in order to remove the stalks, other foreign material, stones and dust in advance. Cumin may also pack in tissue, paper or polythene bags depending on the requirements of the buyer. It is preserved at least one to two feet away from the walls in order to save it from moisture in humid countries. The bags should be sealed to prevent moisture entering or exiting. Labels should be applied to the products. The label needs to contain all relevant product and legal information such as the name of the product, brand name, names, address and date of manufacture, expiration date, weight of the contents, added ingredients (if relevant) plus any other information that the country of origin and of import may require.[22,28] The essential oils of Cumin should be conserved in the amber and tight closed glass or aluminum containers even better to seal by inner epoxy covers. The oil packages should be storage far from the direct sunlight and temperate places.[2]

Adulterants

Regarding to this point that the characteristic odor of Cumin is caused mainly by aldehydes which are present the essential oil, synthetic Cumin aldehyde is sometimes added as an adulterant in Cumin oil.[29] Frauds distinguish is very difficult to detect chemically but it is possible because the synthetic Cumin aldehyde may change the refractive index of the oil.[22,30]

Table 1: Physical Properties of the essential oil of Cumin seeds obtained by steam distillation

Physicochemical Properties	Level
Extraction Percentage	2.3-5.7 %
Color	Colorless or pale yellow
Refractive Index (20 ºC)	1.47-1.50
Density (20 ºC)	0.90-0.94
Alcohol solubility (80% v/v)	1:1.3-1:2
Aldehyde percentage (on the basis of Cuminaldehyde)	35-63%
Acidity (on the basis of Cuminic acid)	0.36-1.8
Alcohol percentage (on the basis of Cuminol)	3.5
Carbonyl Index	9.32
Steric Index	19.24

Detection of Purity by Microscope

Many of the Cumin products contain grinded seeds of Cumin. Therefore, microscopic analysis is considered for purity determination. Grinded Cumin is a yellowish-brown powder with a characteristic, aromatic, slightly camphor-like odor and taste. The diagnostic characters are summarized below.[31]

The epicarp composed of a layer of colorless cells, with thin, sinuous walls and a faintly and irregularly striated cuticle. Stomata are fairly frequent and cicatrices may be present. Underlying the epicarp the thin-walled cells of the palisade are sometimes visible. The pale yellowish-brown fragments of the vittae composed of fairly large, thin-walled cells. The fragments are usually wider than the most of the other Umbelliferae fruits. The sclereids from the mesocarp are in two main types. One type occurs as a single layer of longitudinally elongated cells with moderately thickened walls and numerous regularly spaced, well-marked pits. Second type is found in small groups and composed of considerably elongated cells placed more or less end to end in a longitudinal direction. The endocarp composed of a layer of fairly large, thin-walled cellsand arranged with their long axes parallel. The endosperm composed of moderately thick-walled cells containing aleuronic microrosette crystals of calcium oxalate.[31]

Thin Layer Chromatographic Analysis

In this analysis, the extract of cumin, obtained by percolation of 1 g of dried seeds, is concentrated and dissolved in 0.5 ml toluene. Thin Layer Chromatography (TLC) is carried out on a silica gel TLC plates with the solvent system as toluene: ethyl acetate (7:93) alongside the standards of α- and β-pinenes and α- and β-phellandrenes. The spots are detectable by the anisaldehyde-H_2SO_4 spray reagent followed by heating (105-110 °C for 5-10 min). The spots of the above mentioned standards can be visible inside the area of 0.2-0.4 (R*f*), respectively. The pinene spots show the brown color and the phellandrene spots indicate a yellowish-brown color.[32]

CONCLUSION

Cumin is the second most popular spice in the world, after black pepper, and used as a medicinal plant for aromatherapy and various illnesses. For this reason, the standardization of the plant material from cultivation to storage is too important. To insure the achievement of quality, acceptance criteria for plant material and validating of the technical

process in manufacturing are considered. Standardized seeds and essential oils are qualitatively optimized the products or preparations with reproducible content. Determination of the physicochemical characteristics of the oil may establish by measurement of extraction yield, refractive index, density, carbonyl and steric indexes together with aldehyde, alcohol and acid contents. Microscopic analyzing is very important in the products containing grinded seeds. In addition, thin layer chromatography may help to detect the pinenes and phellandrenes in the seeds as the main and characteristic monoterpenes. Cumin aldehyde is not only the active constituent of the Cumin seed and its oil but also sometimes added to the oil as a fraud which can difficulty detected by changing the refractive index.

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Medicinal Plant Diversity and their Pharmacological Aspects of Nepal Himalayas

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ABSTRACT

Background: The Himalayan range of Nepal is affluent with vast diversity of medicinal plants. Due to insufficient supplement of modern allopathic medicine and the traditional believe of ethnomedicinal therapy, still vast majority of Nepalese people are dependent on indigenous use of medicinal plant. Use of Nepalese Himalayan medicinal plants is not only limited to erogenous use of Nepal Himalaya but also regarded as chief ingredients in Eastern medicinal system including Ayurveda of Indian subcontinent, Traditional Chinese Medicine, Korean Oriental Medicine, etc. But due to the lack of efficient pharmacological investigation, Himalayan plant diversity is still limited to their ethnomedicinal uses. Vigorous pharmacological investigation is mandatory to explore their therapeutic potential. **Conclusion:** Here in this review; based on latest published pharmacological research articles, we tried to explore pharmacological aspects of major Himalayan medicinal plant of Nepal for the first time. There is the current need to investigate further pharmacological potency of these medicinal plants in order to explore their therapeutic potential.

Key words: Ethnomedicine, indigenous use, Himalayas

BACKGROUND

Nepal, the Kingdom of Himalaya, is small, landlocked country situated between India and China. Nepal lies on southern slope of central Himalaya and occupies a total area of 147181 sq. km between the latitude of 26° 22' and 30° 27' N and the longitude of 80° 40' and 88° 12' E. The average length of the country is 885 km from east to west and width varies 145 km to 241 km from north to south. About 86% of the total land area is covered by hills and high mountains and remaining 14% is covered by flat lands of Terai. Based on wild altitudal variation (60-8848 m), the climate is broadly classified into cold Arctic/Nival (above 3000 m), cold temperate (2000-3000 m), warm temperate (1500-2000 m), subtropical (1000-1500 m) and tropical (below 1000 m). According to the physiological region, Nepal is divided into 7 regions including Terai, Siwaliks, Mahabharat lekh, Midhills, Himalayas, Inner Himalayas and Tibetan marginal mountain range.[1,2]

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Nepal is blessed with most varied and diverse soil and climate conditions suitable for the growth of veritable plant species. The indigenous people are well acquainted with the properties and uses of plants of their surroundings. Until the middle of the 19th century, plants were the main therapeutic agents used by humans. About 60% of the world population and 60-90% of the population of developing countries rely on traditional medicine[3] and about 85% of the traditional remedies for primary health care are derived from plants.[4]

In Nepal, at least 1,600 to 1,900 species of plants are commonly used in traditional medicinal practices.[5] Traditional medicine in Nepal is used extensively by majority of the population, and includes Ayurveda, Traditional Chinese medicine (TCM), Unani and various forms of indigenous medicine including Tibetan Amchi medicine.[6] Traditional medicine in Nepal comprises those practices based on beliefs that were in existence often for hundreds to thousands of years before the development and spread of modern medicine, and which are still in use today. In the past, many rural areas of Nepal, traditional medicinal knowledge and practice were passed down entirely via oral tradition based on a lineage mode of transmission and personal experience.[7] More recently, however, knowledge transfer has also occurred through formally recognized

school level education. Medicinal plants play vital roles in the Nepalese livelihood and the use of medicinal plants is frequent in several Nepalese regions. The total population of Nepal is 23.1 million and about 90% of the Nepalese people reside in rural areas where access to government health care facilities is lacking. It is estimated that there is one physician for more than 30,000 people whereas there is one healer for fewer than 100 people in Nepal.[8] Nepal is a natural storehouse of medicinal plants. Each year thousands of tons of raw material are exported, mostly to India, but also to other Asian, European and American countries. The government of Nepal aims to promote medicinal plant use and conservation programmes for livelihood improvement and poverty alleviation through various policies.[9]

The Himalayan plant diversity plays pivotal role to fulfill the medicinal demand of Nepalese society. The earliest record of medicinal plant use in the Himalayas is found in the Rigveda (4500 BC and 1600 BC), is supposed to be the oldest repository of human knowledge and describes 67 plants. After the *Rigveda*, *Ayurveda* (the foundation of science of life and the art of healing of Hindu culture) describes the medicinal importance of 1200 plants. The *Charak* or *Charaka Samhita* (900 BC) and *Susruta Samhita* (500 BC) enumerate the art of surgery, therapeutics and medicines in detail on the basis of *Atharvaveda*. The knowledge of using these systems was accessed by Nepali *Vaidhyas* and *Kabirajs* as early as about 879 AD.[10] Therefore, the Ayurvedic physicians were incorporating medicinal plants in traditional Ayurvedic formulations from early on and the Ayurvedic system is reputed all over the Indian subcontinent since time immemorial.[11,12] Almost of the herbs of Nepal Himalayas are considered to contain medicinal properties. Kunwar and Bussmann 2008 reported that 56% of higher plants were ethno botanically important, and 54% were used as ethnomedicine in the Nepal Himalayas. The topographical characteristics of the Himalayas have resulted in a variety of ecological niches that host diverse medicinal plants. It has been estimated that the Himalayan region harbors over 10,000 species of medicinal and aromatic plants, supporting the livelihoods of about 600 million people living in the area.[13-15] This review was carried out by dividing Nepal Himalaya into 3 different region as West Nepal (80°E to 83°E), Central Nepal (83°E to 86°E) and East Nepal (86°E to 88°E), according to Kunwar and Bussmann, 2008.

So many researches are carried regarding to the indigenous use and ethnomedicinal potential of Nepalese medicinal plants till now. However, according to the knowledge of author, regarding the pharmacological aspects very few investigations have been carried out. Here, in this review article we tried to summarize the pharmacological aspects of major Nepalese Himalayan medicinal plants [Table].

Table: List of Medicinal Plants of Nepal Himalayaas based on their latest pharmacological investigation and indigenous use (W = Western, C = Central, E = Eastern region)

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
1.	*Abies spectabilis* (Pinaceae)	Gobre salla (Himalayan silver fir)	Leaves are sniffed for cough and cold	Pentene of *Abies* leaves is anti-inflammatory and antidepressant.[19]	W
2.	*Acacia catechu* (Mimosaceae)	Khair (Cutch tree)	Wood is used as local tea for cough and cold	Cyanidanol, an active ingredient of *Acacia catechu*, is claimed to be effective for treating liver diseases.[20] Catechu has Hypoglycemic, antipyretic and digestive properties. Catechuic acid is valued for expectoration for chest infection.[21]	W, C
3.	*Aconitum ferox* (Ranunculaceae)	Bikh (Himalayan monkshood)	Root paste is taken for joint pain.	Alkaloid extract possess anti-inflammatory properties.[22]	E, C, W
4.	*Aconitum heterophyllum* (Ranunculaceae)	Bish (Aconites)	Rhizome is dried up and taken to relieve body-ache, fever, cold, cough, nose discharge etc.	Ethanolic root extract of *Aconitum heterophyllum* has anti-inflammatory activity against cotton pellet-induced granuloma in rats.[23]	E, C, W
5.	*Aesculus indica* (Sapindaceae)	Karu (Indian horse chestnut)	Seed oil is valued for joint pain and skin problems	Plant is used for delaying Hypersensitivity. Aescin is cardiostimulant and anti-inflammatory.[24]	W

Continued

Table: *Continued*

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
6.	*Ageratum conyzoides* (Asteraceae)	Gnadhe jhar (Ageratum)	Leaf juice is applied externally to heal wounds. Decoction of herb is also given to cure stomach ailments such as diarrhea, dysentery and intestinal colic with flatulence	Hypoglycemic and antihyperglycemic activity on rat[25] also shows anticancer and antiadrenal activity.[26]	E, C, W
7.	*Allium cepa* (Alliaceae)	Pyaj/ Odal (Onion)	Eating raw bulbs reduces fever acting as cooling agent.	It prevents cadmium induced renal dysfunction[27] and has hypoglycaemic effect against type 1 and 2 diabetes mellitus.[28]	W, C, E
8.	*Amaranthus spinosus* (Amaranthaceae)	Bagani dhap (Prickly amaranth)	Root paste is applied on cuts and wounds.	Contains several chemical compounds, including tannins (coagulant), steroids (muscle building), flavonoids (antimicrobial), and volatile oils (antiseptic).[29]	E, C, W
9.	Andrographis paniculata (Acanthaceae)	Kalmegh (Kariyat)	Raw plant root juice is considered as antipyretic and effective in infections	Plant is immunostimulant, anti-inflammatory, antibacterial, analgesic and antiprotozoal.[30]	W, C
10.	*Anisomeles indica* (Lamiacae)	Ratocharpate (Indian catmint)	Leaf extract is useful for urinary complaints	Ovatodiolide and pedallitin of *Anisomeles indica* is good anti-inflammatory. Pre-flowering plant water extract is analgesic. Ethanolic leaf extract is strong antiviral and anti HIV potential.[31]	W
11.	*Artemisia indica* (Asteraceae)	Titepati (Asian mugwort)	Leaf paste is applied on cuts and wounds.	Antimicrobial properties and in vitro antimalarial property.[32]	E, C, W
12.	*Artemisia vulgaris* (Asteraceae)	Tite pati (Fleabane)	Crushed leaves inserted in the nose stop bleeding. Water, mixed with crushed leaves, in taking bath prevents and cures allergy. Raw leaves chewed are good for mouth ulcer; also find uses in rituals.	Has antispasmodic and bronchodilator activity in guinea pigs.[33]	E, C, W
13.	*Asparagus racemosus* (Asparagaceae)	Kurilo (Shatavari)	Tuber paste is used for fever, stomach ache, and diarrhoea	Ethanol and aqueous extracts from the tubers exhibit significant antidiarrheic activity.[34]	E, C, W
14.	*Bauhinia variegate* (Caesalpiniaceae)	Koiralo (Orchid tree)	Flower and floral buds are eaten regularly to cure leucorrhoea and mumps.	Methanol extract of *B. variegata* bark showed the most remarkable activity as antimicrobial and anticancer.[35]	W
15.	*Berberis asiatica* (Berberidaceae)	Chutro (Barberry)	Cambium paste is used for rheumatism and pith paste is used for eye problems.	Widespread use as an extract in eye drops for Conjunctivitis. Effective as an antipyretic, anesthetic, and antihypertensive.[36]	E, C
16.	*Bergenia ciliata* (Saxifragaceae)	Pakhanved (Frilly Bergenia)	Latex is effective in diarrhea, dysentery, stomachache	Aqueous and methanolic extract of *Bergenia ciliata* shows the cytoprotective activity.[37]	W
17.	*Bischofia javanica* (Euphorbiaceae)	Kainjalo (Java sedar)	Chewing raw leaves treat sore throat. Drinking bark juices cure diarrhea.	Betulinic acid derivatives from the bark has the DNA topoisomerase II inhibitory activity.[38]	E, C, W
18.	*Cannabis sativa* (Cannabaceae)	Ganja (Marijuana)	Plant paste is taken for stomach problems	Diuretic, anti-emetic, anti-epileptic, painkilling, anti-inflammatory, and antipyretic properties.[39]	E, C, W

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
19.	*Carum carvi* (Apiaceae)	Jangali jira (Caraway)	Fruit is stomachic and carminative. Seeds are used for their cooling effect.	Aqueous extract of *Carum carvi* (black zeera) seeds has the renal protective activity in streptozotocin induced diabetic nephropathy in rodents.[40]	W, C
20.	*Cedrela toona* (Meliaceae)	Tuni (Indian Mahogany)	Bark is crushed and the paste is applied to cure ulcers. Flower is chewed to promote menstrual discharge in females.	Has antiproliferative and antitumorogenic activity.[41]	E, C
21.	*Celastrus paniculatus* (Celastraceae)	Malkauna, kujur (Staff tree)	Seed paste is applied in case of skin irritation/allergy; good for gout.	Has potent relaxant activity in Human ileum.[42]	C, E
22.	*Cinnamomum tamala* (Lauraceae)	Dalchini, Tejpat (Malabathrum)	Leaves are rubbed on the body surface of the scabies affected person.	Has immunomodulatory activity on rat.[43]	C, E, W
23.	*Cissampelos pareira* (Menispermaceae)	Batulpate (Abuta)	Plant extract is given to treat diarrhea, dysentery, indigestion and urinary disorders. Root is used as antidote.	Roots are proven to have antineoplastic and antiarthritic activiry.[44]	E, C. W
24.	*Citrus medica* (Rutaceae)	Bimiro (Citrus)	Chewing dried fruit peel prevents dysentery. Fruit is good for indigestion. Roots are tied together along with a copper coin and placed in women's naval during child birth, which is believed to expedite the expulsion of the placenta after child birth.	Shows good in-vitro inhibitory activity against diabetes mellitus and Alzheimer's disease.[45]	E, C, W
25.	*Clematis buchananiana* (Ranunculaceae)	Abijalo (Clematis)	Juice extracted by crushing fresh roots is inhaled to treat sinusitis and headache.	Aquous extracts of *Clematis buchananiana* leaf anti-inflammatory, antinociceptive and antipyretic properties in rats.[46]	E, C, W
26.	*Cordyceps sinensis* (Clavicipitaceae)	Yarsagumba (Cordyceps)	Whole plant juice is taken as tonic.	Largely recognized as inducing sexual power and validity.[47]	W, C
27.	*Costus speciosus* (Costaceae)	Betlauri (Wild ginger)	Rhizome mixed with sugar is used to treat venereal diseases. Juice taken before breakfast cures urinary tract infections.	Eremanthin from *Costus speciosus* shows antidiabetic and antilipidemic effect in STZ-induced diabetic rats.[48]	E, C
28.	*Curcuma aromatica* (Zingiberaceae)	Ban haledo (Aromatic turmeric)	Rhizome powder taken with water relieves nausea, stomachache and expels gas.	*Curcuma aromatica* oil has the antineoplastic activity.[49]	E
29.	*Curcuma longa* (Zingiberaceae)	Besar (Turmeric)	Drinking water boiled with root cures throat pain, cold, cough and fever.	More than thousands of researches have been carried out on *Curcuma longa*. Recent interests are on anticancer[50] anti-inflammatory[51] and antioxidant[52] activity	W, C, E
30.	*Cynodon dactylon* (Poaceae)	Dubo (Dog's tooth)	Crushed root juice is taken to relieve piles. Root paste applied heals cuts and wounds. Boiled leaf and root juice help in treating diarrhea and dysentery.	Hydrochloric extract of rhizome shows protective effect against heart failure in rat.[53]	E, C
31.	*Dioscorea alata* (Dioscoreaceae)	Ghar tarul (Winged yam)	Rhizome is eaten raw to relieve throat pain.	This has found to effectively reduced blood pressure of spontaneously hypertensive rat.[54]	E, C
32.	*Drymaria cordata* (Caryophyllaceae)	Chirbire jhar	The plant is warmed while wrapped in a cloth and emanating vapor inhaled in the case of sinusitis, nose blockade and headache. To relive sore throat pain and fever, the plant either eaten raw or cooked.	Hydroethanolic extract shows anxiolytic effect in animal model.[55]	E

Continued

Table: *Continued*

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
33.	*Drynaria propinqua* (Drynariaceae)	Kammari (Dryndria)	Plant is effective in fever and headache.	Propinqualin, 4-O-beta-D-glucopyranosyl caffeic acid, beta-sitosterol-3-O-beta-D-glucopyranoside has been isolated from this plant.[56]	C, E
34.	*Engelhardia spicata* (Juglandaceae)	Mahuwa (Engelhardia)	Flower juice is drunk for abdominal pain.	Engelhardtione possesses antituberculer activities.[57]	W, C, E
35.	*Entada rheedii* (Fabaceae)	Prami (African dream herb)	Body pain, musculo-skeletal problems.	Triterpenes isolated from seed of *Entada rheedii* has antiproliferative and antioxidant activity.[58]	C
36.	*Ephedra gerardiana* (Ephedraceae)	Somlata (Ephedra)	Whole plant is used for respiratory problems.	Ephedrine from *Ephedra gerardiana* stimulates the respiratory centers, uterus, dilates the bronchi and pupils, contracts the intestines and raises blood sugar.[59]	C, E, W
37.	*Equisetum diffusum* (Equisetaceae)	Ankhle Jhar (Horsetail)	Plant stem juice is given for gonorrhea.	Methanolic plant extract shows good free radical scavenging activity.[60]	W, C, E
38.	*Eupatorium adenophorum* (Asteraceae)	Banmara (Sticky snakeroot)	Leaf juice is applied on cuts and wounds.	Methanolic leaf extracts shows the analgesic effect.[61]	E, C, W
39.	*Ficus auriculata* (Moraceae)	Timila (Roxburgh fig)	Stem juice is considered effective against diarrhea and fruits are used in dysentery.	Tannins of the bark extract may reveal anti-inflammatory And analgesic activities.[62] Bark extract shows potential antioxidant activity.[63]	W, C, E
40.	*Ficus hirta* (Moraceae)	Khasreto (Ficus)	Root decoction treats food poisoning.	Aqueous extracts from *Ficus hirta* have hepatoprotective activity against N, N-dimethylformamide induced acute liver injury in mice.[64]	E
41.	*Fraxinus floribunda* (Oleaceae)	Lankuree (Himalayan ash)	Bark infusion is used for body pain.	Anti-inflammatory, anti-oxidative and skin regenerating activities.[65]	C
42.	*Fritillaria cirrhosa* (Liliaceae)	Kakoli (Fertillaria)	Plant juice is taken for stomach disorders	Plant contains steroidal alkaloids effective against stomach disorders.[66]	E, C
43.	*Helianthus annus* (Asteraceae)	Suryamukhi (Sunflower)	Root decoction as a gargle relieves toothache; dried flower chewed cures ulcers, fever, cough and cold. Leaves crushed and mixed with water and taken bath cures allergy and skin diseases.	Terponoids in methanolic and aquous extract of *Helianthus annus* shows anti-inflammatory activity in rat.[67]	E, C
44.	*Hibiscus esculentus* (Malvaceae)	Ramtoriya (Okra)	Fruit mucilage acts as soothing agent on cuts.	Methanol extract of *Hibiscus esculentus* seeds shows antihypoxic and antioxidant activity in male mice.[68]	E
45.	*Hippophae salicifolia* (Elaeagnaceae)	Dale chuk (Sea buckthorn)	Fruit juice is taken for cough, diarrhea, and menstrual disorder.	Contains high levels of flavonoids (with antimicrobial properties and effectiveness against menopausal symptoms), carotenoids and vitamin C.[69]	W, C
46.	*Hippophae tibetana* (Elaeagnaceae)	Bhui chuk, (Tibetean Sea buckthorn)	Fruit juice is taken for stomach disorders.	Contains high levels of flavonoids (antimicrobial), carotenoids and vitamin C.[69]	C, W
47.	*Hordeum vulgare* (Poaceae)	Jau (Barley)	Gruel is made by the powdered grains and given in case of painful indigestion. Barley water with honey is prescribed in bronchial coughs.	Aqueous methanolic extract of this plant shows hepatoprotective activity against acetaminophen induced liver damage in rats.[70]	E, C

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
48.	*Hydrocotyle asiatica* (Mackinlayaceae)	Ghortapre (Pennywort)	Fresh plant parts crushed and ingested orally cure sores of throat and lungs. Leaf juice is used as eye drops to cure eye infection. Dressing with leaf paste reduces swelling or and applied in wounds. Juice of shoots treats gastritis and constipation.	It has neuroprotective, anti-allergic, Anti-pruritic, and anti-inflammatory activities in animal models.[71]	E, C, W
49.	*Lantana camara* (Verbenaceae)	Masino kada, Sitaji phul (Spanish flag)	The juice of crushed leaves is applied to the fresh cut and wounds to heal. Crushed leaves are tied over the sprain to relieve pain.	Ethanolic extract of leaves and roots shows the antibacterial activity against both gram positive and gram negative bacteria.[72]	E
50.	Lichen species (Parmeliaceae)	Jhau (Lichen)	Lichen extract and decoction is applied to treat moles.	Parmelia species are antimicrobial[73] and also used to treat warts and cranial diseases.[74]	W, C, E
51.	*Lindera neesiana* (Lauraceae)	Pahenlo khapate (Spicewood)	Fruit juice taken for diarrhea.	Essential oil extracted from fruits possess significant antimicrobial activity.[75]	E, C
52.	*Lobelia pyramidalis* (Campanulaceae)	Aklebir (Lobelia)	Juice of leaves and flowers is rubbed on body parts during body ache.	Lobeline, the active constituent, may cause nausea, vomiting and diarrhea.[76]	W
53.	*Lycopersicon esculentum* (Solanaceae)	Rambheda (Tomato)	Raw fruit is taken during indigestion and to prevent bleeding from the gums.	It has 5-alpha-Reductase enzymatic activity which enhances the formation of testosterone.[77]	C, E, W
54.	*Lycopodium clavatum* (Lycopodiaceae)	Supari jhar (Groundpine)	Pollen paste is used on cuts and wounds.	Contains anti-inflammatory alkaloids types of compounds.[78]	C, E
55.	*Mentha arvensis* (Lamiaceae)	Pudina (Mint)	Raw leaves chewed help to check stomach related disorders: gastritis, acidity, indigestion etc., also used to flavor chutney.	Various extracts of *Mentha arvensis* clearly shows a protective effect against acid secretion and gastric ulcers in ibuprofen plus pyloric ligation, 0.6 mol/L HCl induced and 90% ethanol-induced ulcer models.[79]	W, C, E
56.	*Mucuna macrocarpa* (Fabaceae)	Baldengra (Mucuna)	Seed powder taken with water helps remove round worm from stomach.	Crude methanolic extract of stem have in vitro and in vivo apoptosis-inducing antileukemic effects.[80]	E
57.	*Musa paradisiacal* (Musaceae)	Kera (Banana)	Person suffering from fever is advised to drink sap released from the plant directly.	Crude aqueous methanolic extract of leaves shows in vitro anthelmintic effect.[81]	E,C,W
58.	*Mussaenda frondosa* (Rubiaceae)	Asari (Mussaenda)	Whole plant is boiled and decoction is given to treat fever, asthma and cough.	Alcoholic and aqueous extract of this plant shows in vitro antioxidant activity.[82]	E
59.	*Myrica esculenta* (Myricaceae)	Kafal (Box myrtle Bay Berry)	Fruits are eaten for dysentery and bark decoction is given for bronchitis.	Crude extract of stem bark shows anti-allergic activity on mice.[83]	W, C, E
60.	*Nardostachys grandiflora* (Valerinaceae)	Jatamansi (Jatamansi)	Whole plant juice is taken to treat headache and high altitude sickness.	Ethanol extract from roots showed anticonvulsant activity and are a nervous system stimulant.[84]	C, E
61.	*Oroxylum indicum* (Bignoniaceae)	Tatelo (Indian trumpet)	Bark and seeds are powdered and mixed with water, and strained; the mixture is fed to patients suffering from high fever or pneumonia, which believed to restore health or brings down fever. Unbroken pod is also used in rituals.	Methanolic extract of root, bark, stem and leaves have the antioxidant activity.[85]	E, C

Continued

Table: *Continued*

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
62.	*Oxalis corniculata* (Oxalidaceae)	Chari amilo (Creeping woodsorrell)	Whole plant is chewed raw and the juice acts as an appetizer; also checks boil. Fresh plant decoction taken treats dysentery. Fruit is consumed to lessen throat pain.	Methanol extract of *Oxalis corniculata* shows in-vitro antioxidant and anti-inflammatory activity.[86]	E, C, W,
63.	*Paederia scandens* (Rubiaceae)	Pat biree (Sewer vines)	Dried fruit is powdered and applied over teeth to relieve tooth ache and prevent tooth decay.	Several pharmacological activities are reported. Most recent are xanthine oxidase inhibitory and uricosuric activity.[87]	E, C
64.	*Paris polyphylla* (Trilliaceae)	Satuwa (Himalayan Paris)	Root paste is taken for fever, vomiting and worms	A methanolic extract is gastro protective. Also possesses anthelmintic properties.[88]	W, C
65.	*Phyllanthus emblica* (Phyllanthaceae)	Amala (Indian gooseberry)	as a tonic to build up lost vitality and vigor and rassayana in Ayurveda. Also considered as a source of vitamin and amino acid.	It has so many action reported included antiviral, antioxidant, etc. recent research shows the antiplasmodic and cytotoxic effect of water extract.[89]	C, E, W
66.	*Picrorhiza kurroa* (Scrophulariaceae)	Kutki (Picrorhiza)	Dried rhizome is boiled in water and taken to cure fever, cough, etc	Methanolic and aqueous extract of rhizome has potent antioxidant and antineoplastic activity.[90]	E
67.	*Piper longum* (Piperaceae)	Pippali (Long pepper)	Dried seed powder paste is applied to reduce sprains; the powdered roots are given to treat cold and cough.	It has insecticidal and acaricidal, antifungal, antiameobic, antimicrobial, antiasthmatic, antidiabetic, analgesic, anti-inflammatory, hypocholesteromic, antioxidant, anticancer, immunomodulatory, antidepressant, antiulcer, hepatoprotective effect.[91]	E
68.	*Plantago erosa* (Plantaginaceae)	Isabgol jhar (Greater plantain)	Leaf paste is applied to heal wounds, cuts, bruises, insect bites, poison-ivy rashes, minor sores and snakebite. Seed powder is with water treats diarrhea and dysentery.	Methanolic extract shows anti inflammatory activity against carageenan induced paw edema in rat and mice.[92]	E, C, W
69.	*Podophyllum hexandrum* (Berberidaceae)	Laghupatra (Himalayan May Apple)	Root juice is taken for liver complaints	Ethyl acetate extract of *Podophyllum hexandrum* rhizome has antioxidant and protective effect on carbon tetrachloride induced rat liver injury.[93]	W, C, E
70.	*Psidium guajava* (Myrtaceae)	Amba (Guava)	Young leaves and tender shoots taken raw cure mouth ulcers, sore throat, cough, toothache. Drinking bark powder mixed in hot water is best local remedy for dysentery with blood in stool; fruits are edible.	Ethyl acetate fraction of *Psidium guajava* leaf extract shows antioxidant and antiglycative potential in streptozotocin-induced diabetic rats.[94]	E, C, W
71.	*Pteris biaurita* (Pteridaceae)	Gulmohar (Fern)	Mashed petiole extract applied on the cuts and wounds stop bleeding and infections	Alcoholic extracts has the antimicrobial activity.[95]	E, C
72.	*Rhododendron arboreum* (Ericaceae)	Lali guras (Tree rhododendron)	Dried flowers crushed and mixed with water stop excessive bleeding in female. Fresh leaves chewed to cure dysentery.	Flower juice has the hypolipidemic effect in experimentally induced hypercholestermic rabbits.[96]	E
73.	*Rhododendron campanulatum* (Ericaceae)	Guras (Bell rhododendron)	Leaves are chewed and the juice from the crushed leaves relieves cough.	Oleamane, the active triterpenoid, has antibacterial and immunomodulatory activities.[97]	E, C

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
74.	*Rhus semialata* (Anacardiaceae)	Arkhar (Sweet sumach)	Sour juice of fruits is boiled with water and raw egg, treats diarrhea and dysentery. It is also used as food preservative.	*Rhus semialata* fruit extract has the antidiarrheic activity in rats.[98]	E
75.	*Rauvolfia serpentine* (Apocynaceae)	Sarpagandha (Snake root)	Use to lower high blood pressure.	Reserpine, the active alkaloid, produced a dose-dependent depression of the central nervous system.[99]	C, E, W
76.	*Rubia cordifolia* (Rubiaceae)	Mangito (Indian madder)	Root decoction with water is given to cure urinary infection; paste is used as an ointment to skin diseases. Root is also used to make dyes.	Mollugin, a bioactive phytochemical isolated from *Rubia cordifolia* L, exhibits antimutagenic, antitumor, antiviral, and inhibitory activity in arachidonic acid- and collagen-induced platelet aggregation. It also has Neuroprotective and anti-inflammatory effects in mouse hippocampal and microglial cells.[100]	E, C, W
77.	*Rubia manjith* (Rubiaceae)	Majitho (manjith)	Root paste is applied over scabies and other skin diseases	Anti-proliferative against epidermal keratinocytes and also has antiseptic properties.[101]	C, E, W
78.	*Rubus ellipticus* (Rosaceae)	Ainselu (Yellow Himalayan raspberry)	Young shoot is chewed raw to relieve sudden stomach pain. Root decoction given to the children to get rid of stomach warm. Root paste is applied on forehead during severe headache; fruit is edible.	Triterpenoid saponins from roots of *Rubus ellipticus* demonstrated inhibitory activities against alpha-glucosidase.[102]	E, C
79.	*Rumex nepalensis* (Polygonaceae)	Halhale sag (Nepal duck)	The root is purgative. Decoction of the root is applied to dislocated bones. A paste of the root is applied to swollen gums. The leaves are used in the treatment of colic and headaches.	Root extracts of *Rumex nepalensis* has anti-inflammatory, cycloxygenase (COX)-2, COX-1 inhibitory, and free radical scavenging effects.[103]	C, W
80.	*Sapindus mukorossi* (Sapindaceae)	Ritho (Reetha)	Scalp is washed with fruit to remove dandruff and lice.	Saponins from *Sapindus mukorossi* has inhibitory effect on bacterial, fungal and viral genital pathogens.[104]	E, C, W
81.	*Schima wallichii* (Theaceae)	Sule-chilauni (Schima)	Bark is rubbed on the caterpillar infected portion removes its hair.	Polyphenolic enriched extract of *Schima wallichii* bark shows anti-inflammatory activity human peripheral blood mononuclear cells (PBMCs) and *in vivo* by carrageenan-induced paw edema assay (acute study) and cotton pallet granuloma assay (chronic study).[105]	E, C, W
82.	*Schleichera oleosa* (Sapindaceae)	Kusum (Kusum tree)	Fruits are eaten as an anthelmintic	Extracts of bark of *Schleichera oleosa* has cytotoxic and hydroxyl radical-scavenging activities.[106]	C, W
83.	*Semecarpus anacardium* (Anacardiaceae)	Bhalaayo (Marking nut)	Root paste is applied externally on the affected portion cures skin diseases. Decoction of the bark is given to the animals to treat worms.	Has hypolipidemic activity in streptozocin induced diabetic rats.[107]	E, C
84.	*Skimmia anquetilia* (Rutaceae)	Narpati (Skimmia)	Leaf infusion is taken for headache and for freshness	Linalool, from this plant, possess anxiolytic effect.[108]	W
85.	*Smilax aspera* (Smilacaceae)	Kukurdaino (Birdweed)	Root decoction is used for venereal disease	Stem juice is used for dropsy and gout. Rutinoside has cancer inhibitory effect.[109]	W, C

Continued

Table: *Continued*

S. N.	Scientific Name (Family)[16,17]	Vernacular Name (English name)[16,17]	Indigenous/Local use	Phytochemical/ pharmacological properties (Literature review)	Distribution[16,18]
86.	*Solena heterophylla* (Cucurbitaceae)	Bankakri (Creeping cucumber)	Fruits are eaten for common cold and pneumonia of child	Plant extract is hepatoprotective and plant coumarin and flavonoids inhibit platelet aggregation.[110]	W
87.	*Spermadictyon suaveolens* (Rubiaceae)	Ban chanp (Forest champa)	Root paste is applied externally to relieve joint pain.	Ethanolic extract of Bark has anti-inflammatory activity on rats.[111] Methanol extract of stem bark has hepatoprotective activity on rats.[112]	E
88.	*Spondias pinnata* (Anacardiaceae)	Amaro (wild mango)	Plant latex is applied for wounds and cuts.	Flavonoids of the plant have been known to inhibit intestinal motility and hydro electrolytic secretion, which are known to be altered for diarrheal conditions.[113]	W, C, E
89.	*Taxus wallichiana* (Taxaceae)	Lauthsalla, Barme salla (Himalayan yew)	Respiratory problems. Leaf juice is used for cancer and bronchitis.	Taxol isolated from the bark of this plant shows the *in-vitro*, *in-vivo* anticancer activity. It also has antifungal, antiviral anticonvulsant, analgesic, and antipyretic and tumor growth inhibitory activity.[114-116]	C
90.	*Terminalia bellirica* (Combretaceae)	Barro (Baheda)	Fruit is used as laxative, in headache, leucorrhoea, liver diseases to gastro-intestinal complaints	Aqueous extract of *Terminalia bellirica* stimulates the secretion and action of insulin and inhibits starch digestion and protein glycation in vitro.[117]	C, E, W
91.	*Terminalia chebula* (Combretaceae)	Harro (Chebulic myrobalan)	Fruit is used for abdominal problem, headache, bronchitis, and several ayurvedic formulation	Hydro alcoholic extract of *Terminalia chebula* fruit shows antiulcerogenic activity in rats.[118]	C, E , W
92.	*Valeriana jatamansi* (Valerianaceae)	Jatamansi (Valerian)	Cuts and wounds, cough and cold	Dried rhizome extract partially reverses the liver cirrhosis and tissue hyper proliferative response in rats.[119]	C, E, W
93.	*Zanthoxyllum alatum* (Rutaceae)	Timur (Prickly ash, Zanthoxylum)	Branchlet used as toothbrush to relieve toothache. Berries taken to cure stomach ache and toothache. Berries are crushed and rubbed on the leg which acts as leech guard.	Crude extract of *Zanthoxyllum alatum* has the spasmolytic activity in gut, airways and cardiovascular diseases.[120]	E, C

DISCUSSION

Though considerable advances are made in the pharmaceutical sciences, especially in synthetic chemistry, plants and their derivatives continue to maintain their significance in medicines. Increased interest in natural drugs than synthetic are because of a high degree of adverse side effects caused by the latter. Nowadays natural medicines are gaining prominence, because they are economical, easily available and relatively free from side effects. It is evident from the present scenario that herbal cure is gaining world wide acceptance and has emphasized on modern scientific exploration, extraction and evaluation of foil medicines from plants. These are either used directly as a plant extract or modified through further synthesis.[121] The Himalayas represent the largest mountain chain in the world, and is famous for its rich plant diversity and varied ecosystem, containing large number of plants. The use of plants in curing and healing is as old as man himself. Plants containing beneficial and medicinal properties have been known and used in some form or other by primitive people. Many plants which are found commonly and are mentioned in above texts are traditional medicine have not been investigated thoroughly. It is necessary to conduct systematic evaluation, standardization, documentation and patenting of these plants. Targeted based studies with concentration on mechanism of action, lethal dose/effective dose and bioavailability mechanisms need to be conducted in future to explore scientifically the hidden potential of these plants so that the ill community gets maximum benefits from

traditional system of medicine.[122] Biodiversity of Nepal-Himalayas is natural wealth and its conservation is important for economic, ecological, scientific and ethical reasons.[123]

CONCLUSION

Although few researches have been carried out, vast majority of medicinal plant species of Himalayan region are still far behind of pharmacological researches in order to prove their therapeutic potential scientifically. Based on indigenous and ethnic knowledge, medicinal plant of Nepal Himalaya has diverse therapeutic potency. Therefore concise and continues research with advanced instruments is necessary to explore their pharmacological property which may act as milestone to decrease the resistance and adverse effect problem of modern allopathic medicine.

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Identification of a Bioactive Compound from *Myrcianthes cysplatensis*

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ABSTRACT

Myrcianthes cisplatensis (Cambess.) O. Berg (Myrtaceae) grows freely in Uruguay especially in the banks of rivers and streams. It is locally known as guayabo colorado and it´s fruits are edible and used for the preparation of marmalades.

In this work we present the results of the bioguided isolation and structural elucidation of the main active compound as well as it´s antibacterial activity. Through repeated chromatography a pure compound could be obtained. The compound was studied by different spectroscopic techniques and could be unambiguously identified as α-methyl-1-(2´, 4´, 6,-trimethoxyphenyl)-1-propanone.

When assaying for antistaphylococcal activity, it showed MICs of 62.5 μg/mL for the sensible strain (ATCC 6538p) and 250 μg/mL for the multirresistant ones (ATCC 43300 and ATCC 700699). This shows that the bioguided fractionation is appropriate even when not very active compounds are isolated

Key words: *Myrcianthes cysplatensis, Staphylococcus aureus*.

INTRODUCTION

In spite of the great advances in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. The World Health Organization[1] states that infectious and parasitic diseases account for nearly 11 million among the 57 million total deaths in 2003.

Although there seems to be a great array of antibacterial and antifungal drugs in clinical use, the appearance of resistant organisms makes them sometimes ineffective or lead to recurrence as stated by the World Health Organization.[2] Amongst some of the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most difficult bacteria to treat.[3]

The use of higher plants and preparations made from them to treat infections is an age-old practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases.[4] This wealth of experience and information about medicinal plants as well as the current problems associated with the use of antibiotics has renewed the interest in plants with antimicrobial properties.[5-11]

In previous work we undertook the biological and chemical prospection of the gallery forest of the northern Uruguay River basin.[12] Plants were selected after an exhaustive review of the available literature according at its ethnopharmacological use and submitted to antimicrobial assays and phytochemical characterization.[13] Among them, *Myrcianthes cisplatensis* extracts showed striking activity with a broad spectrum of activity that deserves further investigation. Many species belonging to the Myrtaceae family(that comprises, *Eucalyptus*, *Psidium* and *Syzygium* genus) have been studied for their antimicrobial properties.[14-16]

Myrcianthes cisplatensis (Cambess.) O. Berg (Myrtaceae) grows freely in Uruguay especially in the banks of rivers and streams. It is locally known as guayabo colorado and it´s fruits are edible and used for the preparation of marmalades.

In this work we present the results of the bioguided isolation and structural elucidation of the main active compound as well as it´s antistaphylococcal activity.

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MATERIAL AND METHODS

Plant material

M. cisplatensis leaves were collected in the banks of Rio Uruguay, Paysandu and identified by Lic. F. Haretche, Museo y Jardin Botanico "Atilio Lombardo", Montevideo. Voucher specimens (N° 26349) were kept in the MVJB Herbarium, Jardín Botánico, Montevideo.

Analytical methods

GC analysis was performed in a Shimadzu GC 14 apparatus with an SE-52 column using a temperature program from 100° to 280° with a 5°/min gradient. A Brucker micrOTOF-Q-TOF with ESI source in positive mode was used for MS spectra and a Shimadzu QP 5050 with a SE 52 column was used for the GC-MS analysis.

TLC was performed on silicagel or RP C18 plates (Macherey Nagel, Dürin, Germany) using $CHCl_3$/MeOH (80:20) or isopropanol/H_2O (50:50) as solvent respectively and H_2SO_4/heating or anisaldehyde as detection reagents.

^1H NMR and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz reapectively, on a Brucker Avance DPX 400 spectrometer, using $CDCl_3$ as solvent and TMS (δ_H 0.00) and acetone (δ_C 31.00) as references. 2D (different H,H-COSY, HMBC, HSQC) experiments were carried out with programs available in the Brucker software.

Bioautography

Bioautographies were made on developed and dried TLC plates according to the agar overlay method of Rahalison et al.[17] using *Staphylococcus aureus* (ATCC 6538p).

Extraction and isolation

Air dried and coarse milled *M. cisplatensis* leaves were twice extracted with dichloromethane for one week in the dark. The combined extracts were evaporated under vacuum and used for the following procedures.

The extract was dissolved in a minimum volume of methanol and submitted to column chromatography on Polyamide (Macherey-Nagel, 815600) with MeOH and acetone as eluents. The second MeOH fraction was submitted to vacuum column chromatography (VLC) on flash Silicagel (Macherey-Nagel, 815380) with CH_2Cl_2/MeOH (100:0 to 90:10) as eluent and the active fraction (95:5) was further purified a C_{18} cartridge to give a single compound (by TLC and GC)

Conglomerone (1)

$C_{13}H_{18}O_4$, dark yellow oil. UV (CH_2Cl_2) λ_{max}.= 279 nm. EI-MS *m/z*: 238 $[M]^+$, 195 $[C_{10}H_{11}O_4]^+$. HR-ESI-MS *m/z*: 239.2879 $([M+H]^+$, 261.2694 $[M+Na]^+$. ^1H NMR ($CDCl_3$): 1.13 (6H, d, 8Hz) α-Me and 3-Me, 3.03 (1H, m) H-2, 3.78 (6H, s) 2´ and 6´OMe, 3.84 (3H, s) 4´OMe, 6.12 (2H, s) H-3´and H-5´. 13 C NMR ($CDCl_3$): 18.0 C-1 and α-Me, 41.7 C-2, 55.4 4´-OMe, 55.8 2´and 6´-OMe, 90.0 C-3´and C-5´, 113.0 C-1´, 158.0 C2´ and C-6´, 161.0 C4´, 208.0 C-1.

Antibacterial analysis

Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and Laboratory Standards Institute (CLSI, 2006) using sensitive (ATCC 6538p) and resistant (ATCC 43300 and ATCC 700699) *Staphylococcus aureus* strains.

RESULTS AND DISCUSSION

Repeated column chromatography of the dichloromethane extract of *M. cisplatensis* leaves gave a compound (**1**) that showed only a spot in TLC and one peak in GC.

The ESI mass spectrum of **1** showed ions at *m/z* 239.2879 and 261.2694 ($[M+H]^+$ and $[M+Na]^+$, respectively) indicating a molecular formula $C_{13}H_{18}O_4$ (needs 238,2801). In the GC-MS spectra a prominent ion at *m/z* 195 is shown along with the 238 ion. The UV spectrum showed a maximum absorption at λ 279 nm indicating the presence of an aromatic group.

The ^1H NMR spectra showed few signals, with a doublet (6H) at 1.13 ppm, a septuplet at 3.03 ppm and singlets at 3.78, 3.84 and 6.12 ppm. In the ^{13}C NMR spectra 9 signals could be identified corresponding to 5 methyl, 3 methine and 5 quaternary carbons according to DEPT. Using a combination of 2D (H,H COSY, HSQC, HMBC) experiments all the signals can be assigned.

Especially useful were the correlations between the protons at δ_H 1.13 (d, 6H) with the signal at 3.03 and carbons at δ_C 18.0 (*via* HSQC) and 41.7 (*via* HMBC) defining a isopropyl group that in turn is correlated to the carbonyl carbon at 208.2 ppm as can be seen in Figure 1. This carbon did not have any other correlation suggesting that is directly linked to the phenyl moiety. This suggestion is further supported by the presence of the peak at *m/z* 195 in the GC-MS characteristic of a trimethoxyphenyl-carbonyl ion.

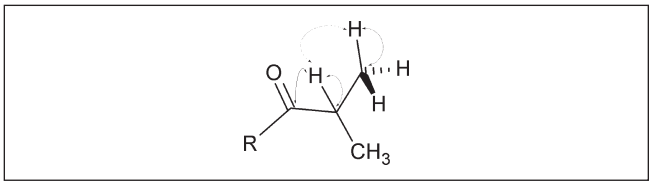

Figure 1: Main correlations in the isopropyl moiety.
Key to the figure:
COSY HSQC HMBC

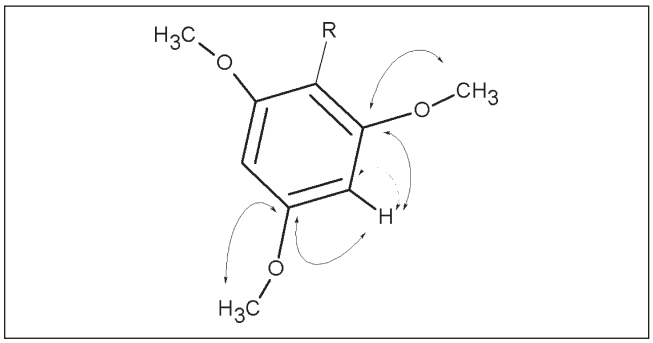

Figure 2: Main correlations in the aromatic moiety.
Key to the figure:
COSY HSQC HMBC

In the same way the correlations between the aromatic protons at δ_H 6.12 ppm with carbons at 158.0 and 161.0 ppm and the absence of correlation with carbon at 113.0 ppm determined the 2´, 4´, 6´ pattern of substitution in the aromatic group (Figure 2). Thus the compound could be unambiguously identified as α-Methyl-1-(2´, 4´, 6´-trimethoxyphenyl)-1-propanone.

When assaying for antistaphylococcal activity, Compound 1 showed . a MIC of 62.5 μg/mL for the sensible strain (6538p) and 250 μg/mL for the multirresistant ones (43300 and 700699). This shows that the bioguided fractionation is appropriate even when not very active compounds are isolated.

CONCLUSIONS

The bioguided fractionation of *M. cisplatensis* dichloromethane extract gives a pure compound which using different spectroscopic techniques could be identified as a propiophenone derivative: α-Methyl-1-(2´,4´,6´-trimethoxyphenyl)-1-propanone. From a biosynthetic point of view the compound could be rationalized as a product of the polyketide pathway with an isobutirylCoA starter and the usual malonylCoA prolonger units through Claisen reaction.[19]

The compound has been previously isolated by Lahey from *Eucalyptus conglomerata* who named it conglomeone.[20] Conglomerone was also proposed by Ricciardi as a phyletic marker for chemosystematics studies in the Myrtaceaee family.[21] However this is the first complete spectroscopic study of the compound as well as the first antibacterial activity reported.

Both the extract and the pure compound showed antibacterial activity against methicillin-sensitive and resistant *Staphylococcus aureus* strains

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Comparative Standardization and Physicochemical Evaluation of the Leaves of *Stevia rebaudiana* Bertoni from Different Geographical Sources

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ABSTRACT

Stevia rebaudiana Bertoni, a natural non-caloric substitute to conventional sugar, is also popular as the "sweet herb of Paraguay". It is a storehouse of various bioactive constituents mainly, the ent-kaurene diterpene glycosides namely- stevioside, rebaudioside A, B, C, D and E. The plant is known to exhibit a wide range of biological activities like hypoglycemic, anti-oxidant, anticancer, antibacterial activities. The present research is based on a comparative standardization and physicochemical analysis of the dried leaves of five varieties of *Stevia rebaudiana* procured from five different geographical locations of India viz., Delhi, Surat, Kangra, Bangalore and Indore. Fluorescence analysis of the powdered leaves was carried out as a means for identification. The standardization parameters included determination of foreign matter, ash values, loss on drying, extractive values. Preliminary phytochemical screening was also performed. The results from the current study can prove to be an indicator to differentiate the five varieties based on their standardization parameters.

Key words: *Stevia rebaudiana*, non-caloric substitute, ent-kaurene glycosides, comparative standardization.

INTRODUCTION

The modern era faces a number of growing ailments and diseases that are a serious concern to normal sustenance of an individual in this scenario. These include hypertension, diabetes mellitus, premature aging, cancer, dental caries, skin diseases like acne and pruritis, bacterial and fungal infections and many more. Control and cure of these diseases require a source that can overcome these health concerns and that has a minimal potential to cause adverse effects.

This situation and need has brought "*Stevia rebaudiana*" (Family:-Asteraceae) into the picture which is a substitute to conventional sugar existing in nature. It is a non-caloric sweetener which is consumed in many countries.[1] It is a small perennial shrub growing upto 1m tall and with leaves 2-3cm long[2] and native to regions of Paraguay and Brazil. It is popular as the "sweet herb of Paraguay", as the leaves have been traditionally used by natives of Paraguay and Brazil for hundreds of years to sweeten local teas, medicines and as a 'sweet treat'. The plant is also known as sweet herb, honey leaf, or sweet chrysanthemum as it possesses sweet tasting glycosides.[3] It is a storehouse of various bioactive constituents mainly, the ent-kaurene diterpene glycosides (the sweet tasting glycosides) namely- stevioside, steviolbioside, dulcoside A and rebaudioside A, B, C, D and E.[4] These compounds stevioside and rebaudioside are 250-300 times sweeter than sucrose, heat stable, pH stable, and non-fermentable. With reference to its sweetening power, it is estimated that 30ml of *Stevia* extract is equivalent to 3 kg of sucrose.[3] The plant's leaves, the aqueous extract of the leaves, and purified steviosides are used as sweeteners. The sweetener extractives have been known to exert beneficial effects on human health- antihypertensive,[5,6] antidiabetic,[7-10] non-carcinogenic,[11,12] antioxidant,[13,14] anti-inflammatory activities.[15,16] They are also thought to effect glucose metabolism and renal function.[17] Apart from these it also exhibits antimicrobial activities.[18,19] It also plays a beneficial role as a dentifrice as it inhibits the development of plaque and cavities.[20]

The current investigation is aimed at a comparative standardization of five varieties of *Stevia rebaudiana* procured from five different geographical locations of India viz.,

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Delhi, Surat, Kangra, Bangalore and Indore to find out which variety best complies with the standardization parameters so that it can be effectively used in manufacturing of various *Stevia* based products with maximum quality.

MATERIALS AND METHODS

Collection

Dried leaves of *Stevia rebaudiana* were procured from different suppliers of India: Saico Healthcare Pvt. Ltd. (Delhi), Keshal Nursery (Surat), Deepak Trading Co.(Bangalore), Shri Krishna Herbal (Indore) and locally field grown leaves from Chachiyan Village (Kangra) between the months of September to November, 2010. The identity of the leaves was verified by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi and a voucher specimen for the leaves was deposited at the Herbarium of National Institute of Science Communication and Information Resources, New Delhi respectively.

Fluorescence Analysis

1-2 mg of the dried leaf powder of all the five varieties of *Stevia* were taken and placed on a microscopic slide and

Table 1: Fluorescence analysis of powdered leaves of *Stevia rebaudiana* from Delhi

Treatment	*Stevia rebaudiana* (Delhi)		
	Day light	UV light	
		254 nm	366 nm
Powder as such	Light green	Greyish brown	Dark brown
Powder + 1N NaOH (aq.)	Brownish yellow	Bright green	Blackish green
Powder + 1N NaOH (alc.)	Yellowish green	Yellowish green	Brownish green
Powder + 1N HCl	Yellowish green	Bright green	Greenish black
Powder + NH_3	Dark green	Blackish green	Purplish black
Powder + 5% iodine	Greyish green	Silvery green	Blackish green
Powder + 5% $FeCl_3$	Blackish green	Greenish black	Dark green
Powder + acetic acid	Light brown	Blackish green	Blackish brown
Powder + 1N H_2SO_4	Yellowish green	Bright green	Blackish green
Powder + 1N HNO_3	Yellowish green	Light green	Blackish green

Table 2: Fluorescence analysis of powdered leaves of *Stevia rebaudiana* from Surat

Treatment	*Stevia rebaudiana* (Surat)		
	Day light	UV light	
		254 nm	366 nm
Powder as such	Dark green	Dark green	Brownish green
Powder + 1N NaOH (aq.)	Dark brown	Light green	Dark brown
Powder + 1N NaOH (alc.)	Dark green	Dark green	Brownish green
Powder + 1N HCl	Brownish green	Light green	Purplish black
Powder + NH_3	Blackish green	Blackish green	Black
Powder + 5% iodine	Blackish green	Dark green	Purplish green
Powder + 5% $FeCl_3$	Dark green	Blackish green	Brownish green
Powder + acetic acid	Dark green	Light green	Brownish green
Powder + 1N H_2SO_4	Dark green	Blackish green	Blackish green
Powder + 1N HNO_3	Orange green	Dark green	Black

Table 3: Fluorescence analysis of powdered leaves of *Stevia rebaudiana* from Bangalore

Treatment	*Stevia rebaudiana* (Bangalore)		
	Day light	UV light	
		254 nm	366 nm
Powder as such	Yellowish green	Yellowish green	Brownish green
Powder + 1N NaOH (aq.)	Brownish green	Dark green	Purplish black
Powder + 1N NaOH (alc.)	Brownish green	Bright green	Brown
Powder + 1N HCl	Brownish yellow	Light green	Grey
Powder + NH_3	Blackish green	Dark green	Black
Powder + 5% iodine	Greyish green	Bright green	Purplish grey
Powder + 5% $FeCl_3$	Yellowish green	Dark green	Purplish black
Powder + acetic acid	Dark brown	Dark green	Purplish brown
Powder + 1N H_2SO_4	Yellowish green	Light green	Black
Powder + 1N HNO_3	Orange brown	Blackish green	Black

Table 4: Fluorescence analysis of powdered leaves of *Stevia rebaudiana* from Kangra

Treatment	*Stevia rebaudiana* (Kangra)		
	Day light	UV light	
		254 nm	366 nm
Powder as such	Dark green	Light green	Blackish green
Powder + 1N NaOH (aq.)	Blackish green	Blackish green	Black
Powder + 1N NaOH (alc.)	Dark green	Dark green	Black
Powder + 1N HCl	Light green	Light green	Blackish green
Powder + NH_3	Blackish green	Greenish black	Brownish green
Powder + 5% iodine	Dark green	Dark green	Black
Powder + 5% $FeCl_3$	Blackish green	Brownish green	Blackish green
Powder + acetic acid	Dark green	Dark green	Purplish green
Powder + 1N H_2SO_4	Light green	Light green	Blackish green
Powder + 1N HNO_3	Dark brown	Dark green	Blackish green

Table 5: Fluorescence analysis of powdered leaves of *Stevia rebaudiana* from Indore

Treatment	*Stevia rebaudiana* (Indore)		
	Day light	UV light	
		254 nm	366 nm
Powder as such	Light green	Yellowish green	Dark green
Powder + 1N NaOH (aq.)	Brownish green	Blackish green	Dark green
Powder + 1N NaOH (alc.)	Light brown	Bright green	Dark brown
Powder + 1N HCl	Yellowish green	Dark green	Brownish green
Powder + NH_3	Dark green	Bright green	Blackish green
Powder + 5% iodine	Brownish green	Light green	Blackish green
Powder + 5% $FeCl_3$	Yellowish green	Blackish green	Black
Powder + acetic acid	Brownish green	Dark green	Blackish green
Powder + 1N H_2SO_4	Yellowish green	Bright green	Blackish green
Powder + 1N HNO_3	Dark brown	Light green	Black

Table 6: Foreign matter of the different varieties of *Stevia rebaudiana*

Stevia rebaudiana	Weight of sample taken (g)	Foreign matter (%)
Delhi	100	2.35
Surat	100	0.58
Bangalore	100	1.65
Kangra	100	1.80
Indore	100	0.85

Table 7: Total ash, acid insoluble ash and water soluble ash of the different varieties of *Stevia rebaudiana*

Stevia rebaudiana	Total ash (%w/w)	Acid insoluble ash (%w/w)	Water soluble ash (%w/w)
Delhi	9.00	1.25	6.25
Surat	13.50	2.25	7.25
Bangalore	12.75	1.25	7.75
Kangra	7.75	0.75	4.25
Indore	11.50	1.75	6.75

Table 8: Loss on drying of the different varieties of *Stevia rebaudiana*

Stevia rebaudiana	Weight of sample taken (g)	Loss on drying (%w/w)
Delhi	10	5.75
Surat	10	7.90
Bangalore	10	5.35
Kangra	10	8.15
Indore	10	7.25

observed in day light as well as in short wave UV light (254nm) and long wave UV light (366 nm). The powdered drugs were then treated with different reagents as 1 N sodium hydroxide (aqueous), 1 N sodium hydroxide (alcoholic), 1 N hydrochloric acid, ammonia, 5% iodine, 5% ferric chloride, acetic acid, 1 N sulphuric acid, 1 N nitric acid[21,22,23] and the results were noted. (Table 1,2,3,4,5)

Standardization and physicochemical parameters

Physicochemical parameters of the leaves which included determination of foreign matter, ash values (total ash, water soluble ash and acid insoluble ash) and loss on drying[24,25,26] and the results were taken. (Table 6,7,8)

Extraction

The five varieties of the leaves collected were taken and subjected to both hot soxhlation as well as cold maceration using petroleum ether (b.p. 40°-60°), chloroform, methanol, methanol:water(1:1) and chloroform:water (1:99) as solvents. The different extracts were concentrated using rota vapor. Extractive values in different solvents (petroleum ether soluble, chloroform soluble, methanol soluble, diluted methanol soluble and water soluble) were then determined

according to the method[24,27] and noted. (Table: 9 and Table 10)

Successive solvent extraction

Successive solvent extraction of the air-dried drug powdered leaves was carried out using the same solvents as earlier successively in increasing order of polarity starting with petroleum ether (b.p. 40°-60°), chloroform, methanol, methanol:water(1:1) and finally with chloroform: water (1:99) by cold maceration.[28] Before extracting with a new solvent, the powdered material was dried in hot air oven at temperatures below 50 °C.[29] The different successive solvent extractive values were then recorded. (Table 11)

Preliminary phytochemical screening

The methanolic extracts of all the five varieties were subjected to preliminary phytochemical screening to judge the presence of various classes of phytoconstituents as per the method.[17,30] The different chemical tests included the tests for alkaloids, saponins, carbohydrates, glycosides (general), anthraquinone glycosides, cardiac glycosides,

Table 9: Extractive values in different solvents by hot soxhlation

Stevia rebaudiana	Petroleum ether (%w/w)	Methanol (%w/w)	Methanol-water (%w/w)	Chloroform-water (%w/w)
Delhi	2.40	31.15	29.50	21.85
Surat	2.55	38.10	33.80	26.50
Bangalore	3.60	35.25	32.00	24.20
Kangra	6.00	28.55	35.40	24.90
Indore	3.25	29.00	32.65	23.40

Table 10: Extractive values in different solvents by cold maceration

Stevia rebaudiana	Petroleum ether (%w/w)	Chloroform (%w/w)	Methanol (%w/w)	Methanol-water (%w/w)	Chloroform-water (%w/w)
Delhi	3.15	11.95	39.95	14.95	18.60
Surat	3.85	10.55	45.30	20.95	25.15
Bangalore	4.90	13.80	47.00	17.05	24.50
Kangra	2.55	10.10	41.95	19.55	20.25
Indore	5.45	14.25	44.30	15.50	21.20

Table 11: Successive solvent extractive values of the different varieties of *Stevia rebaudiana*

Stevia rebaudiana	Petroleum ether (% w/w)	Chloroform (% w/w)	Methanol (% w/w)	Methanol-water (% w/w)	Chloroform-water (% w/w)
Delhi	2.00	4.90	19.20	11.20	10.60
Surat	2.90	4.00	24.50	10.40	12.70
Bangalore	3.00	6.40	22.40	7.20	15.40
Kangra	3.20	6.30	27.20	12.00	11.30
Indore	2.40	5.80	21.20	9.60	12.20

Table 12: Preliminary phytochemical screening of the methanolic extracts of the different varieties of *Stevia rebaudiana*

Test	*Stevia rebaudiana*				
	Delhi	Surat	Bangalore	Kangra	Indore
Alkaloids	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve	+ve	+ve
Glycosides (general)	+ve	+ve	+ve	+ve	+ve
Anthraquinone glycosides	+ve	+ve	+ve	+ve	+ve
Cardiac glycosides	+ve	+ve	+ve	+ve	+ve
Coumarin glycosides	+ve	+ve	+ve	+ve	+ve
Cyanogenetic glycosides	–ve	–ve	–ve	–ve	–ve
Tannins	+ve	+ve	+ve	+ve	+ve
Proteins	–ve	–ve	–ve	–ve	–ve
Steroids	+ve	+ve	+ve	+ve	+ve
Waxes	+ve	+ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve
Amino acids	+ve	+ve	+ve	+ve	+ve
Acidic compounds	–ve	–ve	–ve	–ve	–ve

coumarin glycosides, cyanogenetic glycosides, tannins, proteins, steroids, waxes, flavonoids, amino acids and acidic compounds and the results were taken. (Table 12)

RESULTS AND DISCUSSION

The current investigation assessed in a detailed and comparative standardization and physicochemical analysis of the dried leaves of five varieties of *Stevia rebaudiana* procured from five different geographical locations of India viz., Delhi, Surat, Kangra, Bangalore and Indore. From the current study, it was possible to differentiate the five varieties based on their standardization parameters. The results may prove to be a valuable indicator in finding out a suitable variety that best matches in accordance with the standardization parameters so that it can be effectively used in manufacturing of various *Stevia* based products with reasonable and fair quality.

The various physicochemical parameters carried out for the purpose of standardization and authentication included determination of foreign matter, loss on drying, ash values (total ash, acid insoluble ash, water soluble ash), extractive values in different solvents as petroleum ether (b.p. 40°-60°), chloroform, methanol, methanol-water (1:1), chloroform-water (1:99). Both hot soxhlation, cold maceration and successive solvent extraction were carried out for all the five varieties in all the five solvents and it was found that successive solvent extraction led to lower extractive values compared to hot soxhlation and cold maceration. The foreign matter was found to be the highest in the leaves from Delhi with a value of 2.35% w/w and lowest in the leaves from Surat with a value of 0.58% w/w. Presence of moisture which was determined through loss on drying (LOD) was found to be the maximum in Kangra variety i.e., 8.15% w/w and the minimum in the Bangalore variety i.e., 5.35% w/w. Ash values were mainly determined with the purpose of estimating the inorganic salts naturally occurring in the drug and adhering to it as well as the inorganic matter added for the purpose of adulteration and it was found that the total ash and acid insoluble ash was found to be the maximum in the Surat variety with a value of 13.50% w/w and 2.25% w/w respectively and minimum in the Kangra variety with a value of 7.75% w/w and 0.75% w/w respectively. However, the water soluble ash was found to be the highest in the Bangalore variety i.e., 7.75% w/w and the lowest in the Kangra variety i.e., 4.25% w/w.

Additionally, fluorescence analysis for the powdered leaves was carried out using various reagents in day light and UV light (254 nm and 366 nm) which served as a parameter for identification of the plant material.

Preliminary phytochemical screening was carried out on the methanolic extracts of all the varieties and revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides (anthraquinone, cardiac, coumarin), saponins, carbohydrates, flavonoids, tannins, amino acids, steroids, waxes supporting the reason for its wide range of biological activities.

CONCLUSION

Hence, the current research assists to differentiate the five varieties of *Stevia rebaudiana* based on their standardization and physicochemical parameters. The fluorescence analysis of the powder, various physicochemical parameters like foreign matter, loss on drying, ash values, extractive values as well as phytochemical studies including preliminary phytochemical screening supported the identification and authentification of the five varieties for the present study. The results may thus, be helpful in obtaining the variety of best quality to be used in manufacturing of various *Stevia* based products.

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Pharmacognostical Studies of Leaves of *Lagerstroemia flos-reginae*

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ABSTRACT

Introduction: *Lagerstroemia flos-reginae* (L) Pers. (Hindi-Jarul) belonging to Lythraceae is found throughout India, especially in Assam, Bengal and Deccan peninsula. A decoction of the leaves of *L. flos-reginae* in form of tea is widely used for diabetes mellitus in Philippines. **Methods:** The pharmacognostical investigations of leaves of *L flos-reginae* was done by evaluating its morphological, microscopical studies, leaf constants, phytochemical screening and various physicochemical parameters. **Results:** The microscopical studies revealed presence of epidermis with striated cuticle, bilayered palisade, rannanculaceous stomata, abundant calcium oxalate rosettes and prisms, fragments of bordered pitted xylem vessels and lignified pericyclic fibres in groups. Physical constants of leaf powder showed 14.23% alcohol soluble extractive value, 15.75% water soluble extractive value, 8.94% total ash, 8.65%water soluble ash, 1.94% acid insoluble ash, phytochemical analysis revealed presence of triterpenoid saponins, tannins, alkaloids, steroids, sugars and proteins. **Conclusion:** The above pharmacognostical and preliminary phytochemical studies will be beneficial for proper identification and authentification of leaves of *L. flos-reginae.*

Key words: *Lagerstroemia flos-reginae,* leaf constants, microscopy, physicochemical parameters

INTRODUCTION

Lagerstroemia flos-reginae L. (Syn. *Lagerstroemia speciosa*) belonging to Family Lythraceae is popularly known as Banaba, Jarul in Hindi and Queen's crape myrtle in English. It grows widely in the Philippines, India, South East Asian Countries including Vietanam, Malaysia and south China. In India, it is found especially in Assam, Bengal and Deccan peninsula.[1,2] It is one of the well known ornamental trees and is cultivated widely in gardens as an avenue tree. It is a medium sized to large deciduous tree about 9-18 m high with a rounded crown. Bark smooth, grayish, exfoliating in irregular flakes. Flowers are 5-7.5 cm across in large panicles, sometimes reaching to 30 cm. long; mauve to purple in colour. Capsule ellipsoid or subglobose. Seeds are pale brown in colour.[3] The tea from the leaves of Banaba has traditionally been used in Philippines, as a folk medicine for the treatment of diabetes. The leaves have also been used as purgative, deobstruent and diuretic.[1]

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Phytochemical studies showed presence of triterpene acids like corosolic acid, ursolic acid, oleanolic acid, maslinic acid, asiatic acid and arjunolic acid.[4,5] Leaves also revealed presence of tannin derivatives like lagertanin, lageracetal, lagerstroemin, flosin B, reginin A, reginin C and reginin D.[6,7,8] Besides it also showed presence of sterols like daucosterol, β-sitosterol, phytol, sitosterol acetate.[9] A corosolic acid from alcoholic extract of leaves was reported to have antidiabetic activity when administered *in vivo* as well as *in vitro*.[10,11] Tannins isolated from leaves showed significant hypoglycemic activities in different models as well.[6]

However, there are no reports on the systematic pharmacognostical studies of the leaves of *L. flos-reginae*. Hence, the present investigation is an attempt in this direction and includes evaluation of the leaves of *L. flos-reginae* by its macroscopical, microscopical, physicochemical parameters and preliminary phytochemical screening of different extracts.

MATERIALS AND METHODS

Plant material

The fresh leaves were collected from a healthy and well developed tree of *Lagerstroemia flos-reginae* (L.) Pers from

The Bapalal Vaidya Botanical Garden of Veer Narmad South Gujarat University, Surat, India in June 2007, when the flowering was in bloom. They were identified as leaves of *Lagerstroemia flos-reginae* (L). Pers. by comparing the morphological characters described in the literature[1,12,13] The authenticity of the plant was further confirmed by Dr. Minoo H. Parabia, Head of Department and botanist, Bapalal Vaidya Botanical Research Centre (Department of bioscience) The Veer Narmad South Gujarat University, Surat and voucher specimen number was given as PAH/23082007/01 and deposited at bioscience department of The Veer Narmad South Gujarat University, Surat, India for future reference.

Macroscopic evaluation

The leaves of L. flos-reginae were evaluated macroscopically to photographed to view its extra features.

Microscopic evaluation

i) Sectioning

Transverse sections of fresh leaves of *L. flos-reginae* were taken by microtome and free hand sectioning. Numerous temporary mounts of transverse sections were prepared using lactophenol as a mounting agent and examined microscopically. Histochemical reactions were applied with hydrochloric acid-phloroglucinol to reveal lignified elements, iodine-iodide for starch, Sudan IV for lipophilic substances, Dragendorff's reagent for alkaloidal substances, ruthenium red for mucilage and ferric chloride for phenolic compounds.[14]

Photomicrographs of the microscopical sections were taken with the help of Magnus MLX-DX photomicroscope provided with Honestech software.

ii) Powder characteristics

Microscopical examination of powder of leaves were carried out. Photomicrographs were taken Preliminary examination, behavior of powder with different chemical reagents were performed.[15,16]

iii) Leaf constants

The leaf constants of *L. flos-reginae* were determined by standard methods.[17] Photomicrographs of important microscopical structures were taken with the help of Magnus MLX-DX photomicroscope provided with Honestech software.

iv) Micrometry

The measurements of different cells and cell contents were done with the help of calibrated ocular micrometer.

Physico-chemical parameters

Percentage of total ash, acid-insoluble ash and water soluble ash were calculated. Water soluble and alcohol soluble extractive values of the leaves were determined.[18]

Fluorescence analysis

Fluorescence analysis of powdered leaves was carried out by standard methods.[19,20]

Preliminary phytochemical screening

For the preliminary phytochemical analysis, 5 g powdered drug was extracted in soxhlet extractor with petroleum ether (60-80 °C), ethyl acetate, n-butanol, methanol and water successively. The presence or absence of different phytoconstituents viz. triterpenoids, steroids, alkaloids, sugars, tannins, coumarins and flavanoids, etc. were detected by usual prescribed methods.[21,22]

RESULTS AND DISSCUSION

Macroscopical characters

The leaves are simple, pinnate and opposite. They are elliptical to oblong, 8-22 cm × 3.5-7.2 cm in size. The apex of leaves is subacute. Leaves have entire margin and coriaceous texture. The leaves show fine reticulations both surfaces. Main nerves are in 10-13 pairs, prominent and curving upwards. The odour is characteristic and taste is bitter (Figure 1).

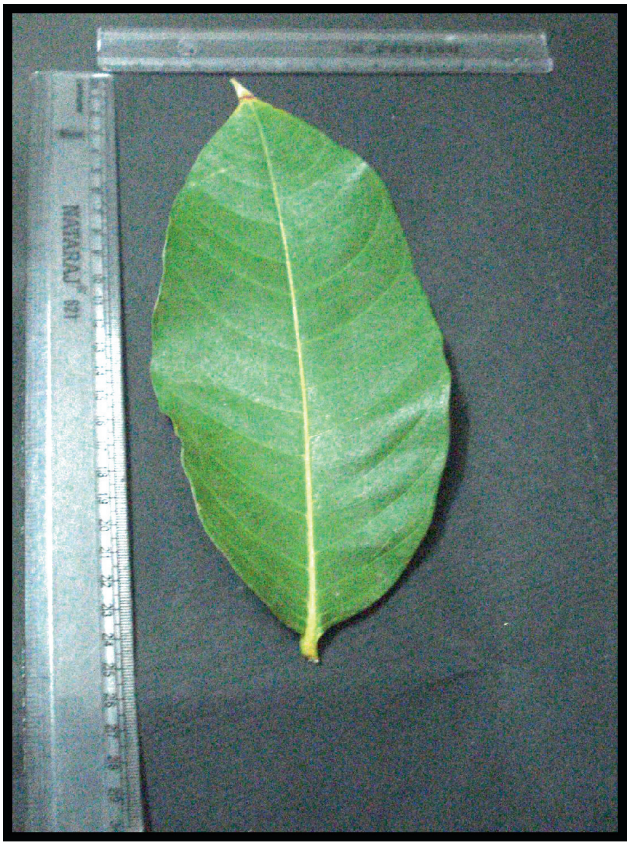

Figure 1: A leaf of *Lagerstroemia flos-reginae*

Microscopical characters

Transverse section of leaf (Figure 2)

Lamina

The leaf of *L. flos-reginae* is dorsiventral with distinct adaxial and abaxial faces.

Epidermis: It consists of single layered rectangular cells, covered with thin and striated cuticle. Some epidermal cells contain mucilage. The adaxial epidermal cells are about twice as large as those of the abaxial epidermis.

Mesophyll: Mesophyll is well differentiated and composed of double layered, compact, radially elongated palisade tissue followed by spongy mesophyll composed of 3-4 layers of loosely arranged parenchymatous cells with scattered calcium oxalate cluster crystals.

Midrib

Transverse section passing through midrib represented concavity on abaxial surface and a convex or rounded adaxial surface.

Vascular bundle: Midrib consists of two bicollateral vascular bundles in which secondary xylem vessels are arranged in form of cup and lid shape. Xylem shows presence of tracheids, xylem parenchyma and xylem vessels. Distinct band of phloem tissue can be seen on either sides of xylem. The central region of vascular bundle is made up of thin walled parenchymatous cells called pith in which sclerenchymatous cells are found in groups. The vascular bundle is encircled by continuous band of the pericyclic sclerenchymatous lignified, thick walled fibres. Pericycle is surrounded by parenchymatous cells of ground tissue. Prismatic crystals and rosettes of calcium oxalate are abundant in midrib and mesophyll.

Microscopical powder studies (Figure 3)

Stomata: Rannanculaceous types of stomata

Epidermal cells: Straight walled epidermal cells with striated cuticle

Palisade: Compactly arranged bilayer palisade cells

Calcium oxalates crystals in form of rossetts and prism

Xylem vessels: spiral and annular

Different leaf constants and micrometric analysis are tabulated in table 1 and 2 respectively.

Results of Physico-chemical parameters for leaf powder of *L. flos-reginae* is shown in Table-3. Quantitative standards revealed that the ash content was 3.56 ± 0.15%. Water soluble ash and acid insoluble ash was 2.51 ± 0.11 and 0.33 ± 0.05% respectively. The water soluble extractive value was 15.75 ± 0.35 indicating the presence of sugar,

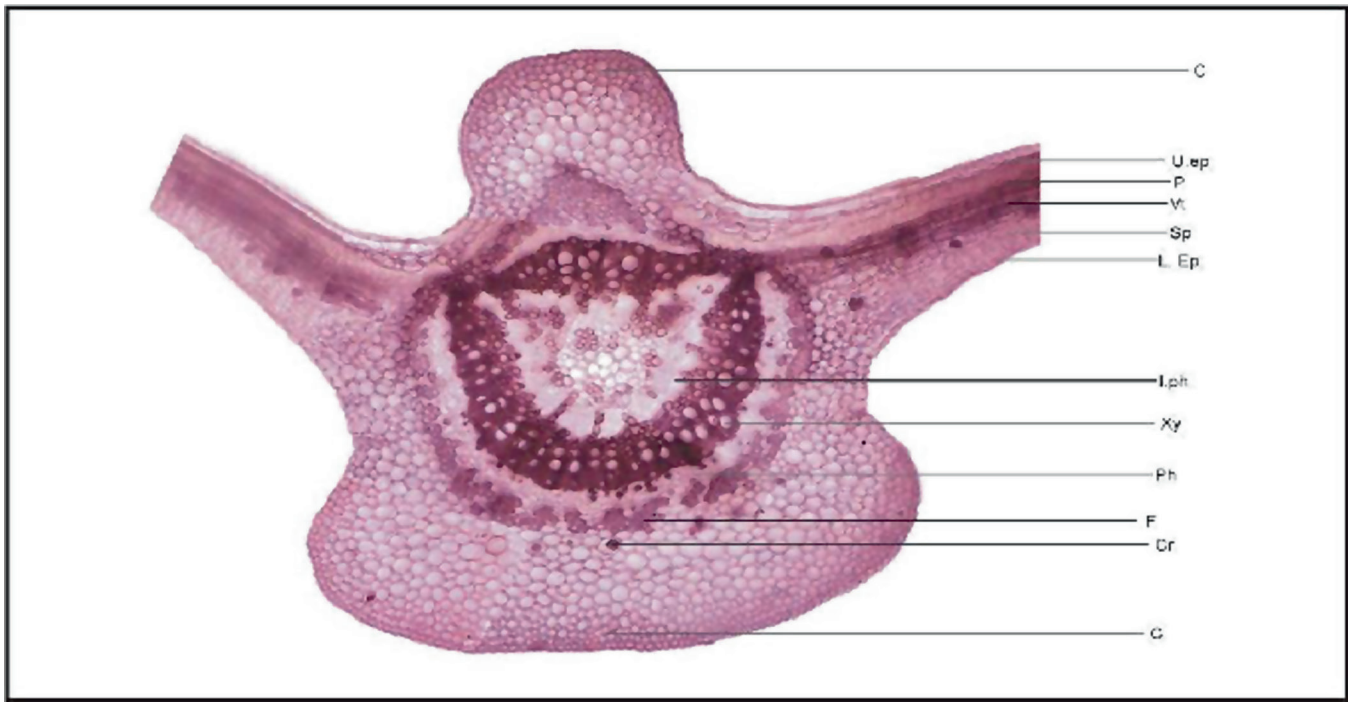

Figure 2: Transverse section of leaf of *L. flos-reginae*
C-collenchyma, Cr-calcium oxalate crystals, F-pericyclic fiber, I.ph-interxylary phloem, L.ep-Lower epidermis, U.ep-Upper epidermis, Ph-phloem, P-palisade, Sp-Spongy parenchyma, Vt-vein

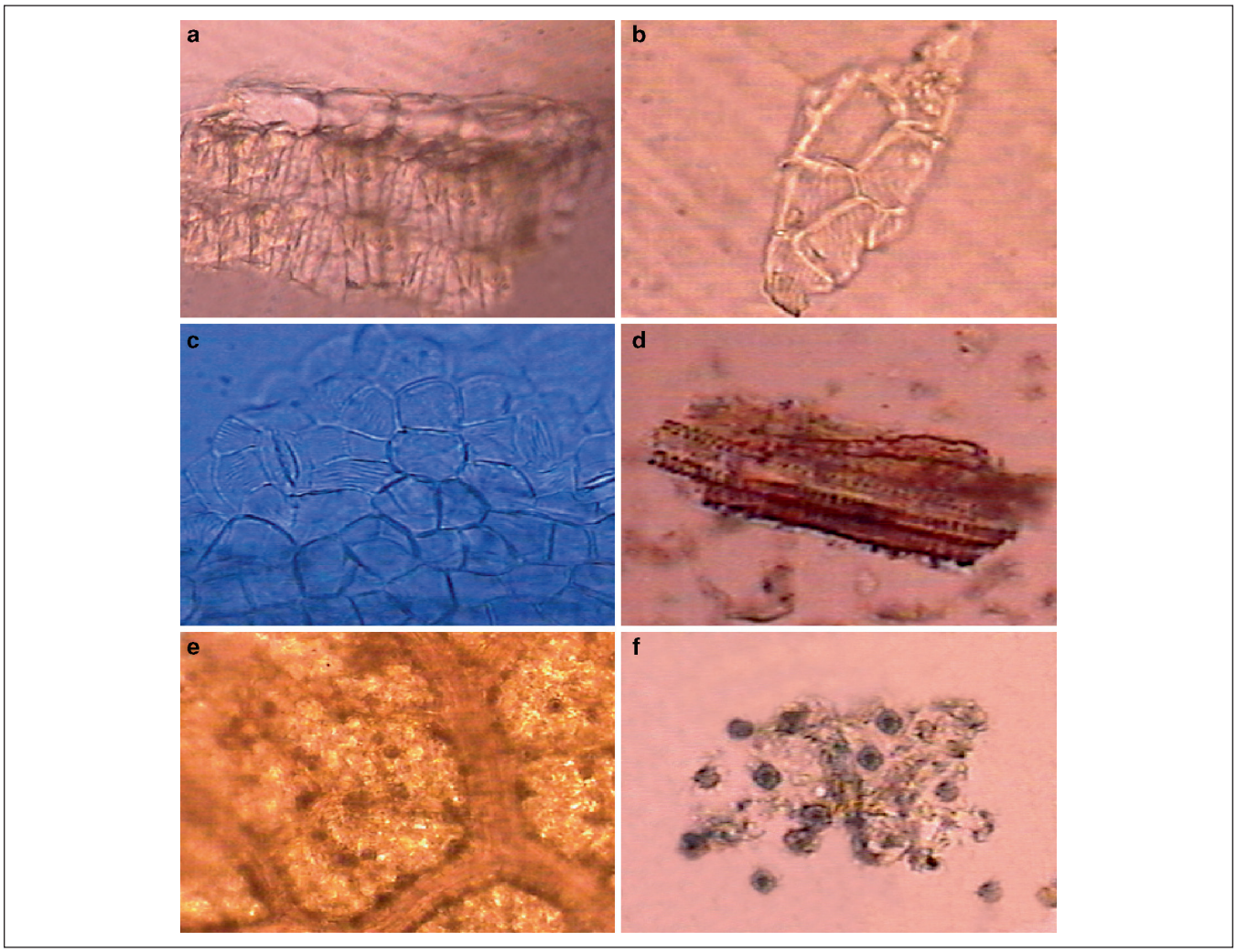

Figure 3: Microscopical powder characteristics of leaf of *L. flos-reginae*
A- bilayered palisade B- striated cuticle C-anomocytic stomata D-Xylem vessel with annular and spiral thickening E- Prisms in veins F Calcium oxalate rosettes crystals

Table 1: Leaf constants of *L. flos-reginae*

Leaf constants	Values
Stomatal number	Upper surface: Nil Lower surface: 400-450
Stomatal index	18.8-21.35
Vein -islet number	9-12
Vein-termination number	10-12

Table 2: Measurement of cells in T.S. of *L. flos-reginae*

Type of cells	Size in µ
Upper epidermis	13.6 × 40.8
Collenchyma	20.4-27.2
Palisade cells	40.8-54.4
Parenchyma	40.8-108.8
Xylem parenchyma	7.8-20.4
Xylem vessels	20.4-108.8
Sclerenchymatous fibres	13.6-149.6
Phloem	13.6-26.4
Calcium oxalate rosettes	13.6-54.4

Table 3: Physicochemical constants for powder of leaf of *L. flos-reginae*

Physicochemical constants	(% w/w)
Total ash	3.56 ± 0.15
Water soluble ash	2.51 ± 0.11
Acid insoluble ash	0.33 ± 0.05
Water soluble extractive value	15.75 ± 0.35
Alcohol soluble extractive value (%w/w)	14.23 ± 0.18
Loss on drying (LOD)	4.55 ± 0.03

Table 4: Fluorescence analysis of powdered leaves of *L. flos-reginae*

Treatment with chemical reagent	Ordinary light	Long UV light
Powder + 1N NaOH in methanol	Greenish brown	Greenish Brown
Powder + 1N NaOH in water	Reddish brown	Reddish brown
Powder + 1N HCl	Light green	Light green
Powder + 50% HNO_3	Reddish brown	Orange red
Powder + 50% H_2SO_4	Green	Brown

Table 5: Qualitative phytochemical analysis of various extracts of leaves of *L. flos-reginae*

Constituents	Pet. Ether extract	Ethyl acetate extract	n-butanol extract	Methanolic extract	Aqueous extract
Tannins	–	–	+	+	+
Saponins	–	–	+	+	+
Steroids	+	–	–	+	–
Flavonoids	–	+	–	+	+
Coumarins	–	–	–	+	–
Carbohydrate	–	–	–	–	+
Alkaloids	–	–	–	+	+
Proteins	–	–	–	–	+

'+' indicates presence and '–' indicates absence

acid and inorganic components. The alcohol soluble extractive value was 14.23 ± 0.18 which shows the presence of polar and non polar secondary metabolites present in the plant materials. Loss and drying at 105 °C was revealing 4.55 ± 0.03 the moisture content in the plant.

The results of fluorescence analysis were tabulated in table 4.

The results of phytochemical screening of powder of leaves of *L.flos-reginae* is mentioned in table 5.

CONCLUSION

The present study deals with pharmacognostical study of leaves of *L. flos-reginae*, which include striated cuticle, bilayered palisade, typical vascular bundles arranged in form of cup and lid surrounded by sclerenchymatous fibers on inner as well as outer side, abundant calcium oxalate rosettes, spiral as well as annular xylem vessels. The physicochemical parameters and leaf constants would help in the authentication of this plant. The preliminary qualitative phytochemical screening shows presence of saponins, steroids, tannins, alkaloids, carbohydrates and proteins. The microscopic features, leaf constants and physicochemical parameters would be useful for laying down pharmacopoeial standards. Further studies are in progress in our laboratory to isolate the active constituents.

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Pharmacognostic and Phytochemical Investigation of *Juglans regia* Linn. bark

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ABSTRACT

Juglans regia Linn belongs to family *Juglandaceae*. It is commonly known as Walnut tree. *Juglans regia* bark has been claimed to possess anti-inflammatory, blood purifying, anticancer, depurative, diuretic and laxative activities. The bark is finely powdered and used to prevent bleeding gums and as a mouth rinse. The present investigation deals with microscopic evaluation of bark and establishment of its quality parameters, including physicochemical, phytochemical evaluation, HPTLC analysis & Microbial load. Chief microscopic characters include cork, phloem fibres with stone cells & calcium oxalate crystals. Phytochemical screening revealed presence of reducing sugars; alkaloids; tannins & phenols; steroids & saponins. The bark powder was found to be free from pathogenic organisms. The study will provide referential information for the correct identification of the crude drugs.

Key words: *Juglans regia* Linn, Pharmacognostic study, Phytochemical analysis, HPTLC analysis.

INTRODUCTION

Juglans regia Linn known as Akhort in India, a native of Eastern Europe to North Asia i.e. China, Iraq, Mexico, Spain, Turkey, Nepal, India (forests in Himalayas) is a member of *Juglandaceae* family. It is a woody, deciduous and frost-tender tree growing to 20m height. The wood is heavy, durable and polishes well. The bark is resinous and scented. This valuable tree has a long history of medicinal use to treat a wide range of health complaints. Almost all parts of the plant are medicinally important. The root and stem bark are anti-helmentic, astringent and detergent. The stem bark is dried and used as a tooth cleaner. The decoction of leaves and bark is used with alum for staining wool brown.[1]

Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need are easily accessible and inexpensive.[2] Human population in countries around the world has been using plants from thousands of years for treating various ailments of humans & animals.[3]

Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained.[4- 8]

Though the traditional Indian system of medicine has a long history of use, they lack adequate scientific documentation, particularly in the light of modern scientific knowledge.[9] To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physic chemical analyses are few of the basic protocol for standardization of herbals.[10-11]

The numbers of reports of patients experiencing negative health consequences caused by the use of herbal medicine has increased in recent year. Analysis & studies have revealed a variety of reasons for such problem. One of the major

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cases of reported adverse events are directly linked to the poor quality of herbal drug and raw medicinal plant materials.[12]

This traditional knowledge about the plants can be transferred to several generations only by proper documentation of their botanical, physicochemical, phytochemical characters and along with their medicinal uses in the form of monographs. The monograph of these plants are prepared according to the WHO guidelines and presented as herbal pharmacopoeia. These guidelines enable to identify, authenticate, detect adulterants and standardize the plant material.[3]

This present work, thus aims to standardize *Juglans regia* Linn bark by pharmacognostic and preliminary phytochemical analysis.

MATERIALS AND METHODS

Collection and authentication

Juglans regia Linn dried bark was procured from the local market in Mumbai. It was identified & authenticated by Prof. Bindu of Botany Department of SVKM'S Mithibai College of Science & Commerce, Vile Parle (West), Mumbai. The dried bark was used for section cutting & the bark powder was used for phytochemical analysis.

The morphological studies such as colour, odour and taste of *Juglans regia* bark were studied.

Microscopic sections were cut by free hand sectioning method. The sections of bark were cleared with chloral hydrate solution & then stained with phloroglucinol & HCl & mounted in glycerine. Numerous mounts of the microscopical sections of the bark specimens were made and examined microscopically. Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provide with MOTIC IMAGE PLUS 2.0 software.

Phytochemical analysis

The powdered bark was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents. The dried and coarsely powder (50 g) was extracted in (300ml) methanol by Soxhlet method (Hot methanolic extraction) & for cold maceration (cold methanolic extraction) 25g powder in 150 ml methanol. The concentrated extracts were evaporated to dryness and the extracts were then weighed. Their percentages were calculated in terms of initial air dried plant material. The colors of the extracts were observed. The extracts as mentioned above, were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material as per standard procedure.[13-17]

Physiochemical analysis

Physicochemical properties such as the percentage of total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive & water soluble extractive values were determined as per the standard procedure.[14] Percentage of ash value is indicative of the purity of the drug and extractive values represent the presence of polar and non polar compounds in the extract.

Fluorescence Analysis

Fluorescence study is an essential parameter for first line standardization of crude drug. The crude powders were subjected to these studies & their fluorescence patterns were noted. The powder material were treated separately with different reagents & exposed to visible, ultraviolet light to study their fluorescence behaviour.[18] The colors obtained by application of different reagents in different radiations were recorded

HPTLC analysis

Chromatographic finger-printing of phytoconstituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern. The fingerprint has potential to determine authenticity and reliability of chemical constituents of herbal drug and formulations.

Chromatographic separation of hot & cold methanolic extracts of *Juglans regia* bark were performed on 10 cm × 10 cm aluminum-backed HPTLC plates coated with 200 µm layers of silica gel 60GF254 (Merck, Darmstadt, Germany). Standard solution of Gallic acid & Methanolic extracts (10 µL each) were applied on to HPTLC plate as 8 mm wide bands and 12 mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying of bands, by means of a CAMAG Automatic TLC Sampler 4 (ATS4). A constant spot application rate of 10 µL/sec was used. Plates were developed to a distance of 80 mm at room temperature (28 ± 2 °C) with $CHCl_3$: Ethylacetate: Formic acid (7.5:6:0.5) (v/v) as mobile phase in a CAMAG glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. Chromatography was performed in CAMAG'S twin-trough chamber. After development, the plates were dried in air & then scanned at 340nm with CAMAG TLC scanner with CAMAG winCATS planar chromatography manager software (version 1.4.2). The plate was later on derivatized with anisaldehyde sulphuric acid & heated at 105 °C till bands develop.

Determination of Microorganisms

Medicinal plant materials normally carry a great number of bacteria & moulds, often of soil origin. While a large

range of bacteria & fungi form the naturally occurring microflora of herbs, aerobic spore forming bacteria frequently predominate. Current practices of harvesting, handling & production often cause additional contamination & microbial growth. Determination of Total Viable count & detection of pathogens was performed as per the method in WHO guideline on "Quality Control methods for medicinal plant materials".[19]

RESULTS AND DISCUSSION

Morphology

Bark of *Juglans regia* was dull blackish brown in colour. It was Thin with whitish epidermal layer tough and fibrous and somewhat mealy. Inner fibers were tough and flattened; the outer ones were white and silky. The taste of bark slightly Bitter and astringent

Microscopy

The transverse section of *Juglans regia* showed one cell layer thick cork on the outermost side of the bark. It also showed presence of phloem fibres with stone cells present in them. Crystals of calcium oxalate were found to be scattered amongst the stone cells (Figure 1-3).

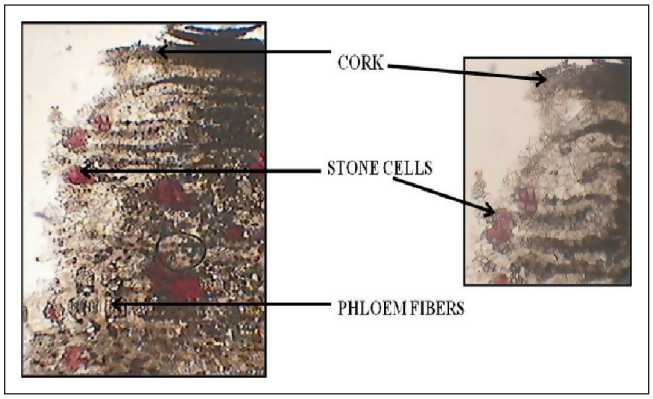

Figure 1: Transverse Section of dried bark of *Juglans regia* Linn

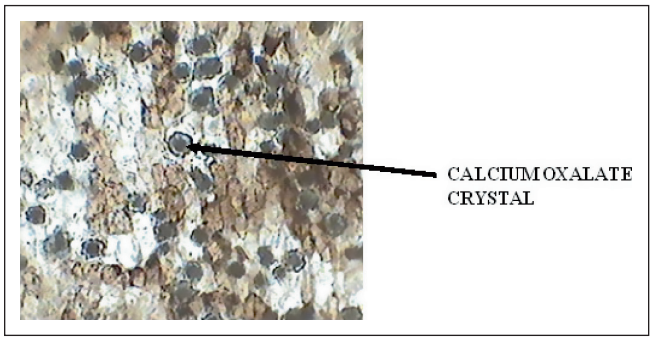

Figure 2: Transverse Section of dried bark of *Juglans regia* Linn showing calcium oxalate crystals

Powder Microscopy

Juglans regia powder was brown in colour & showed presence of stone cells, fibers & calcium oxalate crystals (Figure 4).

Preliminary phytochemical test

Preliminary phytochemical test for hot & cold methanolic extract of the drug was carried out. Both the extracts showed the presence of reducing sugars; alkaloids; tannins & phenols; steroids & saponins (Table 1).

Physico-chemical constants

The powdered bark of *Juglans regia* was studied for their physico-chemical constant which included percentage of

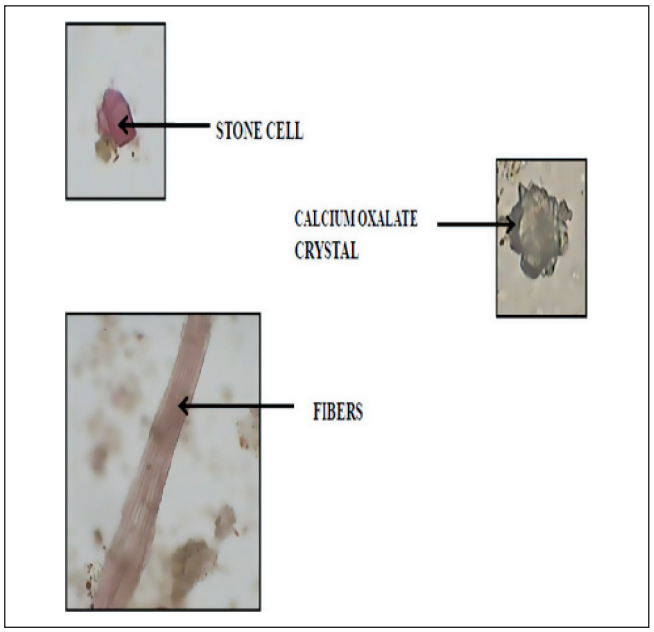

Figure 3: Powder microscopy of dried bark of *Juglans regia* Linn

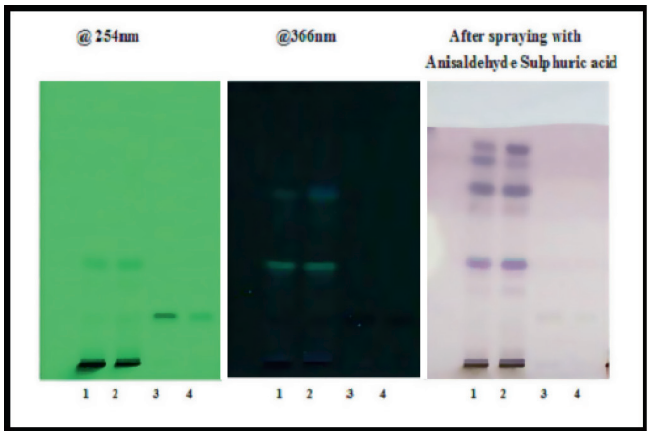

Figure 4: HPTLC profile of methanolic extracts of *Juglans regia* Linn. bark *1- Jugalns regia Cold Methanolic extract (10 mg/ml); 2- Jugalns regia Hot Methanolic extract (10 mg/ml) & 3, 4- Standard Gallic acid (0.5 mg/ml).*

total ash, acid-insoluble ash, water-soluble ash, alcohol soluble extractives (Table 2).

Fluorescence analysis of extract and drug powder

The fluorescence analysis of the powdered drug of *Juglans regia* in various solvents and chemical reagents were performed under normal and UV light. There was no fluorescence observed under UV long (365nm) with any of the chemicals (Table 3).

HPTLC analysis

HPTLC analysis of methanolic extracts was carried out using $CHCl_3$: Ethylacetate: Formic acid (7.5:6:0.5) (v/v) as a mobile phase. HPTLC screening of the extracts was established to substantiate the standardization data on *Juglans regia* Linn (Figure 5). As Gallic acid was used as standard, quantification of gallic acid in the extract was carried out. Hot & cold methanolic extract of *Juglans regia* showed 1.4% & 1.08% of gallic acid respectively.

Table 1: Results of phytochemical screenings of extract of *Juglans regia* Linn

Test	*Juglans regia bark*
Reducing sugars	+
Amino acids	–
Flavonoid	–
Alkaloid	+
Tannins and Phenols	+
Steroids	–
Saponins	+

Key: + = Present, – = Not Present

Table 2: Results of Physicochemial properties of dried bark powder of *Juglans regia* Linn

Physicochemial properties	Result (% w/w)
Total ash	9.51%
Acid insoluble ash	0.125%
Water soluble ash	1.035%
Alcohol soluble extractive	6.03%
Water soluble extractive	4.02%

Table 3: Fluorescence analysis of dried bark powder of *Juglans regia* Linn

Treatment	Day Light	UV (254 nm)
Powder + 1N NaOH (aq.)	Light brown	Black
Powder + 1N NaOH (alc.)	Chocolate brown	Black
Powder + Conc. HCl	Chocolate brown	Black
Powder + Conc. H_2SO_4	Yellowish brown	Black
Powder + Conc. HNO_3	Yellowish black	Black
Powder + Chloroform	Orange	Black
Powder + Glacial Acetic acid	Yellow	Yellow
Powder + 5% NaOH	Brown	Black
Powder + 5% KOH	Chocolate brown	Black
Powder + 5% $FeCl_3$	Chocolate brown	Black
Powder + Ammonia	Greenish black	Black

Determination of Microorganisms

Total aerobic plate count of *Juglans regia* bark powder was found to be 2.41 × 10^5 cfu/ml & no fungal propogules were observed in total fungal count (Table 4). The bark was also found to be free from objectnable pathogens.

DISCUSSION

The information obtained from preliminary phyto-chemical screening will be useful in finding out the genuity of the drug. Ash values; extractive values & fluorescence analysis are few parameters, which normally are adopted to get the qualitative information about the purity & standard of the crude drug. The percent extractives indicate the quantity and nature of constituents in the extracts. Morphological and anatomical studies discussed can be considered as a distinguishing parameter to identify & decide the authenticity of this drug. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy.

CONCLUSION

The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias. Also the manufacturers

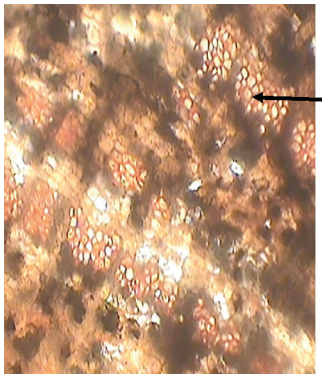

Figure 5: Transverse Section of dried bark of *Juglans regia* Linn showing phloem fibers

Table 4: Total Viable Count of dried bark powder of *Juglans regia* Linn

Microbial method	Medium for plating	Microbial Counts
Total Aerobic Plate count	Letheen Agar	2.41 × 10^5 cfu/ml
Total Fungal count	Sabourauds Dextrose Agar	No fungal propagules were observed

can utilize them for identification and selection of the raw material for drug production.

ACKNOWLEDGEMENT

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Preliminary Phytochemical Evaluation of *Euphorbia Fusiformis* Buch-Ham.

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ABSTRACT

The present investigation was undertaken to analyze the physicochemical and chromatographic profile of dried tuberous roots of *Euphorbia fusiformis*. Physicochemical parameters like loss on drying, total ash value, acid insoluble ash, water insoluble ash, various extractive values, pH etc., were carried out. Further, qualitative tests for various functional groups like alkaloids, glycosides etc., were carried out in methanol and water extracts. The results of the preliminary phytochemical screening indicated the presence of carbohydrates, starch, flavanoids and steroids. Thin layer chromatography was carried out using different solvent systems which revealed two common spots indicating the presence of some common phyto-constituents. The parameters of present study can be used as a reference for further scientific investigations.

Key words: *Euphorbia fusiformis*, Euphorbiaceae, chromatography, total ash, caudicifolin

INTRODUCTION

The use of medicinal plants still plays a vital role to cover the basic health needs in both developed and developing countries.[1,2] The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. Each and every plant has got its own chemical characteristics which help in separating it from other closely related species.[3] To explore the hidden secrets of the plant kingdom such as their complex compounds or active principles which are thought to be responsible for their effectiveness, it is necessary to undertake the analytical evaluation.

Euphorbia fusiformis Buch.-Ham. (Family: Euphorbiaceae) is a rare medicinal plant found in Tropical Himalaya up to 1500 ft. from Garhwal to Nepal. It is also found in Konkan and Deccan Hills.[4] In Gujarat state it is found in Dang, Rajpippala and Chotaudaipur regions,[5] where traditional healers extensively use this plant to treat abdominal tumors. Further, the ethnobotanical value of the tuberous root of this plant refers to its recognized action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis[6,7] with proven anti-inflammatory[8] and anti-bacterial activities.[9] Previously we have explored analgesic activity of tuberous roots of this plant.[10] Regarding the phytochemical profile only constituents like diterpene lactone caudicifolin, methylellagic acid and euphol were reported.[11,12] However no reports are available regarding physicochemical and chromatographic profiles of this drug till date. Hence the present study was undertaken to evaluate preliminary phytochemical and chromatographic profile of tuberous roots of *E. fusiformis*.

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MATERIALS AND METHODS

Plant materials: The tuberous roots of *E. fusiformis* were collected from Waghai forest, Dang, Gujarat, India in fully matured condition in the month of November and the material was authenticated by the taxonomist of our institute. The tuberous roots were made into slices and shade dried for 12 days. The dried root slices were pulverized to fine powder and utilized for phytochemical analysis.

Analysis of physicochemical parameters: Physicochemical parameters like loss on drying at 105 °C, total ash value,

acid insoluble ash, water insoluble ash, water soluble extractive value, alcohol soluble extractive value and pH value were carried out by referring standard procedure.[13,14]

Qualitative test for various functional groups: Qualitative tests for various functional groups like alkaloids, glycosides etc., were carried out by using the aqueous and methanol soluble extracts of the sample .[15-17]

Chromatographic evaluation: The chromatographic studies were performed using various solvent systems to confirm the phytochemical studies. Silica gel GF 254 (precoated plates) were used for the chromatographic evaluation.[18,19]

Sample preparation

***Methanol Extract*:** About 5 g of accurately weighed powder sample was taken in a conical flask and 100 ml Methanol was added to it, shaken and kept overnight. Next day it was filtered. Then it was concentrated to 5 ml and sample was used for spotting **(a)**.

Petroleum Ether Extract: About 5 g of accurately weighed powder sample was taken in a conical flask and 100 ml Petroleum ether was added to it, shaken and kept overnight. Next day it was filtered. Then it was concentrated to 5 ml and sample was used for spotting **(b)**.

Chloroform Extract: About 5 g of accurately weighed powder sample was taken in a conical flask and 100 ml chloroform was added to it, shaken and kept overnight. Next day it was filtered. Then it was concentrated to 5 ml and sample was used for spotting **(c)**.

Chromatographic conditions:

A. For steroids:

Mobile Phase: Tolune: Ethyl acetate (9:1)

Stationary Phase: Silica gel GF 254 (precoated plates)

Detection: a) Short U-V (254 nm): Figure 1,
b) Long U-V (366 nm): Figure 2,
c) Spraying with Vanillin sulphuric acid followed by heating at 110 °C for 10 min. Figure 5

B. For flavonoids

Mobile Phase: Ethyl acetate: Formic acid: water (6.7:1.5:2.6)

Stationary Phase: Silica gel GF 254 (precoated plates)

Detection: a) Short U-V (254 nm): Figure 3
b) Spraying with Vanillin Sulphuric acid followed by heating at 110 °C for 10 min. Figure 4

RESULTS

The results of physico-chemical parameters have been depicted in table-1. The results of the preliminary phytochemical screening for various functional groups indicated the presence of carbohydrates, starch, flavanoids and steroids (Table -2). The Rf values of different extracts have been tabulated in table 3, where Rf values 0.95 and 0.97 were common to all the three extracts.

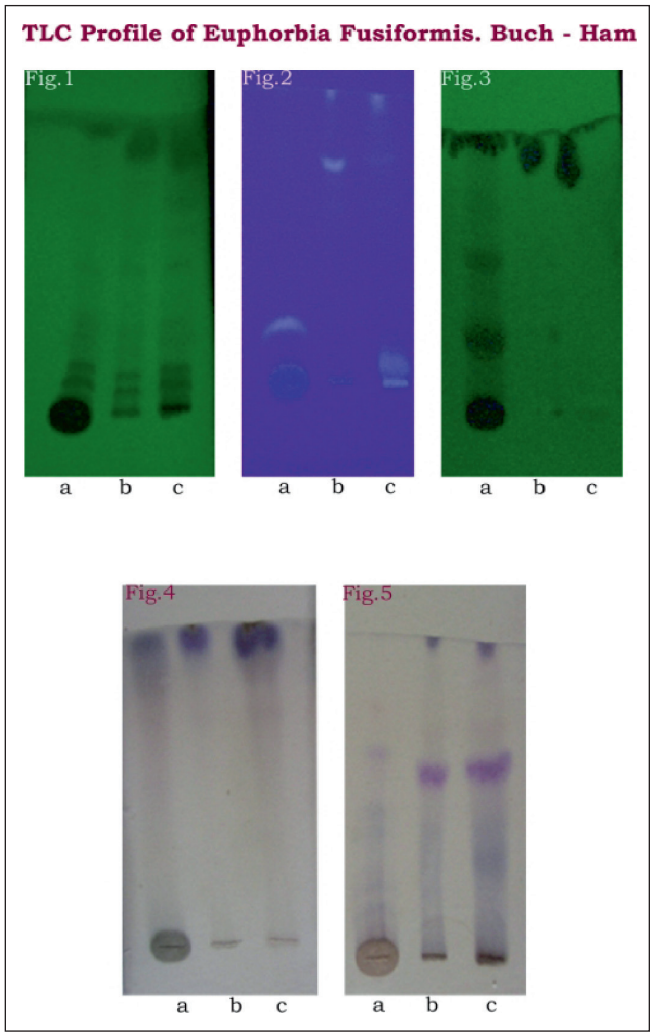

Figure 1: TLC profile of *E. fusiformis* root

Table 1: Physico-chemical parameters of roots of *E. fusiformis*

Parameters	Results
Loss on drying at 105 °C	11.50% w/w
Ash value	7% w/w
Acid insoluble Ash	0.6% w/w
Water insoluble ash	4% w/w
Water soluble extractive	8.38% w/w
Methanol soluble extractive	5.56% w/w
pH	6.9

DISCUSSION

The plants of Euphorbiaceae family are known for their therapeutical interest in both organized (such as Ayurveda and Unani) and un-organized (Folklore) system of medicine.[20-21] They exhibit great chemical diversity and several of them have been listed as source of valuable drugs.[22] One of the genus of this family *Euphorbia* comprises a large and diverse group of plants, which are characterized by the presence of white milky latex and reported to have a number of interesting biological agents.[20,23-25]

Many substances absorb moisture on storage, presence of moisture may affect the preservation quality of the drug. Loss on drying in a sample corresponds to moisture content and volatile matter content in it. The loss on drying at 105 °C was 11.50% w/w, indicative of some moisture content in drug.

Total ash content of crude drug is the inorganic residue remaining after incineration. It represents the inorganic salts occurring naturally in the drug and also inorganic matter from external sources. The ash value is determined to ensure the absence of an undue proportion of extraneous mineral matters introduced accidentally or mixed at the time of collection or in subsequent treatment. In present study test drug have shown total ash content of 7% w/w.

Treatment of ash with hydrochloric acid leaves virtually only silica. Hence it is done to detect the silica in the drug. The ash obtained was further analyzed for acid insoluble particles in ash. In present study values of acid insoluble ash and water insoluble ash were 0.6% w/w and 4% w/w respectively.

The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug and also to find out the phytoconstituent present in the test drug. The results indicated the presence of carbohydrates, starch, flavanoids and steroids which may be responsible for various biological expressions. The preliminary phytochemical test results were rationalized by the thin layer chromatographic studies, which revealed only two common spots in three different extracts, indicating the presence of some common components.

Table 2: Qualitative tests for various functional groups

Functional groups	Tests performed	Results
Carbohydrates	Molish's test	+
	Reducing Sugar test	+
	Keller Kiliani test	+
	Test for starch	+
Alkaloid	Dragendorff's test	–
	Mayer's reagent test	–
Flavonoid	Shinoda test	+
Steroid	LB reagent	+
	Sarkowski reaction:	+
Tannin	Neutral $FeCl_3$	–
	Gelatin test	–
Resin	acetic anhydride	–
Glycoside	Molisch's Test	–
Saponin	Distilled water	–
	Lead acetate	–
Coumarin	Ammonia test	–

CONCLUSIONS

At our best knowledge this is the first preliminary physico-chemical and chromatographic study on dried tuberous roots of *E. Fusiformis* and this will be helpful for the identification of this drug in powder form.

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Table 3: TLC profile of roots of *E. fusiformis*

Conditions	Rf values of samples			Figures
Steroids:	**a**	**b**	**C**	
Short U-V (254 nm):	0.13, 0.19, 0.97 [3]	0.10, 0.15, 0.93 [3]	0.10, 0.16, 0.88 [3]	Figure 1
Long U-V (366 nm)	0.26 [1]	0.17, 0.99 [2]	0.99 [1]	Figure 2
Derivatization: Vanillin sulphuric acid	0.16, 0.24, 0.42, 0.66 [4]	0.13, 0.55, 0.97 [3]	0.33, 0.61 [2]	Figure 5
Flavonoids:				
Short U-V (254 nm):	0.38, 0.63, **0.97** [3]	**0.97** [1]	**0.97** [1]	Figure 3
Derivatization: Vanillin sulphuric acid	0.89, **0.95** [2]	**0.95** [1]	**0.95** [1]	Figure 4

Total numbers of spots are provided in parenthesis next to Rf values.

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Influence of salt stress on phosphorus metabolism in the roots and leaves of one month old *Prosopis juliflora* (Sw.) DC seedlings

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ABSTRACT

A sand culture experiment was designed to study the effect of sodium chloride salinity on phosphorus metabolism in the roots and leaves of one month old *Prosopis juliflora* (Sw.) DC seedlings. It was found that the P level in the roots as well as leaves was decreased with increasing level of salinity in rooting medium. However, the activities of enzymes acid phosphatase and ATPase were increased in both the parts of seedlings grown in saline conditions. The activities of alkaline phosphatase and inorganic pyrophosphatase were found to be decreased in the root and leaves of seedlings grown under saline conditions.

Key words: salinity, phosphorus, enzymes, *Prosopis juliflora.*

INTRODUCTION

The selection and breeding of salt tolerant crops is regarded as one of the main approaches to deal with a serious problem of salt affected soils throughout the world. In order to achieve this strategy it is necessary to identify the mechanisms of salt tolerance in the plant species well adapted to such problem soils. *Prosopis juliflora* is one such plant species which can successfully grow and complete its life cycle in a variety of problem soils. It is noticed that the plant has successfully established in farmlands of Digraj (Dist. Sangli) which are heavily affected by secondary salinization. *Prosopis juliflora* is a multipurpose plant of great economic potential. The ability of this species to grow on the poorest soil, under arid conditions and on saline soil is well known Pasiecznik et al,.[1]

According to Dagar and Tomar[2] in India about 8.53 million ha land is waterlogged, 5.50 million ha land is saline and 3.88 million ha land is alkaline and more and more land is becoming water logged due to several factors. According to CSSRI these soils can be judiciously utilized for raising forestry, agriculture and horticulture crops. Afforestration programme for saline soil requires the proper selection of tree species, as the major problems of such soils are high water table, high salinity impeded drainage and less soil aeration for tree growth, Singh[3]

Phosphorus metabolism occupies a key position in cellular-biochemistry as it is related with energy relation in respiration and photosynthesis. Hence, an attempt has been made to study the phosphorus metabolism in the roots and leaves of *Prosopis juliflora* seedlings grown under salinity stress in laboratory conditions.

MATERIALS AND METHODS

For the experiment, seeds were obtained from the pod of *Prosopis juliflora* plants growing in the salt affected agriculture field in Sangli district in the month of April-May. Mechanically scarified seeds were used to raise the seedlings. After the establishment of seedlings for 5 days, they were treated with increasing concentration of salt (100, 200, and 300 mM NaCl) mix with half strength Hoagland solution. The seedling were grown for one month and then analysed for phosphorus metabolism. The method of Sekine *et al,*[4] was employed for estimation of Phosphorus from the root and leaves. Fresh leaves and roots were used for the assay of enzymes of Phosphorus metabolism. For enzyme acid phosphatase crude enzyme was prepared in 0.1 M acetate buffer (pH 5) and assayed according to the method of Mclachlan[5] The activity of enzyme ATPase was determined following the method

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described by Todd and Yoo[6] and liberated phosphorus was estimated by the standard method.[7] The method described by Weimberg[8] was employed for the study of activity of enzyme alkaline phosphatase. A method by Kar and Mishra[9] was employed for the determination of the activity of enzyme alkaline inorganic pyrophosphatase. The soluble proteins in the enzyme preparations were determined according to the method of Lowry *et al,*.[10]

RESULTS

Phosphorus is an important macronutrient essential for all living organisms. It plays a major role in energy transfer during plant metabolism like respiration, photosynthesis in the form of ATP, NADP and also in cell division and cell expansion. Phosphorus is involved in the formation of cell membrane lipids, which play a vital role in ionic regulation.[11] There are many reports indicating suppression of P uptake due to salt stress.[12,13] Nieman and Clark[14] also found depression of total P in the corn leaves due to salinity at low level of inorganic phosphorus in the nutrient solution. In case of *Prosopis cineraria* seedlings Ramoliya *et al,*[15] noticed that phosphorus content was significantly decreased in the leaves with increase in soil salinity while that was gradually decreased in the stem and root tissues. A decrease in P content of root tissue and that increase in the leaf tissue of salt grown *Poncirus trifoliata* was evident in the experiments by Tozly *et al*.[16]

Prosopis juliflora seedlings have shown a pattern similar to that in *Prosopis cineraria* since in both root and leaves a decline in P content was evident in the seedlings exposed to salt stress (Figure 1) According to Gibson[17] phosphorus deficiency induced by salinity could reduce the cellular ability to accumulate optimum concentration of ion without reduced growth. Thus in contrast to Calcium and Potassium nutrition

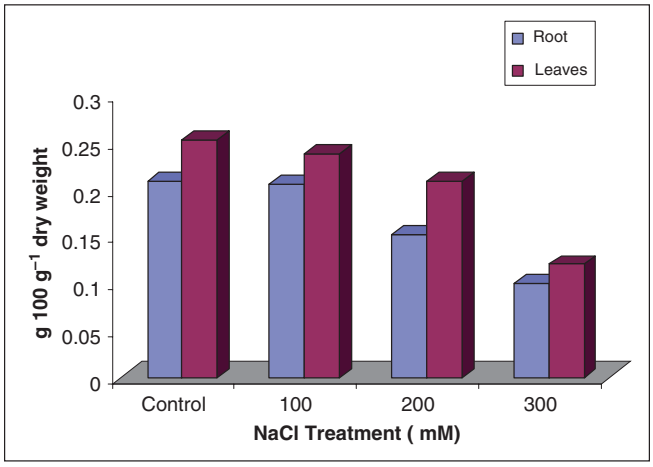

Figure 1: Effect of Sodium chloride salinity on phosphorus content in the roots and leaves of *Prosopis juliflora* (Sw.).

which appears to be quite stable during salt stress in this species, the phosphorus nutrition in -*Prosopis juliflora* seems to be sensitive to salt stress. The disturbance in P nutrition can have significant effects on overall plant metabolism in view of a key role of this element in cellular biochemistry.

Effect of sodium chloride salinity on the activity of enzyme acid phosphatase in the leaves and roots of *Prosopis juliflora* is recorded in figure 2(a). It is evident that the activity of this enzyme in both root and leaves is stimulated at all salinity levels except 300 mM NaCl, at which it has decreased in the roots. Enhancement in the activity of acid phosphatase in the leaves of spinach grown under saline condition has been reported by Pan.[18] Similar observations have been made by Karadge and Chavan[19] in *Sesbania*. Lila Arab and Ehsanpour[20] measured acid phosphatase activity in the leaf and stem of *in vitro* grown *Medicago sativa* under saline conditions and found that the activity was increased due to increasing salt concentration. Chakrabarti and Mukharji[21] have also found that the salt stress caused to increase the activity of acid phosphatase in the leaf and roots of mung bean. Parida and Das[22] studied effect of various levels of salinity (0, 100, 200, 400mM NaCl) on the activity of acid phosphatase in *Bruguiera parviflora* growing under hydrophonic culture. Their experiments also revealed that the salinity causes stimulation of activity of this enzyme.

Effect of NaCl salinity on the activity of enzyme alkaline phosphatase in the leaves and roots of *Prosopis juliflora* is depicted in the figure 2 (b). It is evident that the activity of this enzyme is decreased in the root and leaves with increasing level of salt in the medium. Weimberg[23] noticed a decrease in the level of alkaline phosphatase in pea seedlings due to NaCl salinity. A contrasting behavior of acid and alkaline phosphatases under saline conditions was noticed by Ahmad and Huq[24] in halophytic spinach. In the case of horsegram only lower concentration of salt (25 mM of NaCl) caused the real increase in alkaline phosphatase activity.[25] Parida and Das[22] noticed that the activity of this enzyme in a mangrove, *Bruguiera parviflora* was increased under varying levels of salinity (0, 100, 200, 300 mM NaCl). The effect of salt stress on alkaline phosphatase was studied by Pan[26] in Spinach. He found that the enzyme alkaline phosphatase was inhibited by salinity (> 150 mM NaCl). In case of *Prosopis juliflora* a trend more or less similar to that in Spinach and pea is evident in both root and leaf tissues. Acid phosphatase and alkaline phosphatase in the root and leaves of this plant, however have shown an opposite trend. A difference in ionic balance resulting in a shift in cellular pH might be a reason for such alterations.

Effect of NaCl salinity on enzyme ATPase in the leaves and roots of *Prosopis juliflora* is shown in figure 2(c). It is evident that the activity of enzyme ATPase in the root was

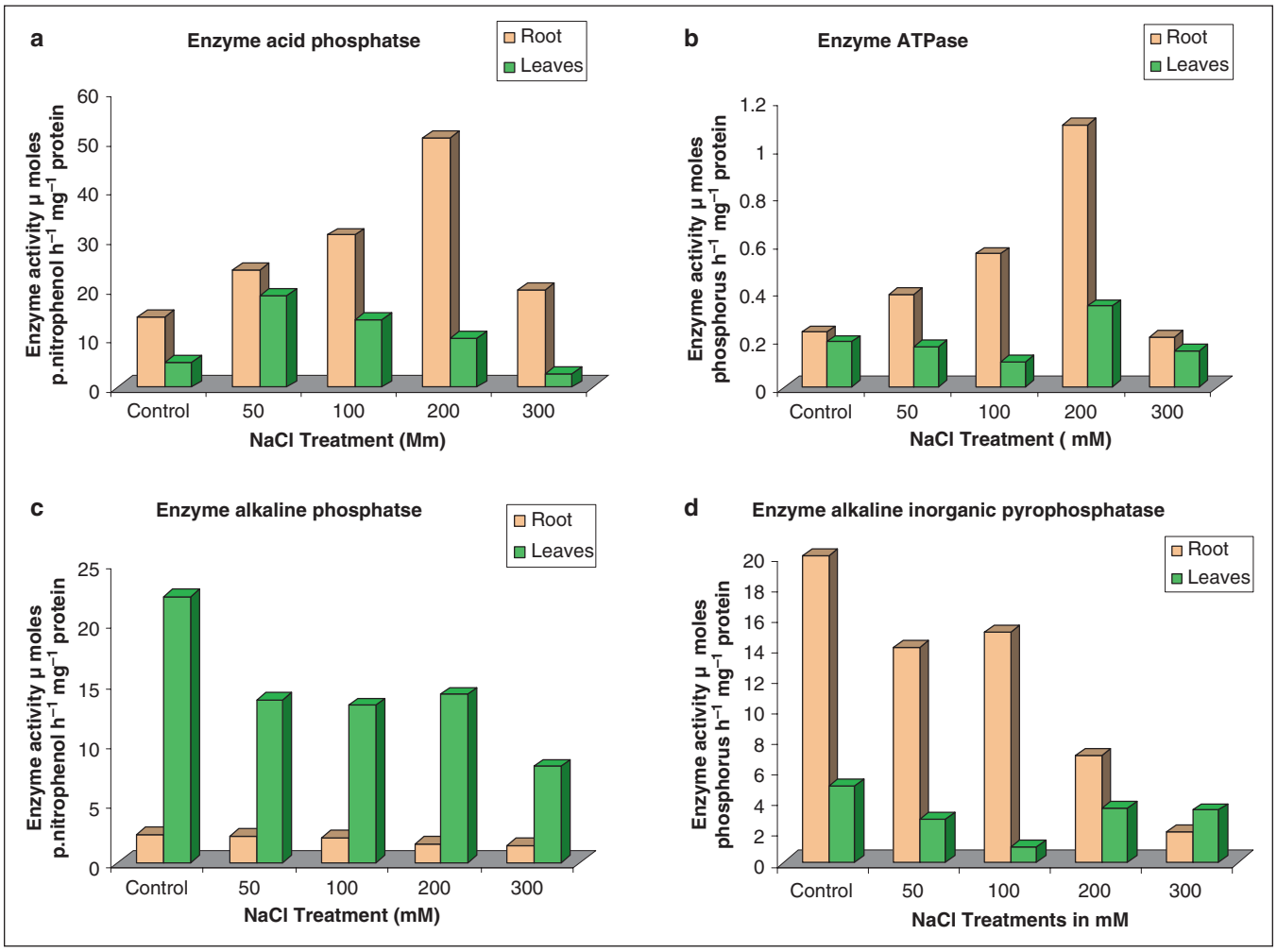

Figure 2: Effect of Sodium chloride salinity on the activity of (a) Enzyme acid phosphatase (b) Enzyme alkaline phosphatase (c) Enzyme ATPase and (d) Enzyme alkaline inorganic pyrophosphatase in the roots and leaves of *Prosopis juliflora* (Sw.).

increases with increasing NaCl treatment upto 200 mM and later decreased significantly at 300 mM NaCl. While, in the leaf tissue its activity was increased with increasing level of salt. Weimberg[8] found that in the seedlings of pea grown under highly saline media, the activity of ATPase was slightly reduced. Kuiper *et al.*[27] noticed that the activities of Mg^{2+} dependent ATPase was increased due to increased mineral level in the root of wheat seedlings and juvenile plants of *Plantago major*. Lin *et al.*,[28] noted that the activity of H^+ ATPase was increased due to 75 mM NaCl in the seedlings of cotton. Horovitz and Waisel[29] reported that this enzyme is associated with salt tolerance with many halophytes. They also observed a stimulation of this enzyme in glycophytic bean and carrot root and inhibition of the same in *Atriplex* and *Suaeda* roots after exposure to salt. Under salinity stress its expression is down regulated in root and upregulated in shoot of pearl millet.[30] Leaf of maize plant treated with 125 mM NaCl showed slight increase in H^+ ATPase.[31] Balasubbramaniam *et al.*,[32] reported a decrease in the activity of ATPase in 3 % NaCl treated *Aster* plant while F-ATPase activity was increased with increase in NaCl concentration. Thus it is clear that this enzyme plays an important role in salt tolerance process. This increase may help in regulation of ion uptake as well as contribute energy to growth processes.

Effect of NaCl salinity on the activity of enzyme alkaline inorganic pyrophosphatase in the leaves and roots of *Prosopis juliflora* is shown in the figure. 2 (d). It is evident that the activity of this enzyme decreases in the root and leaf tissue with increasing salt concentration. This trend is quite prominent upto 300mM NaCl treatment. This enzyme plays important role in regulating the level of pyrophosphate and supplying Pi for various reactions requiring Pi in the cell. Rea and Sander[33] reported that inorganic pyrophosphatase can also acts as proton pump across the tonoplast membrane. Vianello and Macri[34] noted that in higher plants, cell membrane bound proton pumping pyrophosphatase and three moitochondrial H^+ PPiase present in the inner surface of inner mitochondrial

membrane involved in the specific hydrolysis of PPi coupled to proton transport. Simmons and Butter[35] indicated that high activity of this enzyme in certain plants is directly related to high photosynthetic efficiency. Murumkar and Chavan[36] reported that in the leaves of salt sensitive legume *Cicer arietinum,* A stimulation of inorganic pyrophosphatase was evident under saline conditions. In salt sensitive plants such an increase may play same role in energy dependent processes because ATP level is affected due to salt stress. But in salt tolerant *Prosopis juliflora* such situation perhaps may not occur which demands greater breakdown of PPi when ATP level becomes limiting

CONCLUSION

In conclusion it can be stated that due to salinity, there is definite changes in phosphorus metabolism in the salt tolerant species *Prosopis juliflora.* Some of these changes are probably related to mechanisms underlying salt tolerance in this species.

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Comparative Molluscicidal Activities of Fruit Pericarp, Leaves, Seed and Stem Bark of *Blighia Unijugata* Baker

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ABSTRACT

Introduction: The plant is used in ethno-medicine as a fish poison and belongs to family known to contain saponins which are toxic to cold-blooded animals including snails acting as vectors of organisms responsible for many human diseases including schistosomiasis. **Methods:** The mollusccidal activities of 50% ethanolic extracts of the seeds, fruit pericarp, leaves and stem bark as well as the fractions of the fruit pericarp of *B unijugata* Baker were evaluated on *Biomphalaria glabrata*.snails **Results:** The crude extract of fruit pericarp was the most active among the morphological parts tested with LC_{50} of 15 µg/ml while Ethyl- Acetate fractions showed the highest activity of the 3 fractions with a LC_{50} of 7.6 µg/ml and satisfied the condition set by World Health Organization for a potential plant molluscicides either as a crude extract or as a fractions **Conclusions:** the results confirmed the ethno-medicinal uses of the plant and can be so regarded as a potential molluscicides in the snail vector of *schistsomiasis*

Key words: *Blighia unijugata,* Bak, Fruit pericarp, *Biomphalaria glabrata*, fruit pericarp, *schistosomiasis*

INTRODUCTION

Schistosomiasis is a debilitating disease affecting close to 4-5% of the world population[1] and approximately 90% of these estimated cases of human schistosomiasis lives in sub-Saharan Africa. Within the sub-Saharan Africa, Nigeria is the country with the most cases of human *schstosomiass* which is widespread in both the urban and rural communities.[2]

Epidemiological studies showed the prevalence rates to be high, for example 26% of school children were found to be infected in Anambra state, south eastern Nigeria.[3] while 21% of school children, 18.4% of local dry cleaners and 15 .8% of vehicles washers were found to be infected in studied population in Ibadan South western Nigeria while prevalence rates of 26.6-36.8%were found in some localities in Kano, North Eastern Nigeria.[4]

Chemotherapy is one of the most valuable methods in the cure of *schistosomiasis* but chemotherapy provides only temporary abatement of human parasites burden because of rapid re-infection rates subsequent to drug intervention and the fact that the drug is ineffective to immature stage of the parasite.[5,6,7] Experience has shown that in high risk setting, cessation of drug treatment for even a few years can result in recurrence of high level of *Schistosoma* infection among adults and children as if the community had never been treated.[8] Evidence suggest that countries such as China and Philippines controlled their *Schstosomiasis* by combining the destruction of amphibian snails control with treatment of infected humans[9] and without changes in *Schistosomiasis* transmission potential even multiple years of annual drug treatment will not be adequate to prevent *Schistosoma* infection in many high risk areas. and this may lead to onset of both community and donor fatigue in large scale drug treatment projects if disease control is not fully effective and durable over the long term.[8]

This insight couple with the indication that resistance to Praziquantel might develop in future and the fact that some side effects associated with Praziquantel may reduce drug compliance in primary health care[10] has buttressed the view that mollusccide*s* *schistosomes* transmitting snails still has a useful part to play in integrated control schemes for this important disease and a pressing need for more selective and efficient molluscicide for the control of snail vector.[11,12]

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The plant material

The plant *Blighia unijugata* Baker family Sapindaceae is a small to medium-sized tree up to30 m tall widespread in tropical Africa.

Ethno-medicinal uses

Like all other plants used in ethno-medicine the uses varies from place to place but the traditional used related to the present work is the use of macerated twigs, leaves, flowers and fruit as a fish poison and the coastal people in Nigeria.

The leaves are eaten as vegetable and various part of the tree are considered to have sedative and analgesic properties and are used in traditional medicine for the treatment of rheumatism, kidney pain and stiffness. Fruits have also been used for the treatment of nausea and vomiting.[13]

Like all other members of the Sapindaceae family saponins are believed to be present.

Saponins are naturally occurring plant glycosides, which form a soapy latter with water.

There is a high correlation between plants employed as fish poison or soap substances and their molluscicidal activity[14] and many potent molluscicides of plant origin were triterpenoids saponins and some triterpenods have actually been isolated from this particular plant and they include the following triterpenoids friedelin and epifriedelinol.[15]

MATERIALS AND METHODS

Plant Collection

The various parts of the plants were collected along Ologuneru in Iddo Local Government of Oyo State in March 2010 with the assistance of Mr. Benjamin Daramola and Mr. Odewo both member of staff of University of Lagos Herbarium who identified it and a voucher specimen was deposited at the University of Lagos Herbarium with voucher number LUHN 3325.

Each plant part was allowed to dry at room temperature and 50 g of leaves 10 g each of seed, stem bark and fruit pericarp were macerated with 50% Ethanol for 72 hours filtered and concentrated to dryness at 30 °C under vacuum using a rotary evaporator.

The extraction was carried out thrice and the yield for each plant part was calculated.

For the preparation of the fractions from the fruit pericarp, 71 g of the powdered peicarp was macerated for 72 hours with 50% ethanol, filtered and the filtrate concentrated to dryness under vacuum to yield 10.30 g of the dried extract and out of this 9.80 g was dissolved in water and partitioned between ethyl acetate, butanol and water to give 2.77 g of ethyl acetate fraction, 2.81 of butanol and 3.35 of water fraction respectively.

Molluscicidal Screening

Snails for the experiment were collected from streams that has not been subjected to either synthetic or plant molluscicides. The snails were identified by Dr. Olorunmola of Drug Research and Production Unit of Obafemi Awolowo University Ile-Ife Osun State. They were allowed to acclimatize in the laboratory for two weeks before use. The molluscicidal test was divided to two stages;

Rapid Screening Test

The methods described by various authors[16,17,18] were used with slight modification such that concentrations of 1000 and 500 ppm were used. Extract which show 100% activities at concentration 500 ppm were then used for the final screening test. The extract with the activity within the WHO recommended guidelines was fractionated into, Ethyl Acetate, Butanol and Water fractions and each fraction subjected to further screening to determine where activity resides

Final Screening Test

A different method[19] was used for the final screening test but Copper Sulphate was used as positive control at a concentration of 1 ppm and was set up in duplicate which gave 100% mortality and 500 ml de-chlorinated water was used as negative control. The same method used for the crude extracts was also used for the screening of the various fractions.

The lethal concentration that kills 50% of the snails was determined with the use of probit analysis table with value plotted on graph paper to determine the LC_{50}.

RESULT AND DISCUSSON

Results of Rapid Screening Test

Data in Table 1 below from Rapid Screening show that the leaves and the seed gave 100% mortality at 1000 ppm

Table 1: Results of Rapid Screening

Plant parts	Concentration ppm	%Mortality
Leaves	1000	100
	500	0
Seed	1000	100
	500	0
Stem bark	1000	100
	500	100
Fruit pericarp	1000	100
	500	100

Table 2: Results of Mollucsicdal Screening of Stem Bark and Fruit Pericarp

Plant Parts	Concentration (ppm)	% Mortality	LC_{50} ppm
Stem bark	1000	100	
	500	100	
	250	0	
Fruit pericarp	1000	100	
	500	100	
	250	100	
	125	100	
	100	100	
	75	100	
	50	100	
	40	100	
	30	100	15
	20	0	
	10	0	
	5	0	
Positive Control Copper Sulphate	1 ppm	100	
Negative Control	(500 ml) De-chlorinated water	0	

Table 3: Results of Molluscicidal Activity of the Fractions of Fruit pericarp

Fractions	Conentration ppm	%Mortality	LC_{50}
Ethyl-Acetate	30	100	
	20	100	7.6
	10	100	
	5	0	
Butanol	30	100	
	20	100	15
	10	0	
	5	0	
Water	30	100	
	20	0	25
	10	0	
	5	0	

Positive and Negative Control were set up as in above.

but no activity at 500 while the stem bark and fruit pericarp show 100% mortality at both 1000 and 500 ppm and both are then used for the molluscicidal screening

Result from Table II above showed that the fruit pericarp has the highest activity and was subsequently fractionated to Ethyl-Acetate, Butanol and Water

The result of the molluscicdal bioassay showed that of the four morphological parts tested, only the stem bark and the fruit pericarp were active at concentration of 500 ppm and below with the fruit pericarp having the highest activity with LC_{95} and LC_{50} of 15 and 7.6 ppm. The result of the fruit pericarp alone without the seed suggest that the concentration of the active compounds are more in the fruit pericarp and that may be the reason why molluscicidal activity of the powdered dried fruit were found to be lower as carried out by[20] where the LC_{95} values were 98.7 for adult *Bulinus globules* and 98.5 for *Bulinus truncates*. The seed has been shown to be a good source of protein, carbohydrate, minerals and crude fiber and can serve as feed supplement and the oil from the seed can be used in the production of soap and lather shaving cream.[21] This fact should serve as impetus to the local people who can be encouraged to exploit the commercial benefits as well as the health benefits of this plant since *schistosomiasis* has been shown to be both a cause and an effect of continuing rural poverty in endemic areas.[22]

In the case of the 3 fractions of the fruit pericarp activity increases from the Water fraction to Butanol fraction with Ethyl-Acetate fraction having the highest activity with LC_{50} of 7.6 ppm respectively. For a plant to be considered as a potential molluscicide according to the World Health Originations (WHO) guidelines a methanolic or lipopholic extracts should be active at equal to or less than 20 µg/ml to kill 90% of snails exposed for 24 hours.[12] It must also be freely solubility in water since the medium of final usage will be water. It is only the fruit pericarp of *Bl. unijugata* Baker that met this condition and may be a candidate for further studies While the criteria for solubility and concentration were met, other consideration like the effect on non target organisms such as fishes and other amphibian inside the river has to be investigated before it can be declare a candidate molluscicide and this together with the isolation of the compounds responsible for activity will be the focus of future research.

It is interesting to note that in all cases of death, death occurs within 6 hours of application of the extracts to the snails with a reddish fluid around the snails which may suggest heamolysis of the blood and this gives noxious odor the following day.

It was also observed that both the extracts and the various fractions produced what can be call all or none response as efforts to get a concentration that will not give 100% mortality was not successful. This action of the extracts and fractions would be of immense utility if the plant is to be used in the control of the snails as it will be certain that if the appropriate concentration is used total eradication of the snails in the affected community will be accomplished and this will ensure that there will not be residual snails that can serve as intermediate hosts for further infection of humans.

CONCLUSIONS

This study has confirmed the ethno botanical use of the plant as a fish poison and as a potential molluscicidal agent which the people living in endemic areas can be encourage

to use the fruit pericarp after extracting the oil from the seed which can serve as additional source of income but such usage is subject to further research to determine its effect on other aquatic organisms and to isolate the agents responsible for actvity from the various fractions.

Effort is on to isolate the active compounds responsible for activity from various fractions.

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Wound Healing Potential of Extract of *Jatropha curcas* L. (Stem bark) in rats

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ABSTRACT

Introduction: The present study provides a scientific evaluation for the wound healing potential of extract of *Jatropha curcas* L. stem bark. *Jatropha curcas* L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family. **Methods:** Excision and incision wounds were inflicted upon four groups of six rats each. Group I was assigned as control (ointment base), Group II was treated with standard silver sulfadiazine (0.01%) cream, Group III and Group IV was treated with 5% and 10% extract ointment respectively. The parameters observed were percentage of wound contraction, hydroxyproline content and tensile strength including histopathological studies. **Results:** It was noted that the effect produced by the extract ointment showed significant ($P < 0.01$) healing in both the wound models when compared with control group. All parameters such as wound contraction, hydroxyproline content, tensile strength and histopathological studies showed significant changes when compared to control. **Conclusion:** The result shows that extract ointment demonstrates wound healing potential in both excision and incision models.

Key words: Histopathological, Hydroxyproline, Euphorbiaceae, sulfadiazine, tensile

INTRODUCTION

Herbal medicines have been enjoying revitalization among the clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very crucial and needs imperative attention in order to know the value of the plant. The assessment of the plants for their therapeutic activity is done on the basis of either their chemotaxonomic examination or ethnobotanical information for a particular disease.[1]

Jatropha curcas L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family and contains approximately 170 known species.[2] *Jatropha*, a drought-resistant shrub or tree, which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia.[3] It is a multipurpose, drought resistant, perennial plant gaining lot of importance for the production of biodiesel. It has thick glorious branch lets. The tree has a straight trunk and grey or reddish bark, masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The branches contain whitish latex, which causes brown stains. Inflorescences are formed terminally on branches. The plant is monoecious and flowers are unisexual.[4-5] After pollination, a trilocular ellipsoidal fruit is formed. The seeds are black and in the average 18 mm long and 10 mm wide ripe Jatropha fruits.[6] It is a multipurpose species with many attributes and considerable potential. The wood and fruit of *Jatropha* can be used for numerous purposes including fuel. It is used against dermatomucosal diseases, arthritis, gout, jaundice, Toothache, gum inflammation, gum bleeding, diarrhoea and pyorrhea.[7] Plant extract used to treat Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox. Water extract of branches used in HIV, tumor and Wound healing. The plant contains Organic acids, Cyclic triterpenes stigmasterol,[8] Curcacycline A, Curcin,[9] a lectin Phorbolesters Esterases, Sitosterol and its d-glucoside.[10] The leaf and bark have been shown to contain glycosides, tannins, phytosterols, flavanoids and steroidal sapogenins.[7]

The plant is reported to have properties against diseases. In view of these cited activities, observations and traditional uses of plant, the present study was undertaken to explore the wound healing potential of extract of this plant in excision and incision experimental models.

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MATERIAL AND METHODS

Plant material

Fresh stem bark of *Jatropha curcas* L. was collected from a local area of Jaipur were identified in the department of botany, Rajasthan University, Jaipur. A voucher specimen number RUBL20844 was deposited in the department. The fresh stem bark was air-dried to constant weight, pulverized and stored in an air-tight container for further use.

Extraction of plant drug

Powder of dried stem bark was subjected to soxhlet extraction with methanol: acetone: water (70:20:10). The extract was then filtered and the filtrate was concentrated to dryness.

Preliminary Phytochemical Screening

The extract was subjected to phytochemical tests for tannins, steroids, alkaloids and glycosides, flavanoids, carbohydrates, proteins and amino acid using reported methods.[11-12]

Preparation of formulation and standard used

5% (w/w) & 10% (w/w) simple ointment containing the extract of plant was prepared by trituration method in a ceramic mortar and pestle using white soft paraffin base. For this, 5 g & 10 g extract was incorporated in 100 g of the base. Silver sulfadiazine (0.01%) obtained from Rexin Pharmaceutical Pvt. Ltd. was used as standard drug for comparing the wound healing potential of extract in different animal models.

Animals

Albino rats of either sex (150-200 g) were used for the experimental study. The animals obtained from Shree Dhanvantary Pharmaceutical Analysis and Research Centre, Kim, Surat were maintained under standard husbandry conditions in polypropylene cages and provided with food and water ad libitum. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

Grouping of animals

Four groups of animals containing six in each were used for excision and incision wound models. The animals of groups I, II, III and IV were considered as the control, reference standard, (5%) & (10%) extract ointment respectively.

In vivo studies

Excision wound model

The animals were divided into three groups with six in each were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 mm^2 full thickness of skin from a predetermined shaved area.[13] The wounds were left undressed to the open environment. The ointment base, standard drug ointment (0.1% silver sulfadiazine) and extract of plant ointment (5%, w/w) & (10%, w/w) were applied topically to the control group, standard group and treated group respectively, till the wound was completely healed. In this model, wound contraction was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.[14-15]

Incision wound model

In incision wound model,[16] all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. After the incision was made the both edges of skin kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then ointment base, standard ointment and extracts ointment were applied daily up to 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer.[17]

WOUND HEALING EVALUATION PARAMETERS

Measurement of wound contraction

An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated.[18] The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm^2, as 100%, by using the following formula as:

$$\% \text{ wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{initial wound size}} \times 100$$

Measurement of tensile strength

The force required to open the healing action is known as tensile strength. It is used to measure the completeness of healing. It also indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed

on the 9th day after wounding and the tensile strength was measured on 10th day. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer.[19] In this method, wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Hydroxyproline estimation

Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60-70 °C to constant weight and were hydrolysed in 6NHCl at 130 °C for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4M perchloric acid and color was developed with the help of Ehrlich reagent at 60 °C. The absorbance was measured at 557 nm using a spectrophotometer. The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline.[20]

Histopathological examinations

A specimen sample of skin tissues from control, standard and treated groups was taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin[21] and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation and angiogenesis.

Statistical analysis

Results obtained from both wound models have been expressed as mean ± SEM and the treated group was compared with control group. The results were analyzed statistically using Dunnet test followed by one-way ANOVA, to analyze the differences between the treated and control. The data were considered significant at $P < 0.01$.

RESULTS

Wound contraction

A better healing pattern with complete wound closure was observed in standard and treated group (10%, w/w), (5%, w/w) within 18, 19 & 22 days respectively while it was about 26 days in control rats as shown in (Table I).

Tensile strength of incision wound model

Tensile strength for the treated group on 10th day was found to be significant ($P < 0.01$) than control group as shown in (Table II).

Hydroxyproline content

Treated group showed significant increase in hydroxyproline content when compared to control group ($P < 0.01$) as depicted in (Table II).

Histopathological examinations

In standard and treated albino rats with extract (5%) & (10%), excision and incision type of wounds have shown significant healing as in fibroblasts cells (F), collagen fibres (CF) and new blood vesicles (BV) in (Figure I, II and III) respectively while in control rats wounds shown incomplete healing in (Figure IV). Control group has shown to slightest wound healing ability when compared to extract treated and reference ointment group. Fibroblast cells,

Table 1: Effect of methanolic extract of *Jatropha curcas* l. and standard ointment on % of wound contraction of excision wound models in rats

Post wounding days	% of wound contraction			
	control	standard	Extract ointment (5%)	Extract ointment (10%)
2	8.72 ± 1.791%	13.31 ± 1.229%	12.11 ± 1.538%	9.03 ± 3.02%
4	19.13 ± 1.528%	30.59 ± 2.492%	22.45 ± 1.748%	29.14 ± 2.688%
6	30.55 ± 3.055%	51.74 ± 0.564%	45.34 ± 3.332%	49.95 ± 2.164%
8	40.47 ± 2.107%	63032 ± 2.538%	57.56 ± 3.396%	59.05 ± 2.816%
10	47.79 ± 1.51%	81.04 ± 3.016%	65.65 ± 2.068%	67.11 ± 2.729%
12	55.21 ± 2.473%	87.90 ± 2.488%	74.98 ± 1.469%	81.56 ± 2.791%
14	66.70 ± 2.91%	93.49 ± 1.412%	81.38 ± 1.790%	88.11 ± 2.049%
16	74.12 ± 3.276%	97.82 ± 0.311%	86.64 ± 1.331%	93.39 ± 0.723%
18	83.41 ± 3.602%	99.29 ± 0.113%	92.54 ± 1.086%	97.63 ± 0.345%
20	89.95 ± 1.67%	–	96.13 ± 2.49%	99.89 ± 1.452%
22	93.64 ± 4.71%	–	99.58 ± 3.49%	–
24	97.38 ± 1.82%	–	–	–
26	99.91 ± 5.98%	–	–	–

collagen fibres and blood vessels are prominently present in standard and extract treated group as compared to control.

Table 2: Effect of *Jatropha curcas* L. extract and standard ointment on various wound parameters of incision wound model in rats

Groups	Hydroxyproline (mg/g tissue)	Tensile strength (g/mm²)
control	25.76 ± 0.003	413.80 ± 3.665
standard	61.52 ± 0.004*	607.22 ± 3.717*
Extract ointment (5%)	43.39 ± 0.002*	493.75 ± 4.136*
Extract ointment (10%)	55.76 ± 0.003*	582.80 ± 3.665*

n = 6 albino rats per group; values represents mean ± SEM.
*P < 0.01 (comparison of control with standard& extracts).

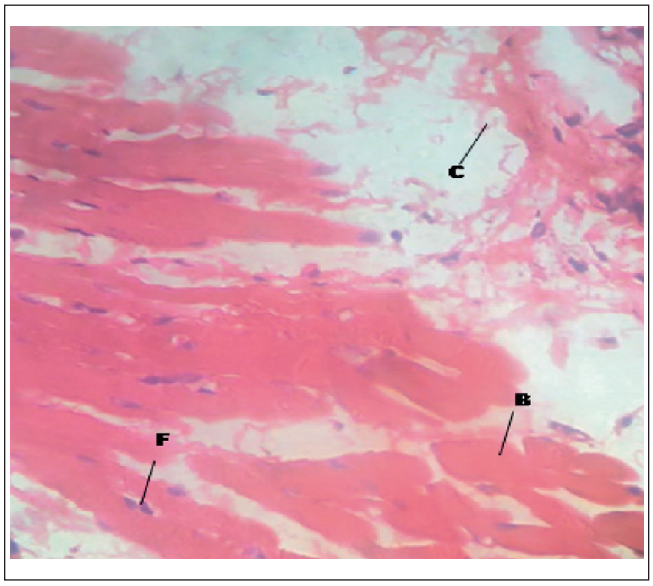

Figure 1: Hepatological characteristics of healed tissue on 18th day by treatment with Standard ointment

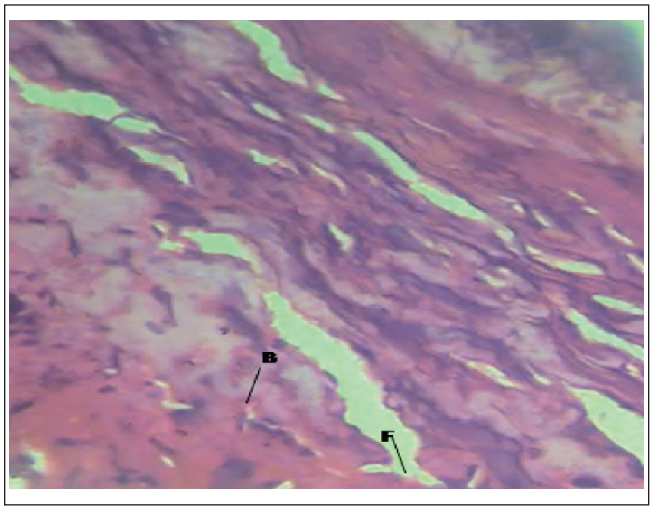

Figure 2: Histopathological characteristics of rat skin on 18th day by treatment with 5% Extract ointment

DISCUSSION AND CONCLUSION

Wound healing is stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair.[22] Hence in this study, excision and incision wound models were used to evaluate the effect of extract ointment on various phases.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres.[23] Increase in blood vessels and role of antioxidants were experimentally proved.[24] In excision wound, the extract showed faster healing with earlier wound contraction compared with control groups.

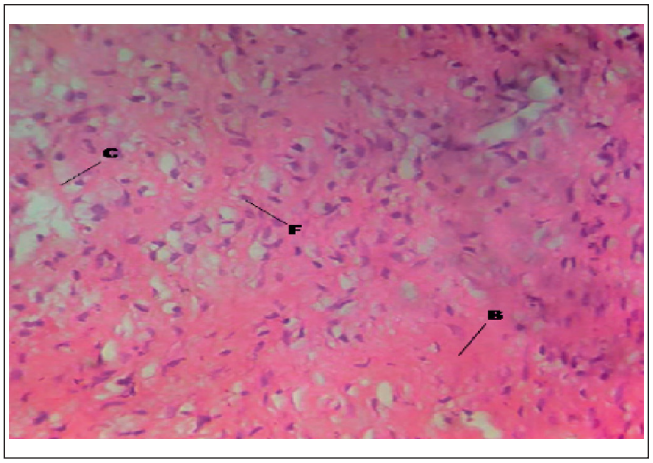

Figure 3: Histopathological characteristics of rat skin on 18th day by treatment with 10% Extract ointment

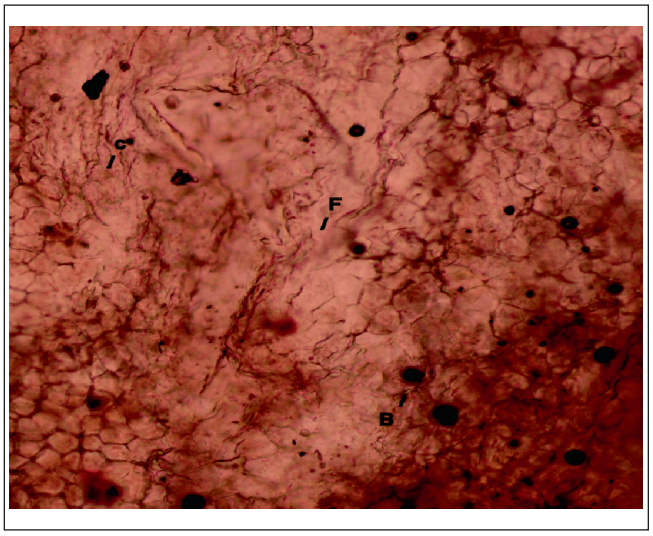

Figure 4: Histopathological characteristics of rat skin on 18th day treatment with ointment base

The earlier wound contraction rate of the extract may be due to stimulation of interleukin-8, an inflammatory a-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue.[25] The extract of plant increased cellular proliferation and collagen synthesis at the wound site as evidenced by increase in total protein and total collagen contents reflected by hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extra cellular matrix of skin, joints, eyes and many other tissues and organs. In spite of its simple structure, it demonstrates remarkable visco-elastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin are due to its interaction with various binding proteins. Due to an influence on signaling pathways, hyaluronic acid and hydroxyproline is involved in the wound-healing process and scarless fetal healing. In clinical trials, topical application of hyaluronic acid has improved the healing of wound.[26] In addition, the muco-polysaccharide hyaluronic acid protects granulation tissue from oxygen free radical damage and thereby stimulates wound healing.[27] Among the glycosaminoglycans, hydroxyproline, dermatan sulfate and dermatan have also been implicated in wound repair and fibrosis. Their ability to bind and alter protein–protein interactions has identified them as important determinants of cellular responsiveness in development, homeostasis and disease.[28]

The results showed that extract ointment possesses a distinct prohealing stroke. This was demonstrated by a significant increase in the rate of wound contraction. Significant increase ($P < 0.01$) in tensile strength, and hydroxyproline content were observed, which was auxiliary supported by histopathological studies. This indicated newly formed fibroblasts cells, collagen fibres and blood vessels. Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids,[29] triterpenoids[30] and tannins[31] are known to promote the wound-healing process.

Preliminary phytochemical screening of extract of *Jatropha* showed the presence of alkaloids, flavonoids and tannins. Its chemical constituents mainly consist of oils and fats, org. acids, flavonoids, triterpenes, steroids, sterols, and proteins. The wound healing action of *Jatropha* may probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents.

Hence, the results obtained from data concludes that extract ointment of plant has properties that render it capable of promoting wound healing activities such as stimulating wound contraction and increasing tensile strength of incision as compared to control.

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Studies on Activity of Various Extracts of *Albizia amara* against Drug induced Gastric Ulcers

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ABSTRACT

Albizia amara is used as a medicinal herb by the tribes of forest regions of Western Ghats. It is used for headaches, backaches, stomach pain, piles and simple ulcers. The anti ulcer activity of various extracts of *Albizia* amara was investigated on ethanol, pylorus ligated and indomethacin induced pylorus ligated ulcer models in mice and rats. The common parameter determined was ulcer index. In the pyloric ligation model and indomethacin induced pyloric ligated models oral administration of both extracts such as petroleum ether and methanol, standard drug ranitidine and control group to separate groups of Wister rats of either sex was performed. Total acidity, volume of gastric juice, pH, percentage protection and ulcer index were assessed. In the case of the 90% ethanol-induced ulceration model in mice, there was a decrease in ulcer score and percentage protection in test groups of petroleum ether (46.72%), methanol (68%) and standard drug ranitidine (85.44%) when compared to the negative control. There was a decrease in gastric secretion and ulcer index among the treated groups i .e. petroleum ether (73.91%), methanol (80.72%) and in standard drug (91.59%) when compared to the negative control in pyloric ligated ulcers. In indomethacin induced pyloric ligated ulcer model in rats there was a reduction in ulcerative score in animals receiving petroleum ether (63.2%), methanolic (62.07%) and standard drug (80.02%) when compared to the negative control. The extract (250 mg/kg) showed significant ($P < 0.01$) reduction in gastric volume, free acidity and ulcer index as compared to control in all models.

Key words: Albizia amara; Pyloric ligation, Indomethacin induced ulcers, ulcer index.

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors.[1] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs.[2] These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.[3] Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide).[4] The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

Albizia amara (Fabaceae) is a plant used in traditional system of medicine in india. The seeds of *Albizia amara* used as an astringent, treating piles, diarrhoea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses.[5] The flowers have been applied to boils, eruptions, swellings, ulcers, also regarded as an emetic, to tackle hair-fall and dandruff on the scalp and as a remedy for coughs and malaria. It is also known as "Kaunthia", a native term originated from Hindi language, indicating an age-old usage of those species by indian indigenous communities.[6] Other popular names are oil cake tree. Leaves were used to tackle hair-fall and dandruff on the scalp. It is used to make hair protective oils. A simple application involves soaking the leaves and flowers in water and using a wet grinder to make a thick paste, and used as a natural shampoo. However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practioners.

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MATERIALS AND METHODS

Plant Material

The dried leaves of *Albizia amara* were supplied by Medicinal plants Revitalisation and Rehabilitation Centre, Sevaiyur, Tamilnadu and authenticated by Dr. S. Jha, Professor, Birla Institute of Technology, Mesra, Ranchi, India. The authenticated specimen has been deposited (PHARM/HS/14/09-10) in the department.

Preparation of extract

The crude drugs were dried under shade for 4-6 days. Then the dried materials were milled to powder. This powdered material was again dried in the oven at 40 °C for 4 h. The coarsely dried powdered leaves were extracted with Petroleum Ether (60°-80°) cold maceration for 72 h, and hot percolation by 90% methanol about 72 h. The extracts were recovered and concentrated to dryness. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of petroleum ether extract and methanolic extract was found to be 15.2% w/w and 7.2% w/w respectively and these extracts were used for further studies.[7]

Preliminary phytochemical screening

The phytochemical examinations of the extracts were performed by the standard methods.[7]

Studies of Acute Toxicity

Acute toxicity studies were carried out on Wistar rats according to standard procedures. Alcoholic extracts at doses of 50, 100, 250, 500, and 1000 mg/kg body weight were administered to separate groups of mice and rats (n = 5) after overnight fasting. Subsequent to administration of drug extract, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed for a further week.[8]

Animals used

Wistar albino rats of either sex weighing between 150-250 gm and mice of either sex weighing between 20-50 gm were used. Institutional Animal Ethics Committee approved the experimental protocol (**BIT/PH/IAEC/13/17:02:2010**); animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Albino rats were used in this thesis was obtained from the Animal House of Birla institute of technology, Mesra Ranchi. The animals were housed in Poly propylene cages and maintained at 24 °C ± 2 °C under 12 h light/dark cycle and were feed ad libitum with standard pellet diet and had free access to water. The animals were given standard diet.

Indomethacin plus pylorus ligated induced ulcer in rats

Animals were fasted for 18 h but allowed access to water only prior to the experiment and divided into 4 groups (n = 6). Group I received the vehicle (1% Tween 80) Groups II and III received 250 mg kg−1 of methanolic and petroleum ether extract, while IV received ranitidine (60 mg/kg^{-1}) respectively. Thirty minutes after oral administration of extract, ulcer was induced by oral administration of indomethacin (20 mg/kg^{-1}). After 7 hr, the animals were scarified and the abdomen opened. The stomach was isolated and opened along the greater curvature and rinsed under a stream of water. ulceration on the gastric mucosa were observed with a hand lens (x10)and scored.[9]

Pyloric ligation in rats

Animals are divided into four groups, each consisting of six rats. First group having pyloric ligated. Second and Third Groups received methanolic extract and pet.ether extract in a dose of 200 mg/kg. Ranitidine, in the dose of 20 mg/kg was administered orally for Group Fourth as a reference drug for ulcer protective studies. After 45 min of extracts and Ranitidine treatment, pyloric ligation was be done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. After 7 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed.[10,11]

Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 36 h before administration of ethanol. The animals were divided into four groups, each consisting of six mice. One Group represented the control group, receive ethanol. Second & third Groups received methanolic extract and Pet. ether extract 250 mg/kg respectively and, Ranitidine, in the dose of 60 mg/kg were administered orally for Fourth group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1 ml/200 g.) Orally, after 45 min of methanolic and pet.extract extract and Ranitidine treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hr latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model.[12,13]

Scoring of ulcer will be made as Normal stomach(0), Red coloration (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), Ulcers (2), Perforation (3) .

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

$$\% \text{ Protective} = \left[\frac{(\text{Control mean ulcer index} - \text{Test mean ulcer index})}{\text{Control mean ulcer index}}\right] \times 100$$

Determination of acidity

Gastric juice was collected and filtered through glass wool in a measuring cylinder and the stomach was opened along the greater curvature. The gastric contents were centrifuged at 3000 rpm for 5 min, and the supernatant was used for the estimation of total acidity (pH). The volume of gastric juice was expressed as mL/100 g of body weight.

For estimation of total acidity, 1 ml of supernatant was diluted to 10 ml with distilled water. The solution was titrated against the 0.05 ml/L Sodium hydroxide using phenolphthalein as an indicator. Titration was continued until the color changed to light pink. The volume of Sodium hydroxide required was noted and was taken as corresponding to the total acidity. Acidity was expressed as

$$\text{Acidity} = \left[\frac{(\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100)}{0.1}\right] \text{mEq/L}$$

Statistical analysis

The results are expressed as means ± standard error of mean (S.E.M.). The data were analyzed by one-way ANOVA followed by Dunnett's test for comparing the control and the test groups, using trial version of GraphPad InStat v 3. Statistical significance was assumed at the 0.05 and 0.01 levels.

RESULTS AND DISCUSSIONS

The results of preliminary phytochemical screening of the both extracts of *Albizia amara* revealed that presence of alkaloids, flavonoids, tannins, terpenoids, phenols and steroids.

Effect on Indomethacin plus pyloric ligated ulcer model

The results are depicted in Table 1, which shows a decrease in ulcer score, volume of acid secretion, total acidity and pH in various extracts of *Albizia amara* i.e. methanolic extract and petroleum ether extract. In the group of animals in which ulcers were induced using indomethacin and pylorus ligation, the methanolic extract showed significant activity in all the selected parameters with % inhibition of ulcers and a significant reduction in total acidity, ulcer score and gastric secretion ($P < 0.001$). Standard drug treatment with ranitidine (60 mg/kg) also showed significant reductions in acidity, gastric secretion and ulcer score with a protective index of 66.37% when compared to positive control group. The petroleum ether extract produced protective index of 63.2%.

Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer model, Oral administration of ME in the dose of 200 mg/kg dose showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 80.72% at the dose of 250 mg/kg in comparison to control whereas Ranitidine as reference standard drug was reduction of ulcer 91.59%. (Results are tabulated in Table 2).

Ethanol-induced gastric ulcer

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. Methanolic extract has shown significant protection index of 68% and 46% with the dose of 250 mg/kg respectively in comparison to control, Ranitidine as reference standard drug was reduction of ulcer 85.44%. (Results are tabulated in Table 3)

Peptic ulcer disease is a chronic inflammatory disease characterized by ulceration in the upper gastro-intestinal tract. The pathophysiology of ulcers is due to an imbalance

Table 1: Effect of *Albizia amara* on Indomethacin plus Pylorus Ligated Ulcers

Group	Treatment	Dosage mg/kg	Gastric contents				% Protection
			Vol. of Gastric juice (ml/100 g)	PH	Total Acidity (mEq)	Ulcer index	
1	Control	1% Tween 80	2.13 ± 0.06	2.13 ± 0.06	116.8 ± 1.6	7.91 ± 0.76	–
2	Methanolic Ext	250 mg	1.33 ± 0.04**	3.5 ± 0.03**	75.2 ± 1.6**	3 ± 0.58**	62.07
3	Pet. Ether Ext	250 mg	1.8 ± 0.05**	2.91 ± 0.04**	64.8 ± 3.4**	2.91 ± 0.23**	63.02
4	Ranitidine	60 mg	0.96 ± 0.02**	3.81 ± 0.06**	40 ± 1.01**	1.58 ± 0.23**	80.02

Values are expressed as Mean ± SEM of 6 observations, Statistical comparison as follows, significant at **$p < 0.01$ compared to control group.

Table 2: Effect of *Albizia amara* on Pylorus Ligated Ulcers

Group	Treatment	Dosage mg/kg	Gastric contents				% Protection
			Vol. of Gastric juice (ml/100 g)	PH	Total Acidity (mEq)	Ulcer index	
1	Control	1% Tween 80	4.28. ± 0.09	4.28 ± 0.09	116.8 ± 1.01	6.9 ± 0.78	–
2	Methanolic Ext	250 mg	2.96 ± 0.06**	4.38 ± 0.06**	59.2 ± 1.01**	1.33 ± 0.1**	80.72
3	Pet. Ether Ext	250 mg	2.33 ± 0.10**	3.5 ± 0.03**	72.8 ± 0.8**	1.8 ± 0.30**	73.91
4	Ranitidine	60 mg	2.7 ± 0.06**	4.95 ± 0.02**	49.6 ± 1.60**	0.66 ± 0.1**	91.59

Values are expressed as Mean ± SEM of 6 observations, Statistical comparison as follows, significant at $^{**}p < 0.01$ compared to control group.

Table 3: Effect of *Albizia amara* on ethanol induced ulcers

Group	Treatment	Dosage mg/kg	Gastric contents				% Protection
			Vol. of Gastric juice (ml/100 g)	PH	Total Acidity (mEq)	Ulcer index	
1	Control	1% Tween 80	2.3 ± 0.04	3.13 ± 0.04	130.53 ± 1.23	6.25 ± 0.30	–
2	Methanolic Ext	250 mg	1.63 ± 0.03**	4.6 ± 0.06**	102.4 ± 1.6**	2 ± 0.22**	68
3	Pet. Ether Ext	250 mg	2.03 ± 0.03**	3.5 ± 0.03**	76.8 ± 1.23**	3.33 ± 0.30**	73.91
4	Ranitidine	60 mg	1.2 ± 0.08**	5.26 ± 0.08**	49.6 ± 1.60**	0.91 ± 0.15**	91.59

Values are expressed as Mean ± SEM of 6 observations, Statistical comparison as follows, significant at $^{**}p < 0.01$ compared to control group.

between aggressive factors (acid, pepsin, H. pylori and NSAIDs) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). The integrity of the gastroduodenal mucosa is maintained through a hemostatic balance between these aggressive and defensive factors. The major cause of gastric ulcer is the chronic use of NSAIDs. Therapeutic and adverse effects of NSAIDs have been attributed to the ability of these drugs to inhibit the action of Cyclooxygenase (COX). COX is responsible for the synthesis of prostaglandins that normally inhibit acid secretion, as well as having a protective effect on the gastric mucosa. Infection of the stomach mucosa with H. pylori- a gram-negative spiral shaped bacterium - is now generally considered to be a major cause of gastrointestinal ulcers. Treatment includes H_2-receptor antagonists (Cimetidine), proton pump inhibitors (Omeprazole) and cytoprotectives (Misoprostol). Antacids, like aluminum hydroxide and magnesium hydroxide, are used often to neutralize excess gastric acidity in the stomach. Due to problems associated with recurrence after treatment, there is the need to seek an alternative drug against gastrointestinal ulcers.[14]

The present investigation demonstrated the efficacy of *Albizia amara* plant extract against gastric ulceration induced by 3 experimental models viz., indomethacin plus pylorus ligated induced gastric ulceration, pylorus ligated induced ulceration and 90% ethanol induced ulceration. The plant extract *Albizia amara* and standard drugs produces a decrease in the ulcer number, total acidity, volume of gastric juice and pH in the indomethacin induced pyloric ligation ulcer model in rats. The curative ratio in this pyloric ligation model was 66.37%, 63.2% and 80.02% using methanol, petroleum ether and standard drug ranitidine, respectively. This indicates that the plant has antiulcerogenic, antisecretory and cytoprotective actions. Several investigators have reported the same results after plant extract treatment. Gastric mucus is known to protect the gastric mucosa against tissue damage by HCl produced by parietal cells. It consists of viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that covers the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant thus can reduce mucosal damage mediated by oxygen free radicals. The protective properties of the mucus barrier depend not only on gel structures but also on the thickness of the layer covering the mucosal layer. A decrease in gastric mucus renders the mucosa susceptible to injuries induced mainly by acids, NSAIDs and alcohol.[15]

The effect of *Albizia amara* extracts on the mucosal damage in the Pyloric ligation induced gastric ulcer model in rats reveals the decreases in ulcer scores. Treatment with successive extracts and standard drug shows the decreases i.e. methanol (80.72%) petroleum ether (73.91%), and ranitidine (91.59%). This indicates that the extracts have cytoprotective effects against the irritant actions caused by acids.[16]

CONCLUSION

Peptic ulcer is an imbalance between gastroduodenal mucosal defense mechanisms and offensive factors. Some studies have revealed that reactive oxygen species (ROS) and lipid

peroxidation are implicated in the pathogenesis of ethanol induced gastric lesions and gastrointestinal damage and that they attack and damage many biological molecules such as prostaglandins. After an initial reaction with ROS, a continuing chain reaction causes cell injury and ultimately cell death.[17,18,19] Therefore, treatment with antioxidants and free radical scavengers can decrease ethanol induced gastric mucosal damage. In the present study, a reduction in ulcer number in ethanol induced gastric ulceration in mice was found after various extract treatments, such as methanol (68%), petroleum ether (46.72%), of *Albizia amara* and the standard drug ranitidine (85.44%). This indicates cytoprotective actions in the plant extracts. Plant chemical substances such as favonoids, tannins, terpenoids etc have been shown to scavenger free radicals and therefore are viewed as promising therapeutic drugs for free radical pathologies. Phytochemical tests revealed the presence of flavonoids and terpenoids in the extracts of *Albizia amara*. Some of the triterpenes are known as an antiulcer agents and their action has been mentioned to be due to activation of cellular proteins, reduction of mucosal prostaglandin metabolism, cytoprotective actions and reduction of gastric vascular permeability. However, the mechanism by which this extract produces an antiulcer effect is not entirely clear. The result in present study seems to provide support for the use of *Albizia amara* as an antiulcer drug in folk medicine. Therefore, also in view of its large use in India more detailed phytochemical and pharmacological investigations on the antiulcer effects and toxicity studies are required.

In all three ulcer experimental models the methanolic extract shows the best antiulcerogenic action, due to the presence of tannins and flavonoids, as in literature references. The present data obtained from various extracts of *Albizia amara* showed the presence of a gastro-protective effect and improved ulcer healing properties. The data also confirmed the traditional claim on the use of *A. amara* for treating gastric ulcers in the Indian subcontinent. Although at this time it is difficult to explain the exact mechanism involved with these crude extracts, the effects obtained on acute and chronic gastric lesions suggest a multifactorial mechanism, involving *A. amara* infuence on free-radical scavenging properties, on endogenous prostaglandins and sulphydryl groups.[20]

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Acute Oral Toxicity of *Abelmoschus manihot* and *Wrightia tinctoria* in Mice

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ABSTRACT

Abelmoschus manihot and *Wrightia tinctoria*, belonging to the botanical family Malvaceae and Apocynaceae, have been traditionally used by the locals in India for treatment of various ailments. The current study reports the outcome of acute oral toxicity investigation of *Abelmoschus manihot* and *Wrightia tinctoria*, on ICR mice. No mortalities or evidence of adverse effects have been observed in ICR mice following acute oral administration at the highest dose of 2500 mg/ kg crude extracts of *Abelmoschus manihot* and *Wrightia tinctoria*. This is the first report on the acute oral toxicity of *Abelmoschus manihot* and *Wrightia tinctoria* and the findings of this study are in agreement with those of *in vitro* experiments and thus provide scientific validation on the use of the leaves of *Abelmoschus manihot* and *Wrightia tinctoria.*

Key words: Acute oral toxicity, Malvaceae, Apocynaceae, *Abelmoschus manihot, Wrightia tinctoria.*

INTRODUCTION

Medicinal herbs have always been used as traditional primary healthcare agents, especially in Asian countries. Over the last 20 years, rapid changes have been observed in the popular use of natural products from plant sources for maintenance of health and for alternative therapy, in Western countries.[1]

Abelmoschus manihot commonly known as "Jungli Bhindi" in **India** belong to botanical family Malvaceae, is a large annual erect hairy plant, 1.2-1.8 m. high. It is native to China, was introduced into India, near Calcutta and in coastal areas of Maharashtra. The mucilage contains polysaccharides and proteins.[2] The flower contains quercetin-3-robinoside, quercetin-3'-glucoside, hyperin, myrecetin and anthocyanins.[3] The different chromatographic methods have been developed on the flavones present in the plant.[4,5] The flowers are used in the treatment of chronic bronchitis and toothache. The ethanol extract of flower was screened for antiviral activity, and it was observed that the hyperoside shown significant anti HBV activity.[6] The flavones present in the plant showed preventive effect in the injury.[7] The leaves were tested on bone loss in ovarectomised rats and it was observed that it was able to prevent the ovariectomy-induced femoral osteopenia[8] **whereas the woody stem extracts possess analgesic activity.[9]**

Wrightia tinctoria commonly known as Dhudh Kodi in India *b*elong to the botanical family Apocynaceae**,**[10] is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India. Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol, in addition to several usual phytosterols, were also isolated and identified**.**[11] The wrightial, a new terpene and other phytoconstituents such as cycloartenone, cycloeucalenol were isolated identified by fractionation of methanol extract of the immature seed pods**.**[12] **The hexane extract of seed pods of *Wrightia tinctoria* was saponified and non saponifiable matter was fractionated with methanol gave a colorless substance, oleanolic acid.**[13] The five flavonoid compounds, Indigotin, Indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves**.**[14] The bark is used as stomachic and in the treatment of abdominal pain and skin diseases**,**[15] as antidysenteric, antidiarrhoeal and antihaemorrhagic**.**[16] The bark is used in flatulence and bilious affections. A decoction of the leaves and bark is taken as a stomachic and in the treatment of abdominal pain**.**[17] The dried and ground bark is rubbed over the body in dropsy.

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Although *Abelmoschus manihot* and *Wrightia tinctoria* are reported to be used in a large number of Chinese traditional medicine preparations, there is no published report on the study of acute oral toxicity of both *Abelmoschus manihot* and *Wrightia tinctoria*. The acute oral toxicity test is the simplest, and often the first toxicity test to be conducted on a sample. A single, high dose of the test sample is given to each experimental animal and the mortality is observed; death within the observation period (usually of 14 days duration), whether caused by natural death or humane killing, is studied.[18] The findings of this study corroborated the need for a safety study on both the species used for primary health care in India. Such studies need to be carried out before the continued widespread use of some species provokes long-term and irreversible damage.

MATERIALS AND METHODS

Plant sample collection and identification

The fresh woody stem of *Abelmoschus manihot* and *Wrightia tinctoria* were collected from Toranmal hills of Maharashtra, India in September 2009 and February 2010, respectively. They were identified by Professor Dr. D. A. Patil of the S. S. V. P. S. Institute of Sciences, University North Maharashtra, India, and voucher specimens were deposited in the herbarium of the R. C. Patel Institute of Pharmaceutical Education and Research, University of North Maharashtra, India, with voucher numbers of PSJ/1235/09 (*Abelmoschus manihot*) and PSJ/1236/09(***Wrightia tinctoria***).

Chemicals

Anhydrous sodium sulfate was purchased from the Sigma-Aldrich Company, while Tween 80 and methanol were obtained from the Merck Company.

Preparation of extracts

The crude extracts were prepared as previously described.[19] Briefly, the fresh woody stems of *Abelmoschus manihot* and *Wrightia tinctoria* were washed, dried, and ground to a fine powder, using a blender. The dried, ground stems were then soaked in methanol (1.5 L) for three days, at room temperature. The solvent-containing extract was then decantered, dried with anhydrous sodium sulfate, and filtered. The extraction of the ground leaves was further repeated (2x) with methanol (1.5 L each time). The filtrates from each extraction were combined and the excess solvent was evaporated (Buchi, Rotavapor, Switzerland) under reduced pressure, using a rotary evaporator, to give a dark green crude methanol extract.

Test species

The experiment was performed on healthy ICR mice (five weeks of age, body weight 23-28 g), obtained from the National Toxicological Centre, University of Pune, India. The female mice were confirmed nulliparous and non-pregnant. The mice were assigned to five dosage groups and one control group with 10 mice (five male and five female) for each test group. The weight variation in the mice used did not exceed ± 20% SD of the mean body weight of each sex. The experimental procedures involving the animals were approved by the University of Malaya Animal Experimental Ethnics Committee [Ethical number: RCPIPER/IAEC/2008-09/53(R)] before commencing the study.

Procedure of acute oral toxicity

The acute oral toxicity of the crude methanol extracts of both *Abelmoschus manihot* and *Wrightia tinctoria* species were evaluated in mice using the procedure described by the OECD (Organization for Economic Co-operation and Development), with some modifications. The mice were housed in suspended, stainless steel, wire-mesh cages in an experimental animal room. The temperature was maintained at 23 ± 3 °C and the relative humidity was 50-60% before and after treatment with the extract. The animal room was artificially illuminated (fluorescent light) with an approximate 12-hour light/ dark cycle. The mice were acclimatized to the laboratory conditions for at least five days prior to commencement of the experiments. The mice were randomly selected for use in the study and marked to provide individual identification. Conventional mouse diets, with unlimited supply of drinking water, were available *ad libitum*, except during the fasting period. The mice were fasted approximately 12 hours prior to dosing, but they had free access to drinking water. Before and after treatment with the extract, the mice were caged in groups by sex and dose levels. The extracts were suspended in a vehicle (10% Tween-80 in distilled water). A stock concentration of 200 mg/ml was prepared and the mice were administrated with 0.2 ml of the extract for every 10 g of mice body weight. The mice were administered with doses of 500, 1000, 1500, 2000, and 2500 mg/kg of extracts. Food was started for the animals approximately three to four hours after dosing. The mice were observed carefully for any signs of toxicity in the first four hours after the treatment period, and daily thereafter for a period of 14 days.[20] Observations for mortality, signs of illness, injury, pain, distress, allergic reactions, changes of outer appearance, behavioral alterations (i.e., ataxia, hyperactivity, hypoactivity), and general stimulation or sedation were conducted twice daily. The observations were recorded systematically; individual records were maintained for each mouse.[21]

RESULTS AND DISCUSSION

Extraction yield of *Abelmoschus manihot* and *Wrightia tinctoria*

Solvent extraction is the most popular method used in sample preparation. The yields from methanol extracts of

Abelmoschus manihot and ***Wrightia tinctoria*** are shown in [Table 1]. Before extraction, the plant material needs to be dried to avoid the presence of water in the extracts. The percentage of crude methanol extract yield is based on the weight of dried and ground plant materials. Methanol is used as the extraction solvent due to its polarity and its known ability to extract compounds such as, phenolics, flavonoids, and other polar materials.

Acute oral toxicity assessment of *Abelmoschus manihot* and *Wrightia tinctoria* crude extracts

Investigation of acute toxicity is the first step in the toxicological analysis of herbal drugs.[22] Overall, animal models have a good predictability for human toxicities of around 70-80%.[23] Generally, it is possible to get the first hints on complex toxicities by applying in vivo methods, as information on some toxic manifestations cannot be assessed by in vitro cytotoxicity methods.[24] Toxic manifestations that affect the entire organism such as pain, distress, allergic reactions, changes in outer appearance, behavioral alterations, and general stimulation or sedation can be detected by in vivo assays. However, the detection of effects on vital functions (cardiovascular, central nervous, and respiratory systems) is usually not assessed in acute toxicity studies.

Acute oral toxicity was undertaken in the present study to determine the safety parameters of the leaves of *Abelmoschus manihot and Wrightia tinctoria*. Mortality, clinical signs, gross findings, and body weights of mice were observed and measured for 14 days after the oral administration of crude methanol extracts to both species. The crude methanol extracts were used in this acute oral toxicity study to ensure that all components in the extract were included.

The Table 2 shows the results of the acute toxicity of the crude extracts of *Abelmoschus manihot and Wrightia tinctoria*. For all doses tested for crude methanol extracts of *Abelmoschus manihot and Wrightia tinctoria*, there were no deaths reported. Throughout the 14-day observation period, there were no significant changes in behavior (i.e., ataxia, hyperactivity, hypoactivity) in any of the mice, nor did they produce any variations in the general appearance. They gained weight with no adverse clinical signs of toxicity at any dose.

Traditionally, the aim of the acute oral toxicity study was the estimation of LD_{50}. The LD_{50} value - defined as the statistically derived dose, which when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period is currently the basis for toxicological classification of chemicals. For a classical LD_{50} study, laboratory mice and rats are the species typically selected. Often both sexes must be used for regulatory purposes.

As no deaths were found for all doses tested for crude methanol extracts of *Abelmoschus manihot and Wrightia tinctoria*, the LD_{50} values of crude *Abelmoschus manihot and Wrightia tinctoria* extracts were >2500 mg/kg. This indicated that both species did not cause any acute toxicity. According to the chemical labeling and classification of acute systemic toxicity, based on oral LD_{50} values, which were recommended by OECD,[25,26] the crude extracts of both species were assigned to class 5 (LD_{50} > 2000 mg/kg), which was termed as the lowest toxicity class (no label; unclassified). Oliver[27] pointed out that (i) the LD_{50} value was not an absolute value, but was an inherently variable biological parameter that could not be described in terms of accuracy, but only of precision, (ii) the LD_{50} value referred only to mortality and was illustrative of no other clinical expression of toxicity.

Table 1: Yield of methanol extracts of *Abelmoschus manihot* and *Wrightia tinctoria*

Plants	Sample/extracts	Weight (g) (%)
Abelmoschus manihot	Fresh sample	4525.10
	Dried and ground plant Material	752.52 (16.63)
	Methanol extract	86.53 g (11.50)
Wrightia tinctoria	Fresh sample	4525.10
	Dried and ground plant Material	648.44 (14.33)
	Methanol extract	66.14 g (10.20)

CONCLUSION

In view of the increasing popular consumption of medicinal plants as alternative therapy, it is necessary to conduct research to support the therapeutic claims and also to ensure

Table 2: Results of the potential toxic effect of the crude extracts of *Abelmoschus manihot* and *Wrightia tinctoria* in mice

Plants	Dose (mg/kg)											
	0[a]		500		1000		1500		2000		2500	
	M	F	M	F	M	F	M	F	M	F	M	F
A. manihot	0/5[b]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
W. tinctoria	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

M = Male ICR Mice; F = Female ICR Mice; [a]control group (treatment without extract); [b]Number of animals dead/number of animals used

that the plants are indeed safe for human consumption. The present research findings have clearly met the objectives of the study. The result was in agreement with that of in vitro experiments, whereby, the crude extracts of *Abelmoschus manihot and Wrightia tinctoria* did not show cytotoxicity against normal MRC-5 cells.[19,28] Based on the outcome of acute toxicity in experimental mice, the crude extracts of both species could be regarded as safe in experimental mice. Further toxicity study over a longer period of time involving detection of effects on vital organ functions would ensure that the plants are safe for human consumption.

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Phytochemical Screening, Antimicrobial and *in vitro* Anti-inflammatory Activity of Endophytic Extracts from *Loranthus* sp.

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ABSTRACT

Four different endophytes were isolated from different parts of *Loranthus* sp. Methanol and water extracts of all the endophytes was assessed for its antimicrobial and anti-inflammatory activity and phytochemical screening. Phytochemical analysis revealed the presence of tannins, flavonoids, terpenoids, steroids, alkaloids, phenols and saponins. The antimicrobial efficacy was determined using paper disc diffusion method against different fungi and bacteria. Sensitivity in terms of zones of inhibition and phytochemical composition of the all endophytic extracts were also determined. The results show that, *A. niger, Penicillium* sp. and *Alternaria alternata* extracts effective against all the bacteria and fungi tested, whereas *A. flavus* extract was failure in inhibiting the growth of all bacteria and bacteria. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation, membrane stabilization assay and proteinase inhibitory assay. Aspirin was used as a standard drug for the study of anti-inflammatory activity. *A. niger, Penicillium* sp. and *Alternaria alternata* methanol fractions showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation (87.88, 86.89, 87.03 g/ml) and red blood cells membrane stabilization with 78.42, 77.61, 77.98 g/ml respectively. Proteinase activity was also significantly inhibited by the *A. niger* (85.21 g/ml), *Alternaria alternata* (84.09 g/ml) and *Penicillium* sp. (79.17 g/ml). BSA anti-denaturation and HRBC membrane stabilization assay indicated that the methanol extracts of *A. niger, Penicillium* sp. and *Alternaria alternata* possess constituents with anti-inflammatory properties. From the result, it is concluded that phytochemicals (tannins, flavonoids, terpenoids, phenols, steroids, alkaloids and saponins) present in the *A. niger, Penicillium* sp. and *Alternaria alternata* extract may be responsible for the antimicrobial and anti-inflammatory activity.

Key words: endophytes, antimicrobial, anti-inflammatory, phytochemicals, *Loranthus sp.*

INTRODUCTION

The increase in prevalence of multiple drug resistance has showed down the development of new synthetic antimicrobial, anti-inflammatory drugs and the new drug is necessary to search for new antimicrobial, antioxidant and anti-inflammatory from alternative sources. Phytochemicals from medicinal plants showing antimicrobial, antioxidant and anti-inflammatory activities have the potential of filling this need because of structures are different from those of the more studied and their those of the more action may too very likely differ.[1] In this growing interest, many of the Phytochemical bioactive compounds from a medicinal plants have shown many pharmacological activities.[2,3] Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases.[4] The rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent intensive for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide.[5,6] Endophytic fungi are relatively unexplored producers of metabolites useful to pharmaceutical and agricultural industries.[7] Endophytes are the microorganisms that grow inside the plants; both

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(plant and endophytes) will be beneficial. Fungal endophytes residing within these plants could also produce metabolites similar to or with more activity than that of their respective hosts.[8] Microorganisms are a rich source of biologically active metabolites that find wide-ranging exploitation in medicine, agriculture and industry.[9] Many of the anticancer agents are explored from endophytes rather than host (taxol from *Pestalotiopsis microspora*).[10] Various research groups have identified more than hundreds of endophytic isolates from South Indian medicinal plants that showed promising activity against antitumour and antimicrobial agents.[11,12]

The development of drug resistance in human and pathogenic bacteria and fungi has prompted a search for more and better antibiotics, especially as disease caused by pathogenic microorganisms, now represents a clear and growing threat to world health.[13,14] Many of the endophytic fungal strains have attracted special attention because they have the capability of producing different colored pigments with high chemical stability. Globally, there are at least one million species of endophytic fungi in all plants,[15] which can potentially provide a wide variety of structurally unique, bioactive, natural products.[16,17]

Increasing evidence indicate that Reactive Oxygen Species (ROS), (example, O_2- and OH-) and free radical mediated reactions can cause oxidative damage to biomolecules (for example, lipids, proteins and DNA), eventually contributing to; aging, cancer, atherosclerosis, coronary heart ailment, diabetes, Alzhemier's disease and other neurodegenerative disorders.[18,19]

Loranthus is a genus of parasitic plants that grow on the branches of woody trees. It belongs to the family Loranthaceae (the showy mistletoe family). *Loranthus micranthus* exhibited various degree of antimicrobial[20] and antidiabetic activity.[21] *L. europaeus* have showed hematopoietic activity.[22]

The literature survey indicates that no reports are available from India regarding antimicrobial and anti-inflammatory activity of *Loranthus* sp. endophytic extract. The present study was aimed to examine the total phenolic content and phytochemical analysis of water and methanol extract endophytes of *Loranthus* sp. were screened for antimicrobial and anti-inflammatory properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the plant.

MATERIALS AND METHODS

The plant was collected in November 2010 from our college campus (Shridevi Institute of Engineering & Technology, Sira Road, Tumkur, Karnataka, India). The plant was identified by their vernacular names and later it was compared with the herbarium of Department of Studies in Botany, Manasa Gangothri, University of Mysore, Mysore and Government Ayurvedic College, Mysore, India.

Isolation and identification of endophytic fungi

The protocol for isolation follow methods used in other endophyte study[23] but adjusted for the specific plant tissues used here following pilot experiments. The plant tissues were washed in running tap water for one hour. Fifty segments of leaves from each plant were cut into 5 mm 2 pieces, including a vein (25 samples) and intervein (25 samples). 25 segments of branches were then cut randomly to a length of 5 mm. Endophytic fungi were isolated from the bark of the plant (25 segments). Twenty five segments (5 mm long) were cut from the stems and the roots. The total 150 segments of plant material were treated by triple surface sterilization technique.[24] Each piece was then placed on malt extract agar (malt extract (20 g/l), rose Bengal (0.033 g/l), chloromphenicol (50 mg/l, agar (15 g/l). All plates were incubated at 26 ± 2 °C until mycelium grew out hyphal tips were cut and transferred to Potato Dextrose Agar (PDA). Half strength PDA was used for subculture and stock culture. Identification was based on colony and hyphal morphology of the fungal cultures, characteristics of the spores.[25,26]

Fungal cultivation and extraction of metabolites

The fungal endophytes were cultivated on Potato Dextrose Broth (Himedia, Germany) by placing agar blocks of actively growing pure culture (3 mm diameter) in 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated at 26 ± 2 °C for 1 week with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile cheesecloth to remove the mycelia mats.

Solvents

Identification of the phytochemical active substances carried out using methanol solvent at 5 g/15 ml (W/V).

Phytochemical analysis

Chemical analysis was carried out in the methanol and water extracts of the all endophytes of *Loratnthus* sp. using standard procedures to identify constituents, as described by Harborne (1984), Trease and Evans (1979) and Sofowara (1993).[27,28,29]

Determination of antimicrobial activity

Antimicrobial assay

Bacillus subtilis, *Pseudomonas fluorescens*, *Clavibacter michiganensis* sub sp. *michiganensis*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum* and strains of *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacteria were obtained from stock cultures presented at –80 °C at

Department of Studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of Studies in Biotechnology and Microbiology, Bangalore University, Gnana Bharathi, Bangalore, India respectively. Three Gram positive bacteria tested were *Bacillus subtilis*, *Clavibacter michiganensis* sub sp. *michiganensis*, *Staphylococcus aureus* and six Gram negative bacterias tested were *Pseudomonas fluorescens*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. All bacteria were grown on nutrient agar media.

Fungi (*Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, Aspergillus flaviceps, Alternaria carthami, Alternaria helianthi, Cercospora carthami, Fusarium solani, Fusarium oxysporum, Fusarium verticilloides* and *Nigrospora oryzae* were obtained from Department of studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of studies in Microbiology, Bangalore University, Gnana Bharathi, Bangalore, India respectively. All fungi were grown on potato dextrose agar medium.

Paper disc method

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. (2009) and Adedapo et al. (2008).[30,31] A swab of the bacteria suspension containing 1×10^8 cfu/ml was spread on to Petri plates containing nutrient agar media. Each extracts were dissolved in ethanol to final concentration of 10 mg/ml. Sterilized filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37 °C for 24 h. The methanol served as negative control while the standard streptomycin (10 µg) discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

Methods of Mizushima and Kobayashi (1968) and Sakat et al. (2010)[32,33] followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37 °C HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min. after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as fallows,

$$\% \text{ inhibition} = \left[\frac{\{Abs_{control} - Abs_{sample}\}}{Abs_{control}}\right] \times 100,$$

Where $Abs_{control}$ is the absorbance of the DPPH radical+ solvent, Abs_{sample} is the absorbance of DPPH radical+ sample extract/standard.

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.[33]

Heat induced hemolytic

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned above.[33]

Protein inhibitory action

The test was performed according to the modified method of Oyedepo and Femurewas (1995) and Sakat et al. (2010).[34,33] The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

BSA anti-denaturation assay

Five ml of each extract was dried in vacuum oven and redissolved in 5 ml of isosaline. Then, 1 mg/ml of all extracts were made from the abovementioned stock solution. To 1.8 ml of 1% of BSA solution, 0.2 ml of extract solution in isosaline was added. The pH was adjusted to 6.5 using 1N HCl. This solution was incubated at 37 ºC for 20 minutes and then heated to 57 ºC for 10 to 15 minutes. After cooling, turbidity was measured at 660 nm. Control was taken without the extracts.[35]

HRBC membrane stabilization assay

Blood was collected freshly and mixed with equal volume of Alsever solution. It was then centrifuged at 3000 rpm for 15 minutes. The packed cells were washed with isosaline and a 10% suspension was made with isosaline. To 0.5 ml of extract, 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC suspension were added. This was incubated for 30 minutes at 37 ºC and then centrifuged at 3000 rpm for 20 minutes. Absorbance was measured at 560 nm. Control was taken without the extract.[36]

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups ($p < 0.05$). Means between treatment groups were compared for significance using Duncan's new Multiple Range post test.

RESULTS

Phytochemical analysis

Loranthus sp. was collected from neem plants (Figure 1). All the incubated parts exhibited the presence of four different endophytic fungal species viz., *Aspegillus niger, Aspergillus flavus, Penicillium* sp. and *Alternaria alternata* (Table 1). In the phytochemical screening of endophytes, *Aspergillus flavus* has showed only presence of carbohydrates and cardio glycosides in methanol extracts whereas no phytochemicals was observed in water extracts. Other three endophytic extracts yielded all the phytochemicals in both methanol and water extracts viz., carbohydrates, tannin, steroids, cardiac glycosides, flavonoids, terpenoids, alkaloids, phenol, saponins and anthraquinones (Table 2).

Antimicrobial assay

The antimicrobial activities of methanol and water extracts of endophytes of *Loranthus* sp. gave different zones of inhibition on the organisms tested (Table 3). The ethanol *Aspergillus niger, penicillium* sp. and *Alternaria alternata* extract inhibited the growth of all most all the bacteria and fungal species significantly. *E. coli*, *Pseudomonas fluorescens, Xanthomonas oryzae* pv. *oryzae, A. helianthi* and *Cercospora carthami* are inhibited by methanol extract of *Aspergillus flavus* minimally, in water extracts there is no activity against all the bacteria and fungi (Table 3).

Anti inflammatory properties

Inhibition of albumin denaturation

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table 4). Maximum inhibition 87.88% was observed from methanol *A. niger* extract

Figure 1: A: *Loranthus* sp on Neem plant, B: Flowers of *Loranthus* sp. C: Different endophytes from different parts of *Loranthus* sp.

Table 1: List of endophytes from different parts of *Loratnthus* sp on PDA media

Types of endophytes	Leaves		bark	stem	root	petiole
	vein	Inter-vein				
Aspergillus niger	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+
Penicillium sp.	+	+	+	+	+	+
Alternaria alternata	+	+	+	+	+	+

Experiments were repeated for thrice for each sample, + = presence

Table 2: Phytochemical analysis of ethanol extract of different plant parts

Tests	Methanol extract				Water extract			
	1	2	3	4	1	2	3	4
Carbohydrates	+	+	+	+	–	+	+	+
Tannin	–	+	+	+	–	+	+	+
Steroids	–	+	+	+	–	+	+	+
Cardiac glycosides	+	+	+	+	–	+	+	+
Flavonoids	–	+	+	+	–	+	+	+
Terpenoids	–	+	+	+	–	+	+	+
Alkaloids	–	+	+	+	–	+	+	+
Phenol	–	+	+	+	–	+	+	+
Saponins	–	+	+	+	–	+	+	+
Anthraquinones	–	+	+	+	–	+	+	+

Experiments were repeated for thrice for each sample, +ve: positive, –ve: negative, 1-*Aspergillus flavus*, 2- *A. niger*, 3-*Penicillium* sp., 4-*Alternaria alternata*

followed by *Penicillium* sp. (86.89%) and *Alternaria alternata* (87.03%). Aspirin, a standard anti-inflammation drug showed the maximum inhibition 76.69% at the concentration of 200 µg/ml. In water endophytic extract, maximum inhibition 77.33% was observed from *A. niger* followed by *Penicillium* sp. (76.54%) and *Alternaria alternata* (77.21%)(Table 4).

Membrane stabilization test

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different methanol and water extracts of different endophytes. All the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect. This effect may possibly

Table 3: *In vitro* inhibition assay from methanol and water extracts of endophytes

Species	Methanol extract				Water extract			
	1	2	3	4	1	2	3	4
Bacterial pathogens								
E. coli	++	+	++	++	–	++	++	++
Pseudomonas aeruginosa	++	–	++	++	–	++	++	++
Staphylococcus aureus	++	–	++	++	–	++	++	++
Klebsiella pneumonia	++	–	++	++	–	++	++	++
Pseudomonas fluorescens	++	+	++	++	–	++	++	++
Clavibacter michiganensis sub sp. *michiganensis*	++	–	++	++	–	++	++	++
Xanthomonas oryzae pv. *oryzae*	++	+	++	++	–	++	++	++
Xanthomonas axanopodis pv. *malvacearum*	++	–	++	++	–	++	++	++
Fungal pathogens								
Aspergillus flavus	++	–	++	++	–	++	++	++
A. niger	++	–	++	++	–	++	++	++
A. nidulans	++	–	++	++	–	++	++	++
A. flaviceps	++	–	++	++	–	++	++	++
Alternaria carthami	++	–	++	++	–	++	++	++
A. helianthi	++	+	++	++	–	++	++	++
Cercospora carthami	++	+	++	++	–	++	++	++
Fusarium solani	++	–	++	++	–	++	++	++
F. oxysporum	++	–	++	++	–	++	++	++
F. verticilloides	++	–	++	++	–	++	++	++
Nigrospora oryzae	++	–	++	++	–	++	++	++

++ = average, + = minimum activity, – = No activity, 1-*Aspergillus flavus*, 2- *A. niger*, 3-*Penicillium* sp., 4-*Alternaria alternate*, Repeated the experiments three times for each replicates

Table 4: Effect of methanol and water extracts of different endophytes on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition

Test sample	Albumin denaturation	Membrane stabilization	Proteinase inhibition
Methanol extract			
Aspergillus niger	87.88 ± 0.006[a]	78.42 ± 0.03[a]	85.21 ± 0.03[a]
Aspergillus flavus	44.76 ± 0.006[a]	54.29 ± 0.03[a]	53.34 ± 0.03[a]
Penicillium sp.	86.89 ± 0.006[c]	77.61 ± 0.03[b]	79.17 ± 0.03[b]
Alternaria alternata	87.03 ± 0.006[a]	77.98 ± 0.03[a]	84.09 ± 0.03[a]
Water extract			
Aspergillus niger	77.33 ± 0.006[a]	72.54 ± 0.03[a]	82.04 ± 0.03[a]
Aspergillus flavus	39.41 ± 0.006[a]	54.81 ± 0.03[a]	50.19 ± 0.03[a]
Penicillium sp.	76.54 ± 0.006[c]	71.87 ± 0.03[b]	77.89 ± 0.03[b]
Alternaria alternata	77.21 ± 0.006[a]	71.95 ± 0.03[a]	81.86 ± 0.03[a]
Aspirin (200µg/ml)	75.89 ± 0.006[b]	85.92 ± 0.03[a]	92.87 ± 0.05[a]

Repeated the experiments three times for each replicates, According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at $P \leq 0.05$, SE-standard error of the mean.

inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree (Table 4). The maximum inhibitions 78.42% from methanol *Aspergillus niger* extract followed by *Penicillium* sp. (77.61%) and *Alternaria alternata* (77.98%). The aspirin standard drug standard drug showed the maximum inhibition 85.92%. In water endophytic extract, maximum inhibition 72.54% was observed from *A. niger* followed by *Penicillium* sp. (71.87%) and *Alternaria alternata* (71.95%)(Table 4).

Proteinase inhibitory activity

The different endophytic ethanol extract exhibited significant antiproteinase activity. The maximum inhibition was observed from methanol *A. niger* extract (85.21%), in decreasing order was *Penicillium* sp. (79.17%) and *Alternaria alternata* (84.09%). The standard aspirin (92.87%) drug showed the maximum proteinase inhibitory action. In water endophytic extract, maximum inhibition 82.04% was observed from *A. niger* followed by *Penicillium* sp. (77.89%) and *Alternaria alternata* (81.86%)(Table 4).

BSA anti-denaturation assay

The inhibitory effect on protein (BSA) denaturation by the water and methanol extracts of endophytes is shown in Figure 2. All the extracts were tested at 200 µg/ml concentration. The *A. niger*, *Penicillium* sp. and *Alternaria alternata* water and methanol fractions showed good activity, whereas the *A. flavus* extract showed comparatively lower activity. At 200 µg/mL concentration, *A. niger* methanol extract showed 79% inhibition of denaturation followed by *Alternaria alternata* (78.6%) and *Penicillium* sp. (65.84%). The water endophytic extracts also showed significant inhibition of denaturation by *A. niger* (76%), *Alternaria alternata* (76.1%) followed by (75.3%) (Figure 3). Denaturation of proteins is well documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation.[37] When BSA is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and

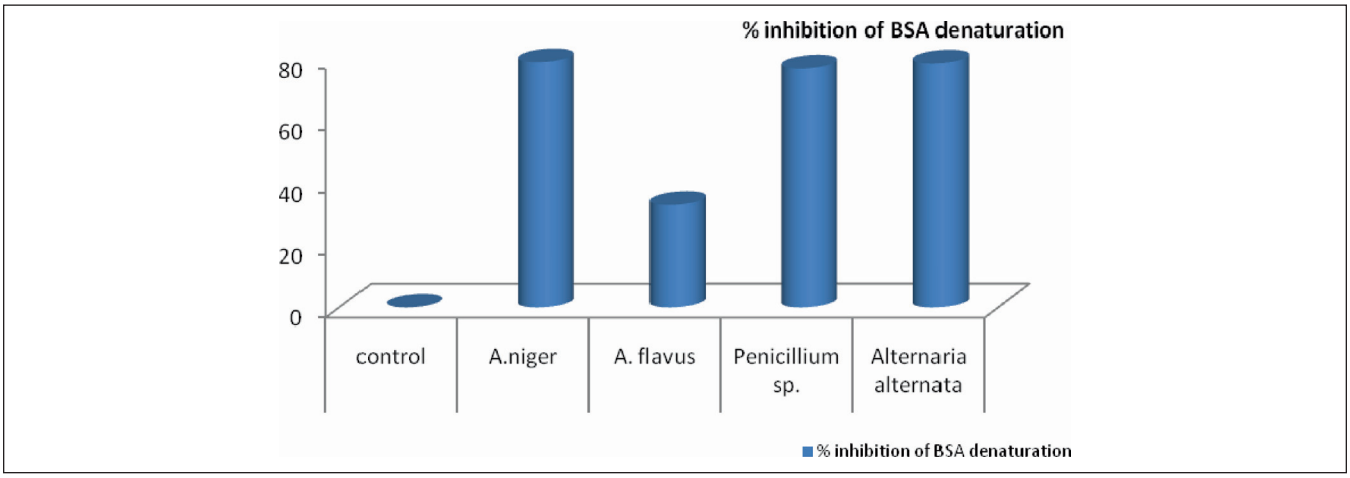

Figure 2: Per cent inhibition of BSA denaturation from methanol extract of endophytes

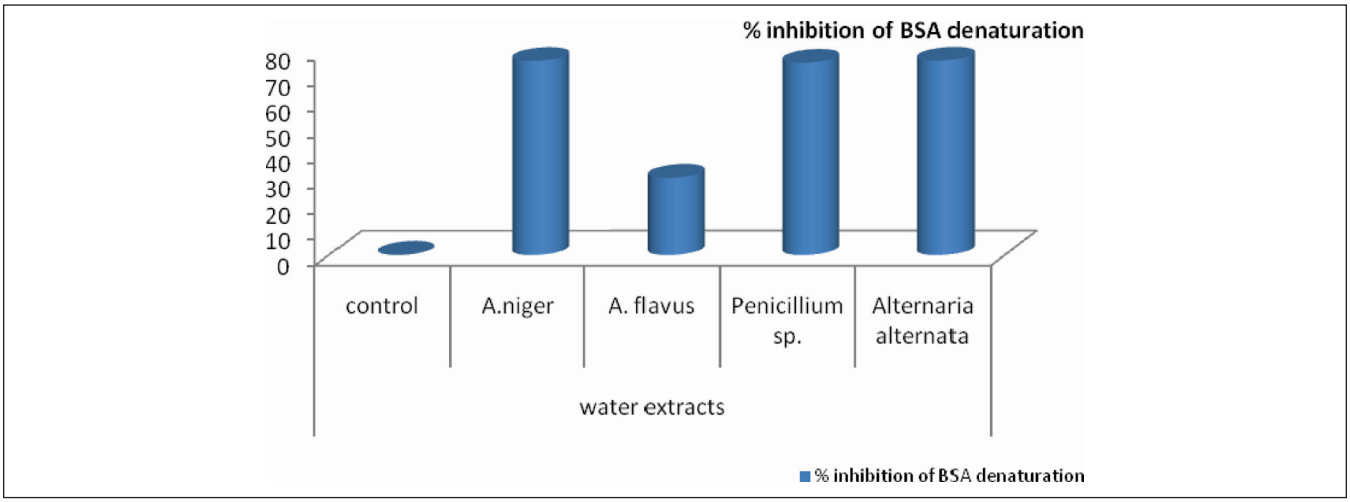

Figure 3: % inhibition of BSA denaturation from water extract of endophytes

Table 5: HRBC membrane stabilization of endophytic extracts, standard drug

Concentration	% stabilization by								by Diclofenac
	Methanolic extract				Water extract				
	1	2	3	4	1	2	3	4	
50 µg/m	79.6	36.1	78.9	79.1	77.4	32.4	76.6	77.1	68.09
100 µg/ml	80.4	47.4	79.6	80.2	79.8	45.7	78.8	79.5	80.48
250 µg/ml	81.6	49.3	80.7	81.1	80.7	46.8	80.2	80.3	82.74
500 µg/ml	68.7	34.3	67.2	69.4	68.8	33.1	65.9	68.1	88.21

Repeated the experiments three times for each replicates,

systemic lupus erythematosus. Thus, this assay was applied for the detecting compounds, which can stabilize the protein from denaturation process. Several nonsteroidal anti-inflammatory drugs such as Indomethacin, Ibufenac, Diclofenac sodium, salicylic acid and flufenamic acid prevent denaturation of BSA at pathological pH (6.2-6.5).[38]

HRBC membrane stabilization assay

After the initial screening of endophytes, it was found that the methanol extract showed activity similar to Diclofenac, the standard anti-inflammatory drug used, for treating inflammation. Various endophytic methanol extracts in isosaline were tested and it was observed at 250 µg/ml both Diclofenac and the endophytic extracts showed similar effects (Table 5). The analogous activity makes the extract a potential candidate for further studies.

DISCUSSION

In recent years, the search for phytochemicals possessing antimicrobial and anti inflammatory properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc.[39] Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes.[40]

Results of our findings confirmed the use of endophytes, *A. niger, Penicillium* sp. and *Alternaria alternata* as traditional medicine. We found strong antimicrobial and anti-inflammatory activities specifically in the methanol extract of *A. niger, Penicillium* sp. and *Alternaria alternata*. Plant phenolic compounds have been found to possess potent antimicrobial[41,42] and anti inflammatory activity.[33,43]

The flavonoids from extracts have been found to possess antimicrobial and anti inflammatory properties in various studies.[44,45] The presence of terpenoids have shown as antimicrobial[46] and anti-inflammatory properties.[47]

Strong presence of tannins in all extracts may explain its potent bioactivities are known to possess potent antimicrobial activities[41] and anti-inflammatory properties.[48] The Saponins have already shown as antimicrobial activity[49] and anti-inflammatory activity.[50]

In vitro anti inflammatory properties

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation.[42] Similar results were observed from many reports from plant extract.[33] The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutraphils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage.[51] The precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the endophytes, *A. niger, Penicillium* sp. and *Alternaria alternate* of *Loranthus* sp. produced this effect surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins.[52]

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors.[53] Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the antioxidant and anti-inflammatory activities of many plants. Hence, the presence of bioactive compounds in the methanol extracts of different endophytes, *A. niger, Penicillium* sp. and *Alternaria*

alternata of *W. trilobata* may contribute to its, antimicrobial and anti-inflammatory activity.

The present investigation has shown that the *A. niger, Penicillium* sp. and *Alternaria alternata* extracts have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The methanol extract fractions showed significantly antimicrobial activity against all Gram-positive and Gram-negative bacteria and different fungi tested. Strong anti-inflammatory properties were confirmed in the methanol endophytic extract fractions. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols and saponins. The anti-inflammatory activity was comparable with standard ascorbic acid, BHT and aspirin. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial and anti-inflammatory agent from *A. niger, Penicillium* sp. and *Alternaria alternata*. These endophytes by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antimicrobial and anti-inflammatory drugs.

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Diuretic Activity of Alcoholic Extract of *Musa sapientum* L. Flower

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ABSTRACT

The present study was designed to investigate the diuretic activity of *Musa sapientum* L. (family- Musaceae) flowers. The dried powder of the flower was subjected to Soxhlet extraction with alcohol and this extract was used for diuretic activity in Wistar albino rats using Lipschitz method. The diuretic activity was assessed in terms of urine output and, concentration of Sodium, Potassium and Chloride ions in urine. The result obtained revealed that the alcoholic extract showed significant diuretic activity at a dose of 250 and 500 mg/kg body weight by increasing the total volume of urine and, concentration of Sodium, Potassium and Chloride ions with respect to standard drug furosemide.

Key words: Diuretic, Sodium, Potassium, Lipschitz method, Furosemide.

INTRODUCTION

The modern era of diuretic therapy began in 1949 when sulphanilamide was discovered to possess diuretic and natriuretic properties. A diuretic is an agent that increases the rate of urination thereby decreasing body fluid, especially the extracellular fluid. Diuretics play an important role in situations of fluid overload, like acute and chronic renal failure, hypercalciuria, cirrhosis of liver and also act as an antihypertensive agent.[1] A number of diuretics like mannitol, thiazides, furosemide, ethacrynic acid are used in practice. Still there is a need for more effective and less toxic diuretic.

That's why there is a great need to search of safer and less toxic diuretic drug from natural resources. There are so many natural diuretic herbs like Aadraka (*Zingiber officinale*), Brahmi (*Centella asiatica*), Gokshura (*Tribulus terrestris*), Ikshuraka (*Saccharum officinarum*), Kantakaari (*Solanum xanthocarpum*), Punarnava (*Boerhavia diffusa*), Sariba (*Ichnocarpus frutescens*), Satavari (*Asparagus racemosus*), Vacha (*Acorus calamus*), Banana (*Musa sapientum*) etc. reported in different traditional literature and practices by natural healers.

Musa sapientum is a tree like perennial herb that grows 5-9 m in height, with tuberous rhizome, hard, long pseudo stem.

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The inflorescence is big with a reddish brown bract and is eaten as vegetables. The ripe fruits are sweet, juicy and full of seeds and the peel is thicker than other banana.[2]

The fruit *M. sapientum* is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout, hypertension, cardiac disease.[3,4] It is also used in the treatment of excess menstruation with *Canna indica* L. var. speciosa.[5] Banana leaves (ashes) are used in eczema,[6] as cool dressings for blister and burns.[3] Flowers are used in dysentery and menorrhagia. Stem juice of fruited plant is used for treating diarrhoea, dysentery, cholera, otalgia, haemoptysis, dysentery, diabetes and menorrhagia.[3] The root is used as anthelmintic,[4] blood disorders, venereal diseases.[3] The plant is also used in inflammation, pain and snakebite.[7]

Banana has played interesting and important roles in the history of human civilizations. Banana is very rich in carbohydrates, vitamin C, A, B and several important minerals, including potassium, copper, magnesium, calcium, and iron. The banana "tree" grows in humid lowland to upland tropical areas; these plants die if they are exposed to cold temperatures.[8] Carbohydrates have been isolated from *M. sapientum*.[9] Catecholamines such as norepinephrine, serotonin, dopamine,[10,11] tryptophan, indole compounds,[12] pectin have been found in the pulp. Several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-Ogalactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) were isolated from the unripe pulp of

plantain.[13,14,15] Serotonin, nor-epinephrine, tryptophan, indole compounds, tannin, starch, iron, crystallisable and non-crystallisable sugars, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp of *M. paradisiaca* and *M. sapientum*.[3]

The review of the scientific literature did not expose any data on the diuretic activity of banana flower. In this study, an attempt was made to assess the efficacy of this indigenous plant for its diuretic activity in terms of urine output and, Sodium, Potassium and Chloride ions concentration in experimental animals with respect to standard.

MATERIALS AND METHODS

Materials

Wistar albino rats 150-170 gms, 36; Standard Furosemide (20 mg/kg); Control Normal Saline (5 ml/kg); Test Solution Alcoholic extract of *M. sapientum* flower (500 mg/kg).

Collection and Authentication of plant

The flower was identified and authenticated as a flower of *Musa sapientum* L. by Dr. Netrabhanu Pradhan Botanist, Prof. and H.O.D. Dept. of Botany, Panchayat College, Bargarh, Orissa and a specimen of flower was deposited in the Herbarium museum of college.

Musa sapientum L. flowers were collected in the month of Nov.-Dec. 2008 from the cultivar of Dularpali, Mahasamund Chhattisgarh. Care was taken to obtain best condition of *M. sapientum* flowers and it was subjected to dry under shade, powdered with laboratory mixer and sieved.

Methods

Extraction

The dried flower powder was Soxhlet extracted with alcohol. The obtained solvent extract was concentrated using rotary vacuum evaporator and dried in desiccators.

Animal

Healthy Wistar albino rats of either sex approximately of same age and weighed about 150-170 g were used for the study. They were fed with standard Indian diet and water *ad libitum*. The animals were housed in polypropylene cages maintained under environmental conditions (12 h light and 12 h dark cycle; 25 ± 3°C). The animals were treated strictly according to the CPCSEA guidelines.

Acute toxicity

The rats were fasted overnight, divided into groups (n = 3) and were orally fed with increasing doses (250, 500, 750 and 1000 mg/kg body weight) of alcoholic extract suspended in Tween 80. After administration of the extracts, the animals were observed during first 3 h for their gross behavioral changes and once in 30 min for next 5 h, then once in 24 h for next 72 h to find out percentage mortality.[16,17,18]

Diuretic activity in rats

The diuretic activity of the extract was assessed by the method previously described by Lipschitz *et al.* for the assessment of diuretic activity, the urine output, sodium, potassium and chloride ion concentration in urine were measured. The animals were divided into four groups each group containing three animals. The animals were deprived of food and water for 12 h prior to the experiment.[16] Before the oral administration of test drugs, the animals were dosed with 25 ml/kg body weight of normal saline. Among the four groups of animals, Group I received Tween 80 (control, vehicle for the extracts) and Group II received the standard diuretic drug Furosemide at 20 mg/kg body weight. Alcoholic extract was studied at two concentrations. Group III received 250 mg/kg and Group IV received 500 mg/kg body weight of alcoholic extract in Tween 80.[19]

Immediately after administration, the animals were placed in fabricated metabolic cages individually to allow separation of urine and faeces. The bottom of the metabolic cage was fixed with a glass funnel inserted into a measuring cylinder containing mineral oil. The presence of mineral oil in the measuring cylinders prevents loss of urine through evaporation. The urine was collected for six hours after administration of control, standard and extract. The bladder was emptied by pulling the base of tail of each rat.[20] Diuretic assay parameters were observed for each rat. The observed parameters were total urine volume, sodium, potassium and chloride ions concentrations. The concentrations of

Table 1: Diuretic activity of *M. sapientum* flower

Name of the Drug/Extract	Dose (mg/kg)	Urine volume (ml)	Concentration of ions (mEq/l)		
			Sodium	Potassium	Chloride
Tween 80	5 ml/kg	0.74 ± 0.47	51.75 ± 1.67	10.84 ± 0.47	52 ± 1.45
Furosemide	20	2.80 ± 0.60*	71.33 ± 2.31	12.87 ± 0.19	92 ± 2.38
Alcoholic extract	250	1.40 ± 0.10*	56.69 ± 0.92	11.42 ± 0.09	51.42 ± 1.26
Alcoholic extract	500	1.93 ± 0.49*	64.32 ± 096	11.93 ± 0.25	53.02 ± 2.40

*$P > 0.05$, Values are mean ± SEM, n = 3

sodium and potassium ions were measured by flame photometry and chloride ion concentration was estimated by titration with silver nitrate solution (N/50) using 5% potassium chromate as indicator.

Statistical analysis

The results were presented as mean ± SEM. "One-way Anova with Dunnett's post t-test was performed using Graph Pad Prism version 3.00 for windows. Graph Pad Software, San Diego California USA, $P < 0.05$ were considered significance.

RESULTS AND DISCUSSIONS

In acute toxicity study, all the animals were found to be surviving after 72 h. This indicates that the extract was found to be safe up to the dose level studied. Since, all the animals survived at a dose of 1000 mg/kg body weight, the LD_{50} of the extract will be >1000 mg/kg body weight. No major behavioral changes were observed during this period of study.

The result of diuretic activity of *M. sapientum* flower showed significant as compared to the standard drug Furosemide and control. The higher dose of extract (500 mg/kg) showed more significant activity as compared to the lower dose of extract (250 mg/kg).

The diuretic activity of the *M. sapientum* flower can be attributed to its presence of amino acids and proteins[21] that plays an important role in the human body urea cycle, which removes nitrogen from the blood and help it to convert into urine.[16]

Determination of urinary electrolyte concentration revealed that alcoholic extract 500 mg/kg body weight was effective in increasing urinary electrolyte concentration for all the ions tested (Sodium, Potassium and Chloride) in comparison to 250 mg/kg dose and control.

CONCLUSION

On the basis of above results it can be concluded that the alcoholic extract produce dose dependent diuretic effect. The present data support the ethanomedical application of *M. sapientum* flower as diuretic.

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