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Vol-II No.-11 Dec 2011

Contents

Editorial

A new turn in Indian economy may take due to Euro zone crisis, F.D.I. and new companies bill to be tabled in Parliament to replace 55 years old Companies "Act 1956". India being primarily domestic economy India's exports are positively linked to the global economic growth.

Earning from the Indian Herbal Pharma sector continues to be at a much lower rate where as in the soft-ware sector growth is steadily increasing. This indicates Indian herbs, scientific research papers need much more support. MSME's & farmers should grow herbs which will not only help our growth but earn more money and foreign exchange to boost overall economy of the country.

Today if we take just one ailment the Diabetes mellitus, India has become the world capital of Diabetes. Total of anti-diabetic drugs market in India is over Rs. 8000 crores. Affordability of health care in the coming years is a serious challenge in India because more than 75% of expenses on healthcare are through our pockets while per capita disposable income is still low and Allopathic drugs remain out of reach and unaffordable for a large section of the population.

According to International Diabetes Federation 366 million people globally are suffering and 46 million die every year due to Diabetes. It means one diabetic person dies after every seven seconds. The disease is known by the name of Madhumeh in our 5000 year old Vedas & Ayurved text books and can be treated by herbs.

Above details force us to continue our support through the research as our patriotic duty. With this year's "Annual function we will be completing our Decadal release of journal, Souvenir & programs". I congratulate to all those who are associated with us for a long time for a cause to promote Indian herbs and to provide right full place globally due to its therapeutical potential by way of scientific documentation in particular in vitro studies.

My special thanks to Dr. Rajendra Dobhal DG-UCOST, members of Tasmia & ICMS for their monetary help in bringing out this issue.

Dr. S. Farooq
Chief Editor

Comparision between HPLC and HPTLC for the separation and quantification of oleanolic acid in *Achyranthus aspera L.*

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Abstract: A Simple HPTLC method has been established for determination of oleanolic acid in Methanolic extract of Achyranthus aspera roots and leaves using toluene: ethyl acetate: formic acid (4.5: 0.5: 0.1 v/v) as mobile phase. Detection and quantification were performed by densitometry at □=352nm.Validated method show linear response over conc. range of 100ng to 500ng. A new validated quality evaluation method for Achyranthus aspera roots and leaves has been established by a defined HPLC. The mobile phase acetonitrile and water (65: 35 v/v) was pumped through a C18 silica column at the flow rate 0.5ml/min and elute was monitered at 215nm, which gave a sharp and well defined peak of oleanolic acid at the retention time 8.654. Therefore this method can be successfully used for the routine analysis of oleanolic acid in both in roots and leaves.The oleanolic acid content of both roots and leaves extract were compared by HPLC and HPTLC densitometry. The quantitative results of both analytical methods did not show any statistical significance.

Keywords: Achyranthus aspera, Oleanolic acid, HPLC, HPTLC, Quantification

Introduction

Achyranthes aspera Linn. (Amaranthaceae), commonly known as chirchita, is a small herb found all over India possessing valuable medicinal properties useful in cough, bronchitis and rheumatism, malarial fever, dysentery, asthma, hypertension and diabetes[1,2,3]. According to the Ayurvedic formulary, A aspera is an important ingredient of many formulations. The dried root of the plant is used in tumor, pyrexia, heart diseases, disorders of liver and blood, and diseases of abdomen (4-5). A dried leaf powder mixed with honey is useful in the early stages of asthma (6). Oleanolic acid is one of the constituents of Achyranthes aspera L. It can be isolated from different parts of plant. The roots and leaves were found to contain oleanolic acid as the aglycone isolated from the saponin fraction (7). Pharmacological studies indicate that oleanolic acid has protective effects for acute chemically injured liver and chronic liver fibrosis and cirrohsis 8-9. It also inhibits tumor cell differentiation and apaptosis10. These bioactivities can be related to medicinal functions of A. aspera, and oleanolic acid can be suggested as a marker compound for the quality evaluation of A.aspera. The aim of this work was to compare the two chromatographic methods, HPTLC and HPLC, for the identification and quantification of oleanolic acid in Achyranthus aspera L. roots and leaves.

Chromatogram layer and HPLC Column

- TLC plates silica gel 60 F254, 20 10cm (Merck)
- HPLC column Luna C18 (150 4.6mm, 5um, Phenomenex)

Chemicals

Toluene, Ethyl acetate, Formic acid, methanol, Acetonitrile, water.

Standard solutions

Dissolve 10mg of reference compound oleanolic acid in 10 ml of methanol and dilute 1:10 with methanol. 1 µL of the solution contains 100ng oleanolic acid.

Apply 1µl=100ng, 2µl=200ng, 3µl=300ng, 4µl=400ng, 5µl=500ng on HPTLC plate as reference marker.

Sample preparation

1. For HPTLC analysis: Around 500mg of air dried roots and leaves of achyranthus aspera were refluxed with 10ml 2M Methanolic HCL for 30min at 1000C hours. The resulting solution was filtered. This solution was used for HPTLC analysis.
2. For HPLC analysis: Around 500mg of air dried roots and leaves of Achyranthus aspera were refluxed with 50ml of methanol for 30min at 800C. The resulting solution was filtered through 0.4 µm membrane filter. This solution was used for HPLC analysis.

Sample application and HPLC injection

- Band wise with Linomat 5, band length 12mm, distance from lower edge 8 mm and distance from left edge 20mm.
- Injection Volume of 20µl for HPLC analysis.

Application scheme and application volume (HPTLC)

S1	A1	S2	B1	S3	A2	S4	B2	S5.
1	1	2	1	3	2	4	2	5 µl

S = standard oleanolic acid, A = Achyranthus aspera leaves, B= Achyranthus aspera roots

Chromatography

- In the Twin Trough Chamber pre-saturated (for 15min, using wetted filter paper) with toluene: ethyl acetate: formic acid (4.5: 0.5: 0.1 v/v) used as a mobile phase and migration distance 85 mm.
- A SHIMADZU model HPLC (LC-2010C HT,). The column and HPLC system were kept at ambient conditions. The injection volume was 20 µl. The mobile phase was acetonitrile: water (65: 35) with the flow rate of 0.5ml/min. The mobile phase was filtered through 0.4 µm membrane filter.

Densitometry

- Absorption measurement at 352 nm for oleanolic acid with TLC Scanner 3 and winCATS software; Evaluation via peak area.
- HPLC- UV detection at wavelength 215nm for oleanolic acid.

Post Chromatographic Derivatization

For visualization of oleanolic acid, the plate was manually dipped in 10% sulphuric acid in ethanol followed by heating at 110oC for 5 min in oven.

Results and Discussion

Oleanolic acid is cleared as a violet coloured zone after derivatization by sulphuric acid reagent and shows a clear at Rf= 0.21. The chromatograms of standard and test sample are shown in figure-1 and Figure-2.

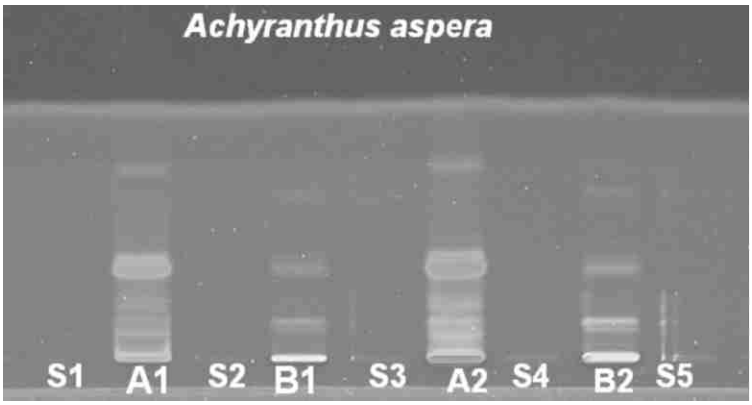

Fig-1- At 366nm

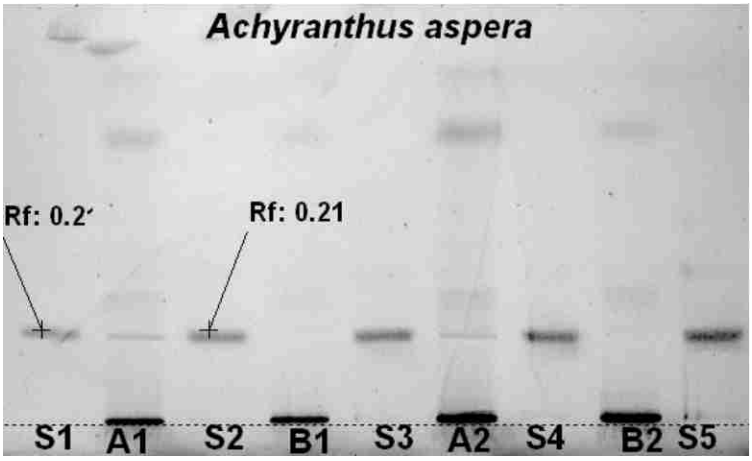

Fig-2- After derivatization with 10% H_2SO_4 in ethanol Chromatogram of A.aspera Leaves(A1 & A2) and Roots (B1 and B2) and standard Oleanolic acid (S1-S5)

Using the proposed HPTLC method the Rf of oleanolic acid was about 0.21. amount of oleanolic acid in methanolic extract of Achyranthus aspera L roots and leaves was found 0.22% and 0.34% w/w. The calibration curve of standard and test sample is shown in figure-3 and Table-1 shows the percentage of oleanolic acid in Different parts of A.aspera.

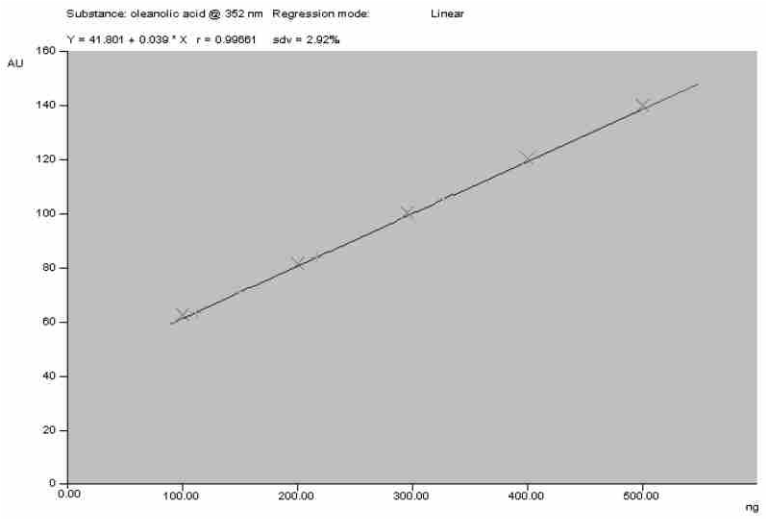

Fig-3. Calibration curve range (100ng-500ng) for oleanolic acid

The calibration curve was linear in the range of 100ng to 500ng for oleanolic acid. Further the correlation coefficient 0.99661 indicates good linearity between concentration and area. The method allows reliable quantification of oleanolic acid from leaves and roots of Achyranthus aspera L.

Table-1: Calculation of Oleanolic acid

Sr. No.	Rf Value	Compound	Plant part used	% of oleanolic acid
1	0.21	Oleanolic acid	leaves	0.34
2	0.21	Oleanolic acid	roots	0.22

The peaks of oleanolic acid from sample solution were identified by comparing their retention times obtained from the peaks with those of standard. HPLC Profile of the methanolic extract of the A.aspera was developed through the same condition as estimation of standard oleanolic acid (Rt 8.624 min). The oleanolic acid was quantified in roots and leaves of A.aspera using regression equation. Oleanolic acid content in leaves and roots was found to be 0.37 % and 0.21% w/w respectively .The values of % RSD were also low, which indicated the suitability of this method for the routine analysis of oleanolic acid.

Conclusion

For both methods, quantification of oleanolic acids by external standard calibration was performed. Using a 20µl volume for injection in HPLC, the oleanolic acid found in leaves and roots was 0.37% and 0.21% w/w respectively.

However in HPTLC just a 1-2µl volume was

applied and the oleanolic acid content was found to be 0.34% and 0.22% in leaves and roots respectively. So, the quantitative results of both analytical methods did not show any statistical significance. Therefore both methods can be successfully used for the routine analysis of oleanolic acid in crude drug and prepared formulation, which can be explored for quality control of raw material and herbal products of traditional system of medicine containing roots and leaves of Achyranthus aspera one of the ingredient.

Acknowledgement

We are highly thankful to DAV (P.G.) College. Department of QA/QC, The Himalaya Drug Company, Dehradun (UK) for providing the lab facilities for this research.

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Gel Protoype Properties of Pectin Derived from *Diospyros peregrina* – A Review

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Abstract

Diospyros peregrina universally well-known as Kalatendu is a temperate sized evergreen tree. It bears dark green foliage & velvety fruits. It is cultivated for ornaments. Its fruit is globular berry with a rubbery peel containing 4-8 seeds implanted in a viscid sticky pulp. It is yellow when ripe & covered with a rusty easily detachable scruffiness; fully ripe fruits have a mawkish sweet taste & are edible. The unripe fruit is acrid, bitter & oleaginous. Diospyros is distributed in the Indian peninsula, extending Northward to Bihar, M.P. & Mumbai, & one of the most characteristic trees of dry, mixed, deciduous forests of these regions. United States is the largest producer, followed by West Germany, Great Britain, Denmark & Switzerland.

Introduncion

Diospyros is a large genus of shrubs and trees comprising of 500 species distributed in the warmer regions. It belongs to the family Ebenaceae. Its generic name is from Greek: dios divine; pyros wheat; it is an allusion to the edible fruits of these plants. About 41 species occur in India mostly on evergreen forests of Deccan, Assam, and Bengal; only few are found in North India. Diospyros peregrina also known as Kalatendu is a moderate sized evergreen tree.

Several species of Diospyros; of which, kaki persimmon (D. kaki) cultivated in South India is best known for edible fruits. D. melanoxylon is distributed in the Indian peninsula, extending northward to Bihar, Madhya Pradesh and Mumbai, and is one of the most characteristic trees of dry, mixed, deciduous forests of these regions. It is a moderate-sized to large tree attaining a height of 60-80 ft. and a girth up to 7 ft., with a straight cylindrical bole of 15-20 ft. under favorable conditions. It bears coriaceous leaves varying in size and form. Under natural conditions seeds germinate in the rainy season and seedling reproduction is often plentiful. The species is the main source of tendu leaves used for wrapping bidis . Their flavour, flexibility, and resistance to decay are properties, which are particularly valued for this use. The fruit is globose and edible . The leaves are diuretic, carminative, laxative, and styptic. Dried flowers are reported to be useful in urinary, skin and blood diseases.

Pectin is a family of complex polysaccharides present in all plant primary cell walls. The complicated structure of the pectic polysaccharides, and the retention by plants of the large number of genes required to synthesize pectin, suggests that pectins have multiple functions in plant growth and development. In this review we summarize the current level of understanding of pectin primary and tertiary structure, and describe new

methods that may be useful to study localized pectin structure in the plant cell wall.

Pectin, an as expected stirring polysaccharide, has in modern years gained gradually more in value. The profits of expected pectin are also additional appreciated by scientists and consumer due to its biodegradability. Pectin is the methylated ester of polygalacturonic acid. It is commercially extracted from citrus peels and apple pomace under slightly acidic conditions. Pectins are divided into two chief groups on the origin of their degree of esterification. The involvement of pectin chains leads to the construction of the three dimensional networks that is to gel formation. The pectin, by itself or by its gelling properties, was engaged in pharmaceutical industry, health promotion and treatment. It has been used potentially as a transporter for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dose form. This assessment will confer the imperative chemistry and universal properties of pectin, and its gel formation method and properties.

Pectin is an evidently taking place biopolymer that is result mounting applications in the pharmaceutical and biotechnology industry. It has been used productively for numerous years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabiliser. Pectin also has several distinctive properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of drugs, proteins and cells. This evaluation will first depict the source and production, chemical structure and general properties of pectin. The methods of gel formation and properties of the gels will then be discussed. Finally, various examples of the pharmaceutical uses of pectin will be prearranged.The plant cell wall represents a major component of the dry mass of plant tissue. It has long been the major source of dietary fibre and is recognised to play a critical role in determining the rheological properties of plants and food texture.

Gel pattern properties of pectin

The most important use of pectin is based on its ability to form gels. HM-pectin forms gels with sugar and acid. This can be seen as a partial dehydration of the pectin molecule to a degree where it is in a state between fully dissolved and precipitated. The particular structure of pectin imposes some specific constraints. HM-pectin, unlike LM-pectin, does not contain sufficient acid groups to gel or precipitate with calcium ions, although other ions such as aluminium or copper cause precipitation under certain conditions.

It has been suggested by Oakenfull (1991) that hydrogen bonding and hydrophobic interactions are important forces in the aggregation of pectin molecules. Gel formation is caused by hydrogen bonding between free carboxyl groups on the pectin molecules and also between the hydroxyl groups of neighbouring molecules. In a neutral or only slightly acid dispersion of pectin molecules, most of the unesterified carboxyl groups are present as partially ionised salts. Those that are ionised produce a negative charge on the molecule, which together with the hydroxyl groups causes it to attract layers of water. The repulsive forces between these groups, due to negative charge, can be sufficiently strong to prevent the formation of a pectin network.

When acid is added, the carboxyl ions are converted to mostly unionised carboxylic acid groups. This decrease in the number of negative charges not only lowers the attraction between pectin and water molecules, but also lowers the repulsive forces between pectin molecules.

Sugar further decreases hydration of the pectin by competing for water. These conditions decrease the ability of pectin to stay in a dispersed state. When cooled, the unstable dispersing of less hydrated pectin forms a gel, a continuous network of pectin holding the aqueous solution. The rate at which gel formation takes place is also affected by the degree of esterification. A higher DE causes more rapid setting. Rapid-set pectins (i.e. pectin with a DE of above 72%) also gel at lower soluble solids and higher levels than slow-set pectins (i.e. pectin with a DE of 58-65%).

LM-pectins require the presence of divalent cations (usually calcium) for proper gel formation. The mechanism of LM-pectin gelation relies mainly on the well-known 'egg-box' model (Grant et al., 1973). The mechanism involves junction zones created by the ordered, side-by-side associations of galacturonans, whereby specific sequences of GalA monomer in parallel or adjacent chains are linked intermolecularly through electrostatic and ionic bonding of carboxyl groups (Fig. 1).

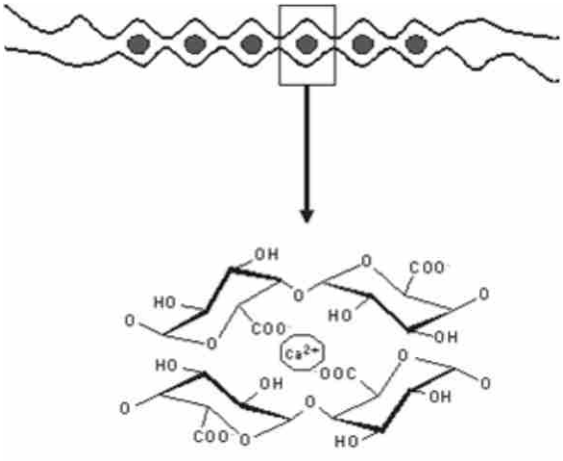

Fig. 1 Schematic representation of calcium binding to polygalactoronate sequences: 'egg box' dimer and 'egg-box' cavity (adapted from Axelos & Thibault, 1991).

It is generally accepted that the junctions consist of dimers in 21 helical symmetry, similar to the 21 model proposed for alginates (Axelos & Thibault, 1991). The oxygen atoms of the hydroxyl groups, the ring oxygen atoms, and the bridging oxygen atoms of the component sugar units participate in the bonding process through their free-electron pairs (Kohn, 1987). The life of the junction depends on the strength of the electrostatic bonds. The bonds are stable when there are at least seven consecutive carboxyl groups on the interior of each

participating chain (Powell et al., 1982). The occurrence of methyl ester groups in the primary backbone limits the extent of such junction zones leading to formation of the gel. Other models for LM-pectin gelation (e.g. 32 helical model) have been proposed (Walkinshaw & Arnott, 1981a,b), but they are currently unconfirmed by experimentation.

Nevertheless, all LM-pectin gels seem to develop similar, if not identical, junction zones (Filippov et al., 1988). Furthermore, amidation increases or improves the gelling ability of LM-pectin: amidated pectins need less calcium to gel and are less prone to precipitation at high calcium levels (May, 1990). Schematic representation of calcium binding to polygalactoronate sequences: 'egg box' dimer and 'egg-box' cavity (adapted from Axelos & Thibault, 1991). Model for the gelation of amidated LM-pectins showing ionic interactions between galacturonic acid residues and hydrogen bonding between amidated galacturonic acid residues (adapted from Sriamornsak, 2002). The gel structure is a net-like formation of cross-linked pectin molecules. The cross-linkages formed by ionic bonds between the carboxyls are strong and produce a rather brittle, less elastic than those formed by hydrogen bonding as in regular pectin. With pectins of lower DE, there is an increasing probability for the formation of cross-links with a given amount of calcium.

As the number of reactive carboxyl groups that can form a salt bridge increases,the greater the chances are that the bridge will be formed. Furthermore, because of the larger amount of charged groups, de-esterified molecules are straighter than the esterified ones, so they will be more likely to form calcium linkages (Thibault & Rinaudo, 1985). Racape and coworkers (1989) suggested that the gelation of amidated pectins could not be explained by the 'egg-box' model alone, as blocks of amide groups along the chain promote association through hydrogen bonding. As expected for any polymer, the lower the molecular weight, the weaker the gel.

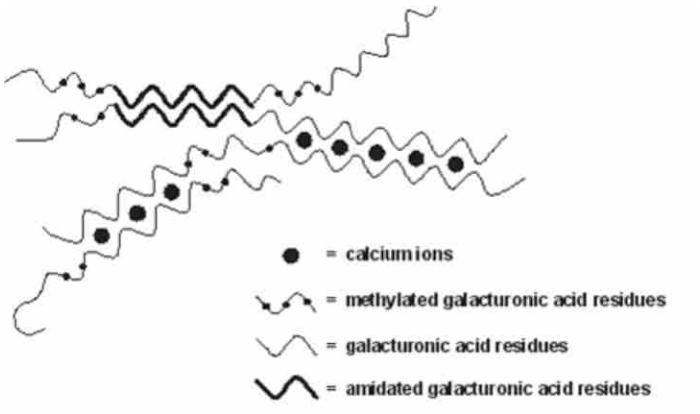

Fig. 2 Model for the gelation of amidated LM-pectins showing ionic interactions between galacturonic acid residues and hydrogen bonding between amidated galacturonic acid residues (adapted from Sriamornsak, 2002).

Fig. 2 illustrated the model for gelation of amidated LM-pectins. Pornsak Sriamornsak The amount of LM-pectin required for the formation of such gel decreases with the DE. The strengths of such ionic bonded gels are strongly dependent on the DE. Monovalent ions such as sodium, which can also react with free carboxyl groups, can affect gel formation because they decrease the cross linking reaction of calcium and improve the solubility of LM-pectin in the presence of calcium (Axelos, 1990). Although sugar is not essential for gel formation with LM-pectins, the presence of small amounts (10-20%) of sugar tends to decrease syneresis and adds desirable firmness of these gels (Christensen, 1986). When some sugar is present, the amount of calcium required to form gel is reduced. High concentrations of sugar (60% or higher) interfere with gel formation because the dehydration of the sugar favours hydrogen bonding and decreases cross-linking by divalent ion forces.

Conclusion

The chemistry and gel-forming characteristics of pectin have enabled this naturally occurring biopolymer to be used in pharmaceutical industry, health promotion and treatment. It has also been used potentially in pharmaceutical preparation and drug formulation as a carrier of a wide variety of biologically active agents, not only for sustained release applications but also as a carrier for targeting drugs to the colon for either local treatment or systemic action. By selection of the appropriate type of pectin, gelation conditions, added excipients, and coating agents, the dosage forms of various morphology and characteristics can be fabricated.

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Heavy Metals Analysis in Different Parts of *Achyranthus Aspera (Chirchita)* Through AAS

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The Himalaya Drug Company Dehradun

Abstract
Heavy metals are a well established cause of severe illness (lung cancer, damage to the brain and nervous system, cause anemia etc.), and these concerns need to be addressed. Lead and Mercury are naturally occurring elements in the earth's crust and are common environmental contaminants. Use of herbs and herbal medicines to relieve and treat many human diseases is increasing around the world due to their mild features and low side effects. It is important to have good quality control for medicinal herbs to protect the consumers from potential contamination of heavy metals present in our environment due to their toxicities to human. Accumulation of heavy metals, namely Pb, Cd, Hg and As was estimated in various parts of the (Achyranthus aspera Linn) known as Chirchita in Hindi. The present paper deals with the study of impact of heavy metals on the plant growth through the environmental pollution. Chirchita (Achyranthus aspera) cures various ailments, but today's scenario of pollution, this plant is also affected.

Certain heavy metals (Ca, Cu, Fe, Zn etc) are nutritionally essential for a healthy life. But some of the heavy metals (Li, Pb, Cd, Hg & As) are toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, fruits, water, air and absorption through skin. The most important vehicle to transport the heavy metal from one place to another is water.

Heavy metal level in Chirchita was estimated in seeds, roots and leaves collected from different sources. An atomic absorption spectrophotometer (GFAAS &tHydrEA mode) is used to determine the levels of metals.

Keywords: Chirchita (Achyranthus aspera) Heavy Metals, Atomic absorption spectrophotometer (GFAAS & Hydr EA mode)

Introduction
Plants are the chief source of medicine since our preliterate mankind generation and the use of herbal food is increasing because of its lesser side effects. Even today medicinally active substances of plant origin, do not find suitable substitutes. There curative properties have been well exploited in the indigenous system of medication. Today India is sitting on the gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. The country is the largest producer of medicinal herbs and is rightly called the "botanical garden of the world".

But in addition to this ever increasing pollution is severely leading to environmental pollution especially water which in turn lead to disruption in life cycles of these plants & its medicinal property. The presence of wide distribution of

heavy metal in soil, due to geographic conditions and environmental pollution is inevitable; therefore, their assimilation in plants is obvious. It has been reported that traces of cadmium and lead can be detected in all plants and food stuffs (Piscator, 1985; Sherlock et al., 1983), and have a toxicological significance too. Due to the use of pesticides and contaminated water the purity becomes affected and finally the level of the contamination of the heavy metals in the human body is increasing and results the various diseases. To explore the possibility of translocation of heavy metals into humans and animals, we have studies the most important medicinal plant Achyranthus aspera "Chirchita" has occupied a pivotal position in Indian culture and folk medicine. It has been used in all most all the traditional system of medicine viz., ayurveda, unani and sidha. Which is used for its various medicinal properties, it is a safe, non toxic and effective phytomedicine with holistic approach is used in the treatment of renal dropsy, ophthalmic and dysentrye. Heavy metal level was estimated in seeds, leaves and roots depending on the medicinal value of the plant portion. (Table-1 shows the medicinal uses of different parts of Achyranthus aspera.)

Table 1. Some medicinal uses of Achyranthus aspera Linn.

Part	Medicinal uses	Reference
Leaves	Syphilitic sores	Nadkarni (2005)
	Gonorrhoea	Rangari(2006)
	Bowel complaint, pile, boil, stomache, skin eruption	Nadkari (2005)
	Early stage of diarrhea and dysentry	Nadarni (2005)
Roots	Pneumonia	Haerdi&Eingeborenosis(1964)
	Astringent and bowel complaints	Quisumbing (1951)
	Stomachic and digestive	Chopra (1933)
	Menstrual disorder	Singh&Singh(1981)
	Leprosy	Rao(1981)
	Antifertility	Malhi& Trivedi (1972)
	Cough,ascites and anasarca	Nandkarni (2005)
Seeds	Expectorants	Nandkarni (2005)
	Brain Tonic	Mishra (1969)
	Bleeding Piles	Mishra (1969)

Material and Method

Different parts (seeds, leaves, and roots) of Chirchita (Achyranthus aspera) were obtained from different sources. The plant parts were washed thoroughly with de-ionized distilled water, dried in a shade, and compressed into a powder with the help of a grinder (Zurera et al., 1987). 1.0 gm of powdered samples were ashed inside the muffle furnace at temperature 4500C±500C and digested in 10 ml of conc. HNO3 and Sulfuric acid (4:1) mixture until a clear solution was obtained. The lead and cadmium content were estimated with GFAAS (novAA 400),analytikjena. The As and Hg were estimated HydrEA technique (novAA 400),analytikjena. Hollow cathode lamps were employed for detection of Pb, Cd, and Hg and As. The instrument was calibrated with standard solutions using the different concentration mode.

Results and Discussion

Heavy metals as environmental contaminants are not a recent phenomenon. They are ubiquitous in trace concentrations in soil and vegetation. In fact many are required by plants and animals as micronutrients. The man made sources of metal contamination are mainly associated with certain industrial activities, agriculture practices, automobile emission, municipal incinerators etc. This paper deals with the heavy metal concentration were estimated in different part of Chirchita collected from the different areas.

Table2, 3 and 4 shows the mean concentration values of various metals, like Pb, Cd, Hg and As, in different parts of the plant collect from different sources.

Table-2: Heavy Metal conc. (ppm) of Chirchita collected from Himalaya Drug company campus Dehradun

Parts used	Pb	Cd	As	Hg
Leaves	3.11	0.180	0.25	0.44
Roots	2.98	0.142	0.32	0.33
Seeds	1.41	0.075	0.18	0.26

Table-3: Heavy Metal conc. (ppm) of Chirchita plant part collected from Trader

Parts used	Pb	Cd	As	Hg
Leaves	5.01	0.21	0.11	0.36
Roots	2.744	0.11	0.42	0.21
Seeds	0.354	0.09	0.31	0.17

Table-4: Heavy Metal conc. (ppm) of Chirchita plant collected from Chamoli district (UK)

Parts used	Pb	Cd	As	Hg
Leaves	2.632	0.15	0.1	0.12
Roots	2.1 36	0.28	0.1 7	0.721
Seeds	1.95	0.10	0.11	0.22

From the present study it was revealed that all the metals accumulated to a greater and lesser extent by different part of Chirchita. The minimum Pb concentration was found in leaves of Chirchita, which is collected from Chamoli & maximum Pb concentration was found in leaves of Chirchita which were collected from the Trader. Results indicate that Cd accumulation is least in the seeds part of the plant. The maximum concentration of Cd found in the sample collected from the trader and minimum in sample collected from chamoli. Accumulation of As is higher in roots part than other parts (Leaves and seeds) in all samples. Results also indicates that Hg content is under the limit however the concentration is increasing as found 0.7 ppm in roots part of sample collected from the chamoli district.

According to World Health Organization (WHO, 1992), permissible limit for medicinal plant based on, ADI (acceptable daily intake) for Pb & Cd are 10ppm & 0.3ppm respectively. The PFA limit for Pb, Cd, and As & Hg are 10ppm, 0.1ppm, 0.1ppm & 1ppm respectively. After comparing these limits with our results, the heavy metal, Pb, accumulation in leaves was found to be near the range. However the Cd concentration in all the parts of A.chirchita studied was found to be out of the acceptance range in all part of plant except the seeds . As concentration in all samples show higher concentration than the permissible limit. Where as Hg concentration in all sample are under limit.

Difference in heavy metal concentration in plants from different region is related to the site from where the samples were collected. However the leaf portion contained higher concentration of heavy metal than bark, furthermore, low concentration of heavy metal was recorded in seeds.

Conclusion

The medicinal plants uses during recent years for the cure of various ailments is obvious from a WHO report, which indicated that many people in developing countries still rely on herbal medicine for treatment of various ailment. From the study it is clear that contamination of heavy metal is increasing and this is the right time to work on this field to come over this problem and to achieve the target. Medicinal plants are easily contaminated with heavy metals during growth, development, harvest, drying, storage and processing To

overcome contaminations from heavy metals, there should be control measures to implement necessary SOP at source. WHO has developed guidelines for the quality control of herbal drugs which provide a detailed description of the techniques and measures required for the appropriate cultivation and collection of medicinal plants, there is still a lacuna between this available knowledge and implementation, because farmers and other relevant persons like producers, handlers and processors of crude herbal plants are not much aware of WHO's guidelines and they continue their work as before without any quality control measures which results in inferior quality of herbal drugs with lots of contaminants like heavy metals, pesticides and microbes. Hence, implementing good agricultural and collection practices (GACP), training for farmers and other relevant persons is an important measure to be taken to ensure good quality of raw herbal drug.

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Standardization of *Emblica officinalis* Garten Fruit (A Neutraceutical Drug Store) from Garhwal Himalayas

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Abstract

Present investigation highlights the standardization of Emblica officinalis Garten from Garhwal Himalayas. All the standardization parameters suggested by WHO were evaluated in the study viz., ash value, acid insoluble ash, water and alcohol extractive values, loss on drying, volatile matter extractives, chromatographic profile, spectroscopic profile, microscopic and macroscopic description. The quantitative determination of active constituent (marker component) was also done by HPTLC study using ascorbic acid as standard.

Key Words: Emblica officinalis, pharmacognostic evaluation, marker compound.

Introduction

Although more than 50% of diseases are curable with allopathic treatment but the goal is not possible without the active participation of the traditional systems of medicine. The worldwide interest on herbal medicines are mainly due to a common belief that anything natural is safer and more in accordance with the health i.e., bio-friendly. This increasing popularity of botanical medicine is due to the ability of curing chronic disease and also due to being devoid of most of the side effects is attracting the interest of most of the pharmaceutical companies1.

But on the other hand this heavy demand results quackery in botanical medicines. It is found that in Indian herbal market in place of genuine crude drug, botanical drugs having similar appearance are being sold. Saraca asoca (Ashoka) is adulterated by the bark of Polyalthea longifolia instead of genuine bark. The Cassia articulata L. leaf adulterates Cassia acutifolia (Senna leaves), which do not have any medicinal value. Such adulterations and substitutions leads to the poor quality of herbal products2,3. Hence strict need of serious measures to check the adulteration is very important.

Emblica officinalis commonly known as Amla in India and stands out as being exceptional for its ethnic, ethno-botanical and ethno-pharmaceutical use4. There is a wealth of technical data to support the safe use of this plant. Almost every part of the plant is reported to have physiological activities. The green fruit is described as being exceedingly acid, dried fruit is sour and astringent. flowers are cooling and aperient while the bark is astringent5. Due to its unique ability of curing disease it has remained the most exploited herb in various herbal medicinal systems (viz., Ayurveda, Traditional Chinese Medicines, Siddha & Unani etc.) worldwide. The chances of adulteration &

substitution of its fruits with other fruits of similar appearance are thus equally exist. Keeping this in mind the present investigation was carried out in which standardization of raw fruit of Emblica officinalis was done. The various parameters under evaluation were total ash value, acid insoluble ash, total alcohol and water extractives, loss on drying, microbial load, presence of heavy metals, pH, volatile oil content, spectroscopic and chromatographic profile and standardization through marker in HPTLC, and micro & macroscopic character. The results of these parameters were also compared with the early reports (if any).

Material and Methods

Plant Procurement and Processing

The fresh fruits of Emblica officinalis were procured from Dr. Shushila Tiwari Herbal Garden, Muni-Ki-Reti, Tehri Garhwal and were authenticated by Dr. SAS Vishwas, HOD, Dept. of Botany, FRI, Dehradun. A voucher specimen was submitted to the herbarium of BSI, Dehradun for future reference. With appropriate storage condition the plant material was weighed and processed for standardization.

Botanical Description

The macroscopic and microscopic examination of raw plant materials were done as per the method suggested in WHO guidelines for quality control of medicinal herbs.

Total Ash Value[6]:

4g sample was placed in a silica crucible and ignited in a muffle furnace at about 400oC for 4 hours. Ash obtained was then cooled in desiccators and weighed to calculate the total ash value using following formula;

$$\% \text{ Total Ash} = \frac{W}{4} \text{ X } 100$$

Where, W is the weight of ash

Acid Insoluble Ash[6]:

The total ash of 4 g of plant material was dissolved in 25ml of dilute HCl and boiled for 5 min followed by its filtration with ashless filter paper. The residue so obtained was washed with hot water, dried and weighed to get the acid insoluble ash.

Determination of extractive values[6,7]:

4.0g of accurately weight coarsely powdered dried material in a glass-stopper conical flask and macerated with 100 ml of the ethanol for 6 hours with frequent shaking and allowed to stand for next 18 hours. It was then filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate was transferred to a tare flat-bottomed dish and evaporate to dryness on a water bath. It was dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter was calculated in mg per g of air dried material.

$$\text{Extractive Value} = \frac{\text{Wt of Extract}}{\text{Wt of Material}} \text{ X } 100$$

Loss on drying[6,7]:

10 g of shade dried fruit of Emblica officinalis was dried at 110 1500C in oven for 1 hr and

then cooled followed by weighing to calculate loss on drying.

Volatile oil[6]

10 g fresh fruits of Emblica officinalis were taken in 250ml round bottom flask of Clevenger Apparatus (Lighter than water) and was processed for next 15 hrs. The oil so obtained from hydro-distillation was extracted out with diethyl ether which was evaporated to get volatile oil.

Heavy Metal Content[6,7]

The standard procedure for determination of zinc in raw plant material was applied using atomic absorption spectrophotometer (AAS).

Determination of Micro-organism[6]

Medicinal plant material normally carries a great number of bacteria and mould, often originating in soil. Presence of micro-organism can be hazardous to health if absorbed even in very small amount.

1g of the plant material was dissolved to 9ml autoclaved water in a test tube. The solution mixed thoroughly to homogenized and 1ml of the solution is diluted to 10ml. In a similar way a series of 12 tubes of serial dilution, each containing 10ml of dilution, was prepared. For the determination of aerobic and anaerobic bacteria, petri dishes of 9-10 cm in diameters were used. 15 ml of liquefied casein soyabean digest agar at a temperature not exceeding 450C was used and the various dilutions were spread on the surface of the solidified medium in a Petri dish. The experiment was performed in duplicate and dishes were incubated at 30-35°C for 2 days, unless a more reliable count is obtained in a shorter period of time. At last the colonies were count by digital colony reader and the results were calculated using the plate with the largest number of colonies, up to a maximum of 300.

Chromatographic Profile[8,9]

TLC Study

Pre coated TLC plates (Al-Silica Gel 60 f 254) were dried in hot air oven for 1hr at 100-1100C. Approximately 5 µg/ml solutions of different extracts in suitable solvent were prepared and spotting at the inert surface of stationary phase is applied with a small capillary. The diameter of spot was followed as per IP and the plates were then placed in a chamber containing small quantity of suitable solvent (known as developer) which serves as mobile phase. As the solvent reached to the top end of the plates, plates were removed from the chamber. The plates were then allowed to stand for 5min followed by spraying of different reagents (Dragandroff, Ninhydrin, and Vanilline sulphate) for the detection of different compounds. The Rf values of the different spots were recorded.

HPTLC Analysis[10]

Different concentration of standard and sample was dissolved in suitable solvents and applied to the precoated aluminum plate by the mean of micro injector. The plate was developed with suitable mobile phase which was selected on the basis of TLC study to provide maximum separation of compounds. Plates were then scanned densitometrically at different wavelengths. The peak areas of each standard and test samples obtained from the system and a calibration graph was plotted between

concentrations & peak area. The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter day and intraday assay precision, repeatability of measurement & repeatability of sample application. The percentage quantities of marker substance in the aqueous extracts were calculated by graph between Rf value and relative height of the peaks followed by application of linear regression equation Y = 33.348 + 0.104 * X (via height).

Spectroscopic Profile

The ultra violet spectrum of the drug is a plot that exhibit how much electromagnetic radiation absorbed at each frequency. This study thus involves the measurement of absorption of light in ultra-violet region (200 800nm) and hence reveals the profile of phyto-constituents present.11

Result and discussion

Macroscopic Description

The fruits of Emblica officinalis was a three chambered capsule measured 2 4 cm in diameter. Immature fruits were fleshy, smoothy, and greenish but change into light yellow when ripe. On ripping it splitted into three one seeded fleshy cocci. The taste of the fresh fruit was astringent and sour.

Microscopic Description

Transverse section of mature fruit of Emblica officinalis showed uppermost epidermis which is covered with thick cuticle layer. Epidermis is single layered made up of tubular cells followed by 3-4 layers of hypodermis consist of thick walled tangentially elongated parenchymatous cells. Below this 5-10 layers of large and ovoid, thin walled parenchymatous cells are present with intracellular space. The parenchymatous cells are iso-diametrically arranged to constitute maximum portion of the fruit. Few silica crystals are unevenly aggregated in the whole mass and a similar un-uniform distribution of fibro vascular bundles appeared between parenchymatous cells that consist of xylem and phloem (Figure 1).

Determination of Physicochemical Constant

The present finding of quantitative analysis involved determination of physicochemical constant, like loss on drying (60.0%), total ash value (1.4%), acid insoluble ash (0.5%), alcohol soluble extractive (4.0%), water soluble extractive (4.9%) and foreign matter (0.8%). These values were in limit as compared with the early reports4. There was no presence of volatile oil and the pH of the water soluble extractive was observed to be 5.45. There is no previous report of confirming these values (Table 1).

Phytochemical Constituents

The results indicated that petroleum ether extract was rich in steroid, fixed oil, and saponins, the chloroform extract was rich in saponin, tannins and carbohydrates, while the acetone extract was observed to possess only penolic compounds and tannins. Two more polar extracts with methanol and water were observed to possess good presence of phenolics, alkaloid, flavonoid, tannin carbohydrate. Amino acids were present only in the aqueous extract (Table 2).

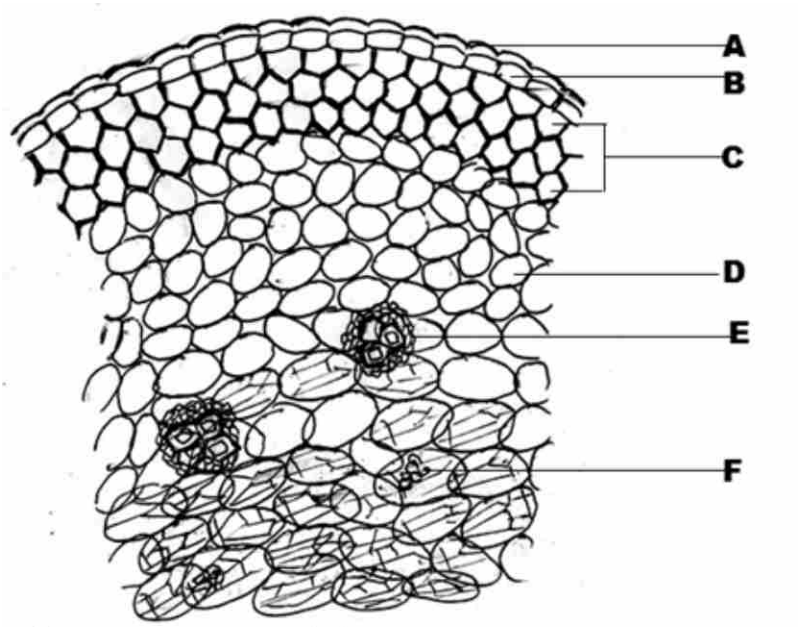

Figure (1) A: Cuticle, B: Epidermis, C: Hypodermis, D: Parenchymatous cell, E: Fibrovascular bundle, F: Silica crystal

Spectroscopic Profile

The present study shows absorption maxima at 254, 273nm respectively for a dilute aqueous solution. There is no earlier report of ultraviolet spectrophotometric studies on the subjected drugs.

Chromatographic Profile

TLC Profile

The chloroform extract was concentrated and applied to precoated (Al Sheet) Silica Gel plates. Toluene and Ethyl Acetate was used as a mobile phase and anisaldehyde sulphuric acid was used as a spraying agent. Three spots at Rf 0.05, 0.12 and 0.17 (all light green) and seven spots at Rf values 0.2, 0.3, 0.45, 0.55, 0.7, 0.75, and 0.97 (all blue) were appeared.

HPTLC Profile

The HPTLC method provides a simple, low cost and a good statistical analysis that proves an efficient method in order to quantitatively determine ascorbic acid concentration in aqueous extract of Emblica officinalis leaves. The sample and standard preparation was done with ethanol and 5 spots of standard 2, 4, 6, 8, 10µg and a spot of test preparation 10µg/ml was tested for the presence of ascorbic acid in aqueous extract of Emblica officinalis leaves. The mobile phase used for this analysis was Ethyl acetate : Acetic acid : Formic acid : Water in a proportion of 51 : 11: 11: 27. The plate was developed and scanned at 280nm. The peak area of each standard and test spot was obtained from the system and a calibration graph was plotted between concentration and peak. The Rf value of the chromatogram was 0.82 for ascorbic acid and the concentration was found to be 2.05% (Figure 2).

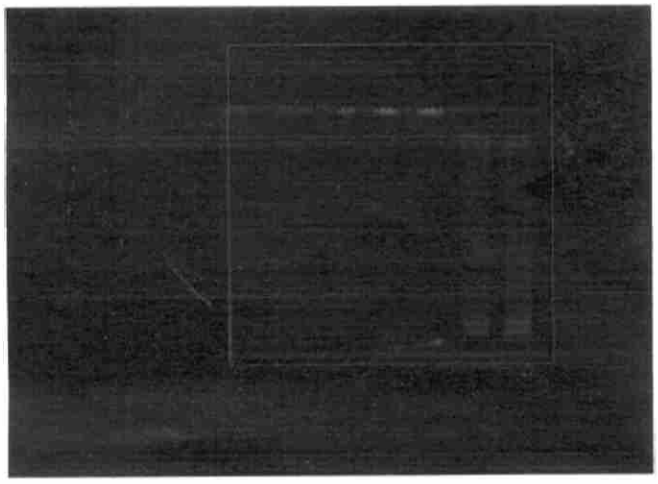

Figure (2): HPTLC chromatogram study of ascorbic acid in aqueous extract of Emblica officinalis Fruit

Conclusion

The present investigation is thus helpful to establish the passport data of Emblica officinalis Garten fruit. It includes taxonomic identification and authentication of the fruit; collection detail location, stage and development of plant, time, pre-processing storage etc.; organoleptic character of the fruit odour, taste, colour, microscopic, macroscopic description, physicochemical constants and chemical composition. Also the results of the present study were compared with the already established limits. Although most of the experimental values were in good agreement with the previous reports however, few reports were found to deviate. The variation on reported values and estimated values in the present study may be due to the various ecological factors. The habitat of earlier studies and present study are different. Moreover, few parameters were not studied earlier hence these results can be utilized to prepare the monograph of the plant.

Table 1: Result of Quantitative analysis of Physicochemical Constant in Emblica officinalis Fruit

Test	Experimental Yield	Earlier Reports	Protocol
Physicochemical Analysis			
Loss on drying(moisture content)	60%	NMT 8%	Quality Control Methods for Medicinal Plant Materials –WHO
Foreign matters	0.8%	NLT 3.0%	
Ash content	1.4	NMT 5%	
Acid insoluble ash	0.5%	NMT 2.0%	
Alcohol soluble extractive	4.0%	22 – 33%	
Water soluble extractive	4.9%	40 – 60%	
pH	4.8	NE	
Volatile oils	Absent	NE	
Heavy Metal Analysis			
Lead	2 ppm	10ppm	- DO -
Cadmium	Absent	0.3ppm	
Arsenic	0.6 ppm	5ppm	
Microbiological Analysis			
Total viable aerobic count	$<10^2 cfu^{-1}$	$<10^4 cfu^{-1}$	- DO -
Total Fungal count	$<10^2 cfu^{-1}$	$<10^2 cfu^{-1}$	
E. coli	Absent	NE	
Salmonella typhii	Absent	NE	
S. aureus	Absent	NE	
HPTLC Analysis			
Aqueous extract with ascorbic acid	2.05%	NE	- DO -

NE: Reports not exist; NMT: Not more than; NLT: Not less than

Table 2: Resutls of phytoconstituents of different extracts of Emblica officinalis Fruit

Tests	Pet. Ether	Chloroform	Acetone	Methanol	Water
Sterols	+	–	–	–	–
Phenolics	–	–	+	+	+
Fixed oil	+	–	–	–	–
Alkaloids	–	–	–	+	+
Glycosides	–	–	–	–	–
Saponins	+	+	–	–	–
Flavonoids	–	–	–	+	+
Tannins	–	+	+	+	+
Carbohydrates	–	+	–	+	+
Amino Acid	–	–	–	–	+

+ present; absent

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Screening of Insecticidal Activity of *Salix babylonica* Roots Against Mustard Aphid

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Abstract

Crude ethanolic root extract of Salix babylonica (Salicaceae) was evaluated against mustard aphid lipaphis erysimi. The plant extract was found very effective and caused 87% mortality at 2.0mg/l in 24hrs and 3 % mortality at 0.5 mg/l in 6 hrs. Lower concentration was also effective in 24 hrs. LC-50 and probit value were also calculated. LC-50 value was came out 3.0, 1.70 and 0.78 mg/l after 6, 12 and 24 hrs respectively.

Introduction

It is well known that the studies on insect and pest behavior and their control are collectively proceeds in entomology [1]. Insects are found in almost all types of environment. They affect man's interest in a number of ways; insects like mosquitoes and housefly spread large number of diseases like malaria, dengue and cholera in addition to painful bites. However, all insects are not harmful; some are beneficial such as honey bee which give us honey and silk worm which provide us silk. Insecticide is mainly a substance intended for killing, repelling or otherwise preventing treated surface from insects [2]. The use of insecticides started with the discovery of Paris green [3], which was a synthetic compound of arsenic, in 1867 to control the colorado potato beetle. Until 1939, most of the insecticides were inorganic chemicals [4]. There are a large number of organic [5] and metal organic [6] compounds as insecticides which have made revolutionary change in the field of chemical control. The phenolics [7] and carbamates [8] are the major organic derivatives used as potent insecticides. But later on the introduction of metal based organic derivatives as insecticides made a new revolution in entomology. Organotin derivatives have emerged as potential biologically active compounds in last 15-20 years. The spectrum of the chemotherapeutic values of organotin compounds has been expanded as they have found their place among a class of potential biologically active compounds [9] exhibiting antimicrobial activity against different kinds of microbial strains [10]. They also show anti-inflammatory and cardiovascular activity [11], trypanosomal activity [12] along with anti-herpes [13] and anti-tubercular activity [14]. Salix babylonica belongs to family Salicaceae is a sub-deciduous or ever green tree up to 15 m. high. Branches glaborous, drooping. Leaves narrowly lanceolate. Commonly found along moist places and oftenly planted or cultivated as an ornamental tree [15]. Most of the members of the genus Salix were analyzed for their flavonoid, terpenoid, xanthone and phenolic constituents of diverse and important biological activities against different pests and insects [16-19].

The present paper deals with the screening of insecticidal activity of alcoholic extract of S. babylonica roots against mustard aphid Lipaphis erysimi.

The present paper deals with the screening of insecticidal activity of alcoholic extract of S. babylonica roots against mustard aphid Lipaphis erysimi.

Material and methods

Collection of plant material and extract preparation

The Leaves (5 kg) was air dried, crushed, powdered and exhaustively extracted with light petroleum ether (60-800 C). The petroleum extract was concentrated and dried. The petroleum free mass then extracted with 90% EtOH repeatedly, until the extractive became colorless. The ethanol extract was concentrated to give a residue (75.0 g) that was used as its insecticidal activity against mustard aphid Lipaphis erysimi.

Insecticidal assay

There were five insecticides tested against Lipaphis erysimi Kaltenback with four treatment of each insecticides i.e. 0.5ml/litre, 1.0ml/litre, 1.5ml/litre, 2.0ml/litre of water repeated four times along with control treatment. The total forty insects were tested in each treatment having four replications. In each replication, ten insects were put in a Petri plate sized 6 inch with mustard leaf and then plant extract were sprayed with chromatographic sprayer. After 6, 12 and 24 hours of spraying, observations were recorded with number of insect mortality (dead insect)

The corrected mortality percentage, statistical probits and LC50 value were calculated. The corrected mortality percentage was calculated by Abbott formula [20] and LC50 value by regression equation.

Results and discussion

The percentage mortality was found to increase with the corresponding increase in dosage, indicating a direct relationship between the two. Highest mortality of 87 % was observed with 2.0 mg/L after 24 hours (Table 3) and lowest mortality was found 3 % with 0.5 mg/L after 6 hours (Table 1). Based on insecticidal data statical probits were calculated the probit range indicates that the activity was significant. The LC50 values were also calculated.

TABLE -1
Insecticidal Activity of ethanolic extract of Salixbabylonica Leaves
After 6 hours

Conc. Mg/L	Total No. of Insect	No. Of dead Insect	% Mortality	Corrected % mortality	Probit	LC_{50} Value(mg/L)
control	40	2	5.00	0.00	0.00	
0.50	40	3	7.50	3	3.12	
1.00	40	3	7.50	3	3.12	3.0
1.50	40	14	35.00	32	4.53	
2.00	40	16	40.00	37	4.67	

TABLE -2
Insecticidal Activity of ethanolic extract of Salix babylonica Leaves After 12 hours

Conc. Mg/L	Total No. of Insect	No. Of dead Insect	% Mortality	Corrected % mortality	Probit	LC_{50} Value(mg/L)
control	40	2	5.00	0.00	0.00	
0.50	40	10	25.00	21	4.19	
1.00	40	14	35.00	32	4.53	1.70
1.50	40	20	50.00	47	5.18	
2.00	40	27	67.50	66	5.41	

TABLE -3
Insecticidal Activity of ethanolic extract of Salix babylonica Leaves After 24 hours

Conc. Mg/L	Total No. of Insect	No. Of dead Insect	% Mortality	Corrected % mortality	Probit	LC_{50} Value(mg/L)
control	40	2	5.00	0.00	0.00	
0.50	40	19	47.50	45	4.87	0.78
1.00	40	23	57.50	55	4.62	
1.50	40	28	70.00	68	5.47	
2.00	40	35	87.50	87	6.13	

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Physico-Chemical and Pharmacognostical Analysis of *Chromolaena odorata* (l) King & Robinson

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Abstract

The plant *Chromolaena odorata* possess anti-microbial, wound healing, anti-gonorrhoeal and anti-larval effect1, 2, 3, 4. But there were no earlier reports on its pharmacognostical analysis. So, we had carried out the pharmacognostical analysis of the leaves of the plant. Leaf constants were determined with the parameters such as stomatal index, vein islet number, vein termination number and their respective values were found out between 37.5-40.2, 8-10, 9-12 / islet. Physico chemical parameters were determined with the parameters such as total ash, water soluble ash, acid insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and the respective values were found out between 18, 9.5, 3.63, 6.21, 15 and 28.

Keywords: Pharmacognostical analysis, Chromolaena odorata, Stomatal Index, Vein Islet Number, Vein termination number.

Introduction

The plant is widely distributed as neo tropical shrub introduced to many parts of the tropics. It forms pure stands in disturbed areas, grasslands, fallows and forestry plantations. Species spread rapidly due to its efficient short and long distance dispersal abilities. The plant is a multi stemmed shrub, 2.5m tall in the open and 10m tall when climbing vegetation (Fig 1). The shoots may root when touching the ground which possesses an underground 'organ' which ensures the plant's survival in case of fire, drought or mechanical damage through coppicing. It grows from sea level to 1000 to 2800 m. It occurs on open freely drained ground and is absent in natural forests.5, 6, 7

Morphological features

The plant is an aromatic, viscid, pubescent erect under shrub growing upto 3 meters. Leaves are simple, opposite obovate to deltoid-ovate chartaceous, basally 3 nerved, puberulous above and pubescent below.8, 9

Experimental

Anatomy of leaf

The leaf has prominent mid vein and lateral veins. The mid vein projects as a hemispherical body on the abaxial side and as a small hump on the adaxial side. The upper epidermis has many long multi cellular uniserate unbranched trichomes. The ground tissues formed the upper and lower portions of the midrib were collen chymatous. The epidermal cells were highly lobed. The mesophyll consists of less distinct short palisade layer of cells. The parenchyma cells were thin walled cells around the vascular bundles. Vascular system was of complex system of both main and accessory vascular bundles

(Fig 2, 3). There was a large, top shaped collateral (main) median vascular bundle with a slightly smaller lateral vascular bundle on either side. On the adaxial part their were two small strands, each with a few xylem elements and a few phloems (3, 4). The canal was circular and was surrounded by 4-6 epithelial cells. The lower epidermis cells were lobed with wavy walls with numerous trichomes. Glandular type and covering type of trichomes were present (Fig 4, 5). The Glandular type was located in shallow pits, mostly on the abaxial side. The gland was sessible, broadly bowl-shaped and consists of two vertical rows of cells. The para dermal section of the gland appears as two semicircular cells with dark contents and prominent nuclei. The covering trichomes were multi cellular, uniserate with tapering ends and the trihomes were straight or bent on side. Stomata were of anomocytic type. No distinct subsidiary cells were evident around the stomata. The stomata were elliptical, oblong or circular (Fig 6, 7, 8). The mesophyll consists of less district short palisade layer of cells and 3-4 spongy mesophyll tissue.

1. Determination of leaf constants

a) Stomatal Index:

The epidermis was peeled off and mounted in saffron. Stage micrometer was focused under 45 x and camera lucida was fixed on the eye piece of the microscope. A square of known dimension (0.4mm) was drawn. The stage micrometer was replaced by leaf preparation. The stomata and epidermal cells in the square were traced and their numbers were counted. A cell being counted if at least half of its area lay with in the square by the following formula

$$\frac{S}{E+S} \times 100$$

Where

S= Number of Stomata/unit area, E= Number of epidermal cells in the same unit area.

b) Vein Islet and Vein Termination Number:

The Epidermal cells were peeled off and mounted on a stage micrometer was focused under 45x. Camera ludica was fixed on the eye piece of the microscope. A Square of known dimension (4 Sq.mm) was drawn. The stage micrometer was replaced by leaf preparation. The views were traced off which were included within the square. The numbers of vein islets in the sq mm. were counted where the islets were intersected by the sides of the square. The numbers of view terminals were counted within the square (Fig 9, 10). The values were tabulated in the table 1.

2. Physico Chemical Standards

a) Determination of total ash

About 2gms of the leaf powder was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red leaf, until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to the air dried powder. The procedure was repeated to get the constant weight and the values were tabulated in table 2.

b) Determination of water soluble ash

Total ash was boiled with 25ml of water was filtered through ash less filter paper (Whatmann 4). It was followed by washing with hot water. The filter paper was ignited in a silica crucible, cooled and the water insoluble matter was weighed. The water soluble ash was calculated by subtracting the water insoluble matter from

the total ash and the values were tabulated in table 2.

c) Determination of acid insoluble ash

The total ash was boiled for 5 minutes with 25ml of 10% w/v dilute hydrochloric acid and filtered through an ash less filter paper. The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed and the values were tabulated in table 2.

d) Determination of sulphated ash

About 1 gm of powder was weighed in a silica crucible. The powder was treated with 1ml of concentrated sulphuric acid and added few drops of concentrated sulphuric acid, ignited and repeated until the difference of weighed was not more than 0.5 gms and the percentage of sulphated ash with reference to air dried drug was calculated and the values were tabulated in table 2.

e) Determination of Alcohol soluble extractive

About 5 gms of the powder was macerated with 100ml of alcohol in a conical flask for 24 hours and shaken frequently during 6 hours. It was filtered rapidly taking precaution against less of alcohol and 25ml of the filtrate was evaporated by dryness in a fixed flat filtered shallow dish dried at 105°c and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried powder and the values were tabulated in the table 2.

f) Determination of water soluble extractive

About 5 gm of the powder was added to 50 ml of water at 80°c in a stoppered flask. It was shaken and allowed to stand for 10 minutes cooled to 15°c and 2gm of kiesel ghur was added to it and filtered. 5 ml of the filtrate was transferred to a flamed evaporating dish (7.5 cm in diameter). The solvent was evaporated on a water bath. Drying was continued for half-an-hour, finally it was dried in a hot air over for two hours and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug and the values were tabulated in the table 2.

Conclusion:

Physico chemical standards such as total ash, water soluble ash, sulphated ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive values were determined in percentage. The leaf constants were determined where the stomatal index was found to be 37.5 40.2, vein islet number was between 8-10 and vein termination number was between 9-12 per vein islet. Based on the earlier reports on the leaves of the plant Chromolaena odorata proposing the one medicinally importance of the leaves the present study proceeded with the physico chemical analysis. This may help in standardization of the drug.

Morphological character of the leaf was studied. The leaf material was taken for anatomical studies. The observed characters were glandular trichomes, covering trichomes, anomocytic stomata and mesophyll.

Table 1

S.No.	Leaf Constant	Values
1.	Stomatal Index	37.5-40.2
2.	Vein Islet Number	8-10
3.	Vein termination number	9-12 / islet

Table 2

S.No.	Physico-Chemical Factor	Values in percentage
1.	Total ash	18
2.	Water soluble ash	9.5
3.	Acid insoluble ash	3.63
4.	Sulphated ash	6.21
5.	Alcohol Soluble extractive	15
6.	Water Soluble extractive	28

Figure 1

Figure 2

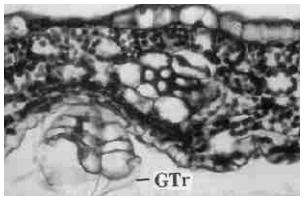


Figure 3

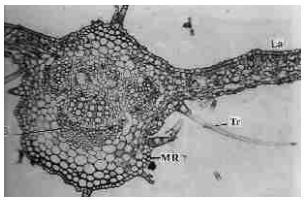


Figure 4

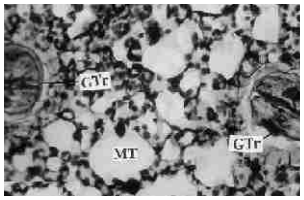


Figure 5

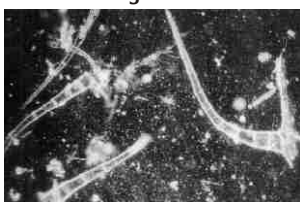


Figure 6

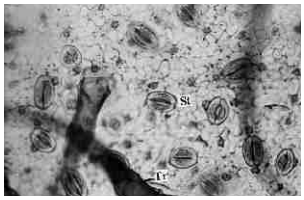


Figure 7

Figure 8

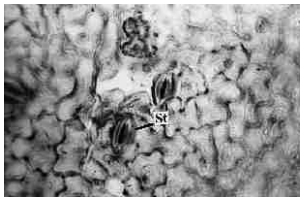


Figure 9

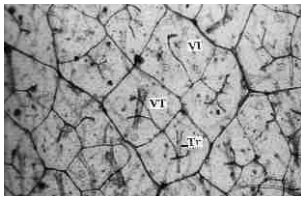
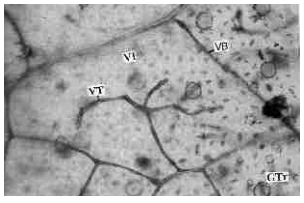


Figure 10



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Two New Compounds from the Stem bark of *Samanea saman*

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Abstract

From the stem bark of *Samanea saman* two new compounds have been isolated and characterized as stigma -7, 22-diene-3- O [α - L- arabinopyranaosyl (1 → 2)] -ß - D - galactopyranoside 1 and 19 - hydroxy - octacos - 5 - ene -3 one 2 by spectral and chemical studies.

Key Words: *Samanea saman*, Mimosaceae, Raintree.

Introduction

Samanea saman (Mimosaceae) commonly called "Raintree" is a useful medicinal plant is native of Yucatan Peninsula Guatemala to Peru ,Bolivia, Brazil, throughout the West Indies, in old world tropics and also in Southern Florida1 but is planted almost throughout India.

The root decoction is used in hot baths for stomach cancer in Venezuela.2 Rain tree is a folk remedy for cold, diarrhea, headache, intestine ailments and stomachache.3 The alcoholic extract of the leaves inhibits Mycobacterium tuberculosis.4 The alkaloid fraction of the leaves is effective on the CNS and PNS. In Colombia, the fruit decoction is used as a CNS-sedative. The leaf infusion is used as a laxative5. In the West Indies, seeds are chewed for sore throat6.

Earlier alkaloids,7 flavonoids, kaempferol8 have been isolated. Ethanolic extract of the stem bark furnished compound 1 and 2 from benzene : ethyl acetate (9:1, v/v). Homogeneity and purity were established by TLC and Co-Pc.

Extraction and Isolation:

The air-dried and finely crushed stem bark (5 kg) of Samanea saman was repeatedly extracted with boiling EtOH, concentrated under reduce pressure in a rotatory evaporator and poured into an excess of cold-water to give water soluble and water insoluble portion. The water insoluble portion was extracted successively with different solvents of increasing polarity over a column. Elution with solvent system C6H6 : EA (9:1v/v) gave two compound 1 (0.4g) and 2 (0.6g) separate by preparative TLC.

Experimental Section:

The stem bark of Samanea saman was collected in April 1999 from the Botany Department, University of Allahabad, Allahabad, U.P., India. Melting points are uncorrected, column, thin layer chromatography analysis were performed at room temperature. Sugars were characterized by descending paper chromatography on Whatmann No. I paper, using n-BuOH : AcOH : H2O (4:1:5) as a

developing solvent.

Phytochemical investigation and results

Compound 1: C40H66O10, m.p. 126oC, non-reducing glycoside of steroid showed absorption in the IR spectra for secondary hydroxyl group

(3460 cm-1) trisubstituted double bond (1649 &t 792 cm-1) and gem-dimethyl group (1385 and 1375cm-l). On acid hydrolysis it gave an aglycone 1a and two sugar moieties. Both sugars were identified as D-galactose and L-arabinose on the basis of co-paper chromatography with their authentic samples.

The IH NMR spectrum of la showed signals for (i) two tertiary methyl group at 0.79 (3H,s) and 0.55 (3H, s) (ii) three secondary methyl group at 1.03 (3H, d, J=6.72Hz), 0.83 (3H, d, J=6.5 Hz) and 0.85 (3H, d, J=6.92Hz) (iii) Two olefinic protons at 5.12 (1H, dd, J=15.39 and 9Hz) and 5.24 (1H, dd, J=15.0 and 8.09 Hz) due to double bond at C-22 (23) (iv) one olefinic proton at C-7 resonated at 5.18 (1H, brs)9 (v) hydroxy group at C-3 resonated at 5.40 (1H, bm) and 3.83 (lH, dd, J= 9.6Hz) showed it equatorial ( ) orientation. All the above data indicated the presence of a tetracyclic steroid nucleus with 3-hydroxy group, double bond at C-7 (8) , methyl goup at C-18 and C-19 and a side chain having double bond and gem-dimethyl group.

In mass spectrum, intense peak at m/z 272 and 140 also confirms the presence of double bond at C-22 (23). which was also supported by 13C NMR spectrum, showed two doublet at 131.8 (C-22) and 135.46 (C-23)9 . see 13C NMR table. Peaks also observed at 254, 272, 232, 85, 43 &t 29. Hence la must be stigma 7,22 - diene -3-ol.

Hydrolysis of the glycoside with enzyme takadiastase liberated free arabinose and no galactose in the hydrolysate indicating the nature of arabinose linkage. After complete takadiastase hydrolysis the glycoside was hydrolysed with emulsin, galactose was observed in the hydrolysate, confirmed the linkage to be in nature with the aglycone. The -and -nature of arabinose and galactose was further supported by its 1H NMR in which the shifts of anomeric protons were appeared at 4.38 (1H, d, J = 7 Hz) and 5.30 (1H, d, J = 8 Hz), respectively10. IH NMR spectrum showed signals at 3.52 - 3.88 (11H) for sugar protons.

This confirmed that galactose was involved in the glycosidic linkage with the aglycone and arabinose involved in inter glycosidic linkage with C-2' of galactose. Easy hydrolysis eliminated the possibility of C-C glycosidic linkage, so the linkage must be of C-O-C type i.e. at C-3.

On the basis of described facts the structure of the compound 1 was identified as stigma7, 22-diene-3-O-[- -Larabinopyranosyl (1 2)]- -D-galactopyranoside.

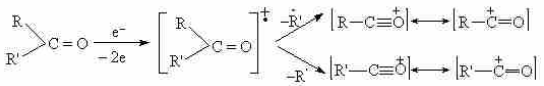
The white coloured compound 2, m.p. 76°C, molecular formula: C28H54O2 was analysed as 19-hydroxy-octacos-5-ene-3-one on the basis of colour reaction and spectral data.

IR spectrum showed absorption bands at 3420 (hydroxyl group), 1720 (carbonyl group), 1632 and 970 (trans double bond) and presence of

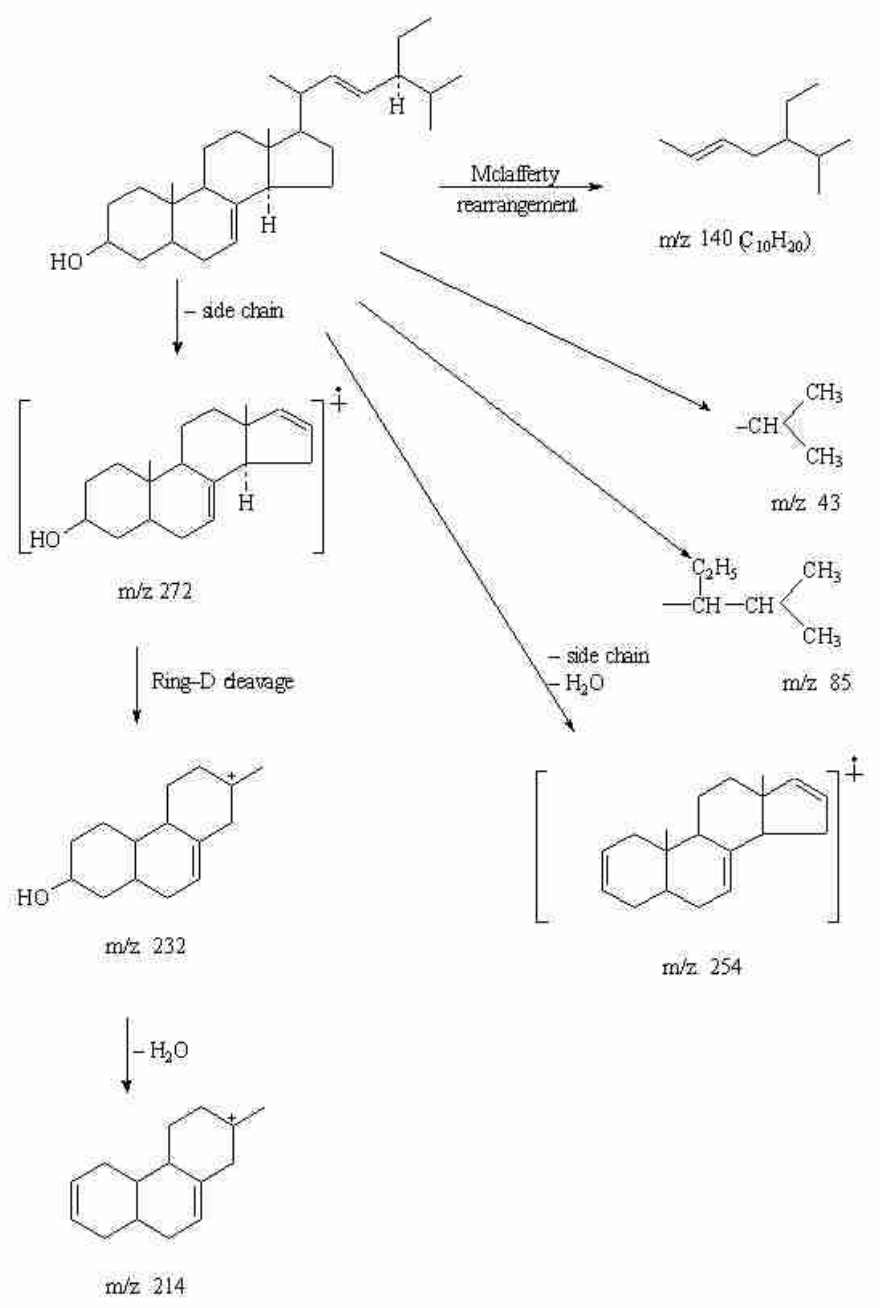
bands at 2930, 2850 and 720 cm-1 and absence of absorption in the aromatic region suggested that it is a long chain aliphatic compound. It decolourized KMnO4 and Br2 solution confirming the presence of unsaturation in the compound. It formed hydrazone with 2,4-DNP confirming the presence of carbonyl group.

The 1H NMR spectrum exhibited absorption peak at 5.20 (2H, t, J=15 Hz) for methine proton (CH=Ch), and at 2.40 (2H, d,-CO-CH2 for two methylene protons at C-4 position. Appearance of a triplet 0.94 (6H, t, 2 CH3) showed the presence of two methyl group. A broad singlet at 1.40 (36H, brs, 18 CH2) indicated the presence of eighteen methylene groups. Signal at 2.26 (2H, q, CH2CO) showed the presence of one methylene group at C-2 position. The 'J' value of the vinylic protons strongly suggested the trans configuration of the double bond. A four proton multiplet appearing at 2.04 (4H, m, -H2CCH(OH)CH2-) was attributed to the two methylene units attached to the carbinolic carbon. Multiplet at 3.60 (1H, bm, -CHOH, unresolved) was due to the proton of a methine group bearing the hydroxy group. Hydroxy proton resonated as a singlet at 1.67 ppm.

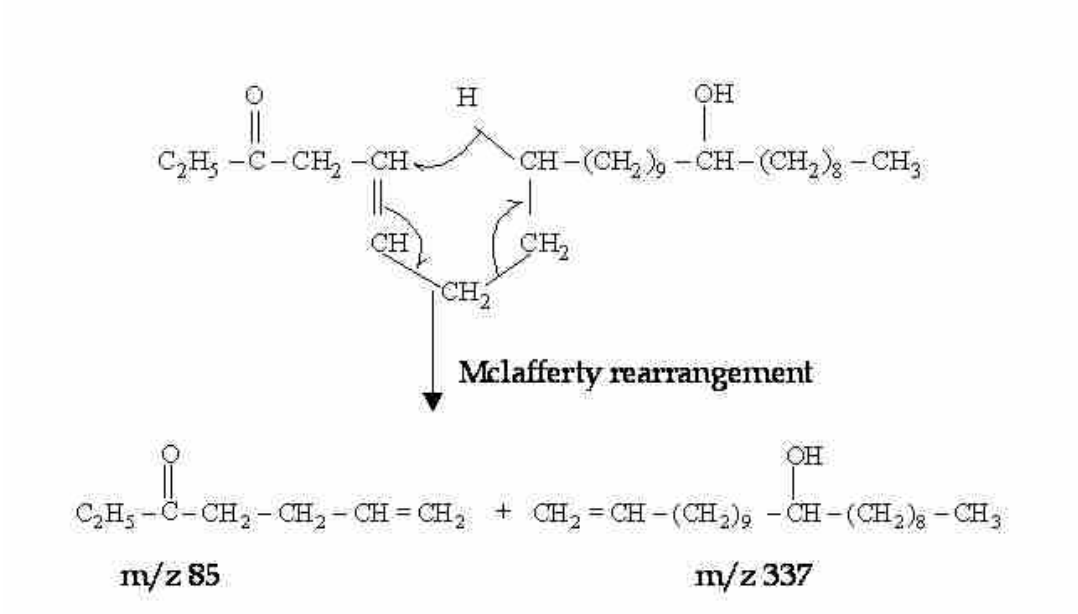
All the above observation indicated the compound to be an unsaturated aliphatic hydroxy ketone having 28 carbon atoms.
The mass spectrum of the compound exhibited a molecular ion peak, at m/z 422. It is commonly observed that major fragmentation peaks arising from aliphatic ketones results by the cleavage at >C=O group with charge retention at the oxygenated fragment as shown:

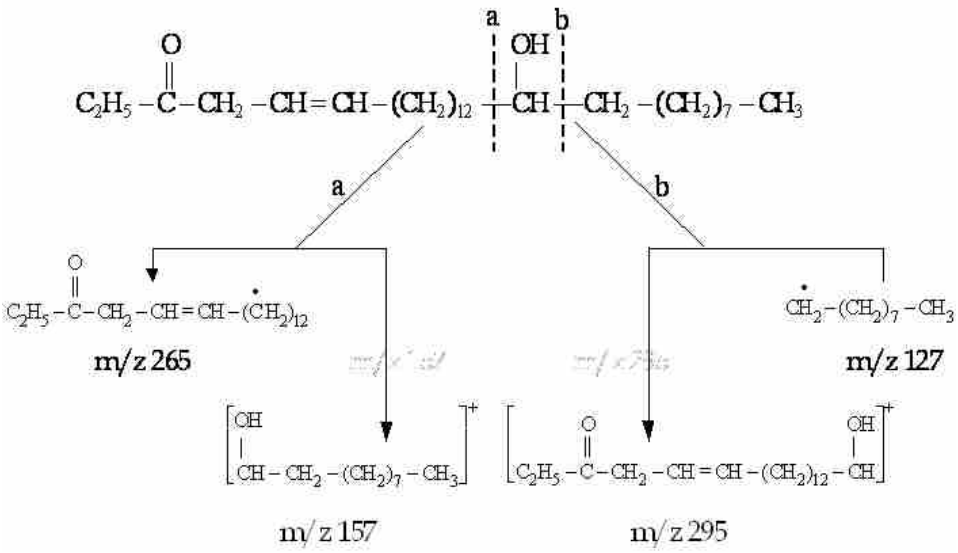


The straight chain nature was confirmed by the absence of [M+15] ion and the presence of [M+1]+ ion peak suggested the unsymmetrical nature of the compound11. The location of >C=O was found to be at C3, from prominent fission ions at m/z 393, 365, 57, 29 and fission ions at 351 and 71. The mass spectral peaks at m/z 295, 265, 157 and 127 showed the presence of hydroxy group at C-19.Thus, the compound 2 was characterized as 19-hydroxy-octacos-5-ene-3-one.

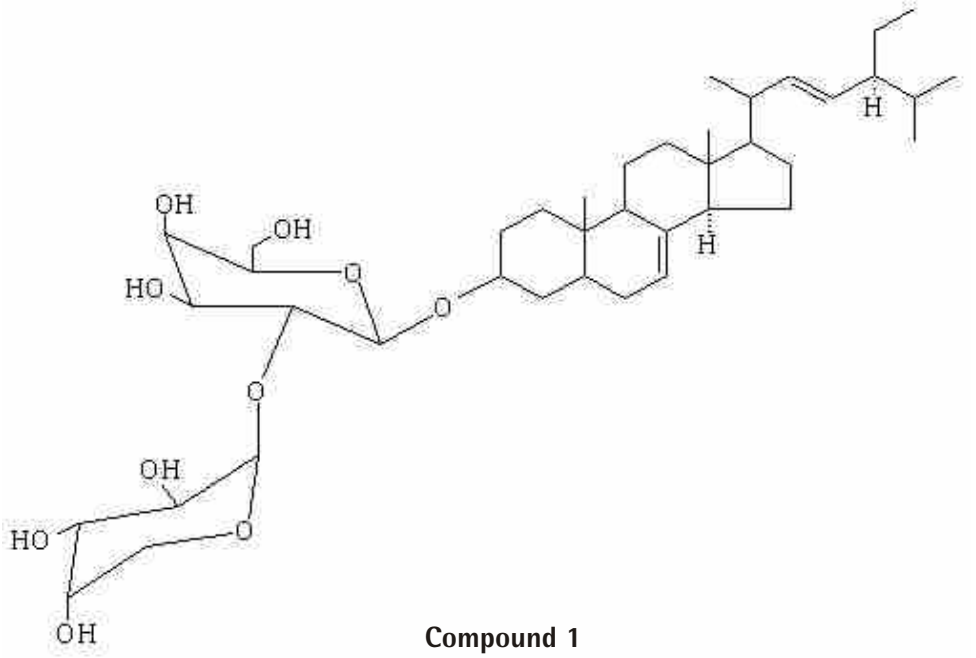


Mass fragmentation of the Compound 1

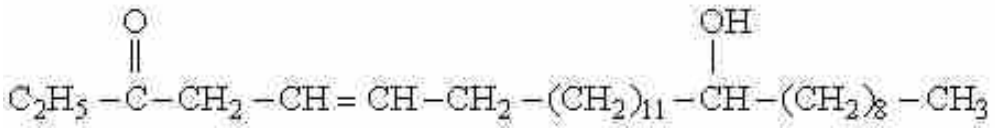




Mass fragmentation of the Compound 2



Compound 1



Compound 2

C NMR(?) values of glycoside Comp -1 and its Aglycone.		
Position Assigned	Glycoside Chemical Shift (ppm)	Aglycone Chemical Shift (ppm)
C-1	35.60 (t)	35.60 (t)
C-2	36.20 (t)	36.20 (t)
C-3	58.23 (d)	49.81 (d)
C-4	34.88 (t)	34.90 (t)
C-5	30.85 (d)	30.83 (d)
C-6	28.30 (t)	28.30 (t)
C-7	136.00 (d)	136.00 (d)
C-8	125.26 (s)	125.25 (s)
C-9	31.82 (d)	31.82 (d)
C-10	52.00 (s)	52.00 (s)
C-11	21.96 (t)	21.96 (t)
C-12	39.59 (t)	39.59 (t)
C-13	42.24 (s)	42.24 (s)
C-14	55.00 (d)	55.00 (d)
C-15	24.16 (t)	24.15 (t)
C-16	28.82 (d)	28.82 (t)
C-17	55.97 (d)	55.95 (d)
C-18	13.40 (q)	13.40 (q)
C-19	17.85 (q)	17.85 (q)
C-20	40.45 (d)	40.45 (d)
C-21	22.65 (q)	22.65 (q)
C-22	131.80 (d)	131.80 (d)
C-23	135.46 (d)	135.46 (d)
C-24	52.29 (d)	52.29 (d)
C-25	31.85 (d)	31.85 (d)
C-26	21.13 (q)	21.12 (q)
C-27	20.00 (q)	20.00 (q)
C-28	26.35 (t)	26.35 (t)
C-29	14.30 (q)	14.30 (q)
C-1'	98.20 (d)	
C-2'	79.80 (d)	
C-3'	71.90 (d)	
C-4'	67.30 (d)	
C-5'	74.60 (d)	
C-6'	60.80 (t)	
C-1''	103.80 (d)	
C-2''	71.60 (d)	
C-3''	75.20 (d)	
C-4''	70.30 (d)	
C-5''	67.20 (t)	

Conclusion

The structures of these compounds have been elucidated by IR, MS and NMR spectral analysis. However to our knowledge from the survey of literature stigma7, 22-diene-3-O-[- -Larabinopyranosyl (1 2)]- -D-galactopyranoside and 19-hydroxy-octacos-5-ene-3-one were previously unknown from Samanea saman.

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Phytochemical Screening of *Bergenia ligulata* Wall

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Abstract
Bergenia ligulata Wall is an important medicinal plant used for treatment of a number of ailments like urinary disorders and cardiac problems. The roots of *Bergenia ligulata* Wall were extracted with different organic solvents in increasing order of polarity. Preliminary phytochemical investigation revealed the presence of phytoconstituents like alkaloids, steroids, flavonoids, terpenoids, tannins, glycosides, carbohydrates and saponins. The successive acetone extract was used for isolation of bergenin derivative. The chemical structures of the isolated pure compound was established by chromatographic & spectroscopic techniques such as UV, IR, NMR & Mass spectroscopy.

Keywords: *Bergenia ligulata* Wall, Acetone extract, Phytochemical and Spectroscopy.

Introduction

Bergenia ligulata Wall is a perennial herb belongs to family Saxifragaceae. This family comprises of 30 genera and 580 species, mostly distributed in South East Asia. In India Bergenia ligulata Wall grows at high altitudes between 1800-5100 meters in Himalayas usually in rocky areas and cliffs. It is popularly known as Pashanbheda in Indian system of medicine.

The rhizomes of Bergenia ligulata Wall are compact, solid somewhat cylindrical. The outer surface is wrinkled furrowed, ridged and is covered with root scars.

Bergenia ligulata Wall is used in different diseases. It is a key tonic for urinary complaints, kidney stones, astringent, bitter, purifies the urinary bladder, cold, hemorrhagic disease, distension of stomach and epilepsy1-2. Bergenia species are used as antilithic, for boils and blisters, in urinary diseases, as antidiabetic, hemorrhoids, stomach disorders and in opthalmia 3-4. The rhizomes of Bergenia species have been traditionally used for treatment of diarrhea, vomiting, cough, pulmonary infection, menorrhagia, excessive uterine hemorrhage, kidney stone and ulcer of large intestine. In addition they have been applied externally for healing wounds, eyesores and boils. Their alcoholic extracts have been shown significant analgesic, anti-inflammatory, diuretic and antibacterial activities 5-7.

The main chemical constituents identified of the plant Bergenia ligulata Wall are bergenin, -sitosterol, -sitosterol-D-glucoside, leucocyanidin, gallic acid, methyl gallate, catechin & catechin from Bergenia rhizomes that has pronounced lipase inhibiting and antioxidative properties 8 -11.

Materials and Methods

The plants of Bergenia ligulata Wall were collected from hilly areas of Dehradun,

Uttarakhand, authenticated by Dr. Santosh Kumar Agarwal, Prof. & Head, P.G Department of Botany, D.B.S (PG) College, Dehradun, Uttarakhand. The roots, rhizomes & leaves of Bergenia ligulata Wall were dried in shade and subjected to reduction to coarse powder using grinding mill. The coarse powder of whole plant was extracted in hot water and then filtered to give the aqueous extract. The coarse powder of roots, rhizomes & leaves were extracted with ethanol (95%) in soxhlet extractor. The coarse powder of roots was extracted successively with different organic solvents in increasing order of polarity (i.e. Petroleum ether, Diethyl ether, Chloroform, Acetone and Ethanol) in soxhlet extractor. The crude extracts were evaporated to dryness in rota evaporator under low temperature and reduced pressure. The yield of different extracts obtained as semisolid mass were subjected to preliminary phytochemical screening by using standard procedure 12-16.

All the chemicals and solvents used were of analytical reagent grade. The eluted pure compound was confirmed by thin layer chromatography. Melting point was determined by an electrochemical micro melting point apparatus. The structure of the isolated compound was established by chemical, chromatographic and spectroscopic analysis. Spectral analysis was carried out at IIT, Roorke & RSIC, Panjab University, Chandigarh The UV spectrum was recorded on ELICO SL-160, India. Infra Red spectrum was recorded in KBr disc on Jasco FTIR and PKIN ELMER FTIR-RX1 Spectrophotometer. NMR spectra were recorded on CPD32 Bruker and BRUKER ADVANCE II in MeOD & DMSO solvents respectively. Mass spectra were recorded on a Waters Q-T of micro MS.

Isolation and Characterization

Acetone extract (25g) of the roots of Bergenia ligulata Wall was subjected to extensive column chromatography over silica gel (60-120 mesh) eluted with solvent Chloroform: Methanol (different ratio). The elution fraction of the column (Chloroform: Methanol, 9.4:0.6) resulted compound (BL-2). Further recrystallized with methanol as light yellowish white crystalline powder (150mg), m.p 190 oC. The compound was identified as 4- methoxy, 7-hydroxy, 11(4'-hydroxy, 5'-methoxy, benzoyl) Bergenin.

Spectral Data of the Isolated Compound

UV (EtOH)λ_{max}: 255 nm.

IR (KBr) $_{max}$ cm-1: 3375, 2922, 1694 & 1608 cm-1.

ES-MS, m/z (rel. intensity %), 508[M]+ ,(10), (Calculated C23H24O13, 508), 360 (100) & 202 (58).

1H-NMR (DMSO 400 MHz) : 7.93 (d, 1H-3'), 7.44 (s, H-4'), 7.20 (s, 1H-5') 6.88(d, H-2'), 4.94(t, 1H-10a), 4.40 (d, d, 4-OH), 4.18 (t, H-4a), 3.60 (t, H-3), 3.94 (3-OMe).

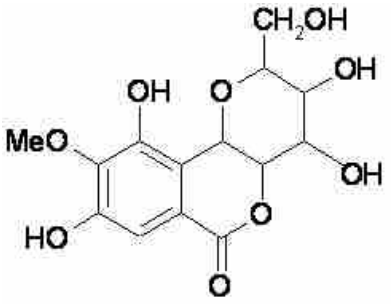
13C-NMR (DMSO, 400 MHz) : 150.7(C-9), 147.5(C-7), 131.3(C-2'), 114.9 (C-4'), 110.0 (C-5'), 79.1(C-4), 77.6 (C-2), 73.7(C-4a), 70.26 (C-11), 59.9 (OMe), 161.7 (C=0).

Results and Discussion

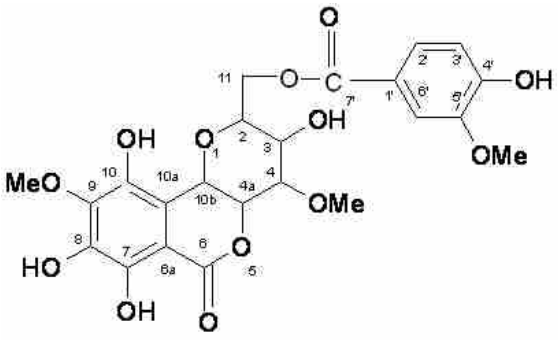
Present study is based on phytochemical screening of Bergenia ligulata Wall that is also known as Pashanbheda. Shade dried coarse powder of plant material was extracted with different organic solvent. The yield of different extract obtained as semisolid mass is shown in (Table 1). Phytochemical screening of various extracts revealed the presence of Alkaloids, Glycosides, Steroids, Carbohydrates, Flavonoids

and Tannins in this plant (Table 2).

The isolated compound shows max at 255 nm indicating the presence of carbonyl group. The IR spectra of the same compound indicated the presence of signals at 3375 cm-1 (hydroxyl), 2922 cm-1 (broad C-H , alkane), 1694 cm-1 (carbonyl) & 1608 cm-1 (aromatic) moieties. The 1H-NMR spectra of compound revealed the presence of signal at 9.75 ppm (R-CHO proton), 6.86-8.70 ppm (aromatic protons), 3.55-4.18 (HC-OR & HC-OH proton) & 0.88 ppm (RCH3 proton). The compound was assigned M.F C23H24O13 by mass measurement studies showed molecular ion peak at m/z 508(M+). Further fragmentation of the compound showed characteristic ion peak at m/z 360 & 202, these are fragments of basic bergenin moieties. The 13C spectra of the above compound revealed the presence of signals at 165.7 ppm (Carbon of carboxyl), 110.9 ppm (Carbon of benzene) & 59.9 ppm (Carbon of methoxy). The chemical structure of isolated compound was confirmed by comparing peak to peak spectroscopic data of earlier reported known compound, Bergenin from the same plant 17-19 . On the basis of the above observation isolated compound was finally identified as 4- methoxy, 7- hydroxy, 11(4'-hydroxy, 5'-methoxy, benzoyl) Bergenin.



Bergenin



4- methoxy, 7- hydroxy, 11(4'-hydroxy, 5'-methoxy, benzoyl) Bergenin

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Table 1 - Showing yield of different extract of Bergenia ligulata Wall.

S.No.	Extract	Yield (% w/w)
01	Aqueous extract of whole plant.	09.21
02	Ethanol extract of leaves.	11.02
03	Ethanol extract of rhizomes.	15.12
04	Ethanol extract of roots.	14.36
05	Successive extract of roots	
(a)	Petroleum ether extract.	0.636
(b)	Diethyl ether extract.	2.026
(c)	Chloroform extract.	0.214
(d)	Acetone extract.	17.792
(e)	Ethanol extract.	12.538

Table 2 Phytochemical screening of different extract of Bergenia ligulata Wall.

S.No.	Different Extract of Bergenia ligulata Wall	Steroids	Terpenoids	Carbohydrates	Alkaloids	Glycosides	Flavonoids	Tannins
01	Aqueous extract of whole plant.	-	-	+	-	+	-	-
02	Ethanol extract of leaves.	+	-	-	+	-	-	-
03	Ethanol extract of rhizomes.	-	-	-	+	-	+	+
04	Ethanol extract of roots.	-	-	+	+	+	+	-
05	Successive extract of roots							
(a)	Petroleum ether extract of roots.	+	+	-	-	-	-	-
(b)	Diethyl ether extract of roots.	+	-	-	-	-	-	-
(c)	Chloroform extract of roots.	-	-	-	+	-	-	+
(d)	Acetone extract of roots.	-	-	-	-	+	+	+
(e)	Ethanol extract of roots.	-	-	+	+	+	+	-

Phytochemical Analysis for the Comparison of Pure Honey with the Nector (Natural Plant Sweetner) Present in Flowers

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Abstract

Honey is the natural sweet substance produced by honeybees from the nector of flowers or from secretions coming from living organisms feeding on plants, that bees gather, transform and combine with specific ingredients, store and leave to ripen in the combs of the hive.'

Honey known as madhu in ayurvedic scriptures is one of the most important medicines used in ayurveda. In ayurveda, honey is used for both internal and external applications. It is mainly used for the treatment of eye diseases, cough, thirst, phlegm, hiccups, blood in vomit, leprosy, diabetes, obesity, worm infestation, vomiting, asthma, diarrhoea and healing wounds. It is also used as a natural preservative and sweetener in many ayurvedic preparations. It is also used as a vehicle along with some medicines to improve its efficacy or to mitigate the side effects of the other medicines it is mixed with. It is also known to mitigate the increased kapha dosha Eight types of honey are described in ayurveda depending on the type of bee which collects it. They are Pouttika, Bhramara, Kshoudra, Makshika, Chatra, Arghya, Oudalaka, Dala. Amongst all the above "Makshika" is considered as the best type with immense medicinal properties.

Ayurveda explains another special quality of honey. Honey is called as "Yogavahi". The substance which has a quality of penetrating the deepest tissue is called as Yogavahi. When honey is used with other herbal preparations it enhances the medicinal qualities of those preparations and also helps them to reach the deeper tissues.

It contains Sugars like fructose, glucose, sucrose, maltose, lactose and other disaccharides and trisaccharides, Proteins, fats, vitamins, minerals, enzymes and amino acids,, Volatile aromatic substances. Ashes and water etc.

Nector is a sugar-rich liquid produced by plants. Although its main ingredient is natural sugar (i.e., sucrose, glucose, and fructose), nectar is a brew of many chemicals. All twenty of the normal amino acids found in protein have been identified in various nectars, with alanine, arginine, serine, proline, glycine, isoleucine, threonine, and valine being the most prevalent. Other substances reported in nectar include organic acids, terpenes, alkaloids, flavonoids, glycosides, vitamins, phenolics, and oils.

Keeping in view of the richness of naturally occurring chemicals in plant nector and honey and their therapeutic importance the present study is undertaken to compare the honey with natural plant sweeteners through phytochemical analysis.

Key words: Antimicrobial, anti-inflammatory, Honey, Phytochemical analysis

Introduction

Honey known as madhu in ayurvedic scriptures is one of the most important medicines used in ayurveda. In ayurveda, honey is used for both internal and external applications. It is mainly used for the treatment of eye diseases, cough, thirst, phlegm, hiccups, blood in vomit, leprosy, diabetes, obesity, worm infestation, vomiting, asthma, diarrhoea and healing wounds. It is also used as a natural preservative and sweetener in many ayurvedic preparations. It is also used as a vehicle along with some medicines to improve its efficacy or to mitigate the side effects of the other medicines it is mixed with. It is also known to mitigate the increased kapha dosha. (Kapha dosha is the ayurvedic category for body constitutions- those with kapha dosha are of larger proportions with robust frame.) It should also be kept in mind that fresh honey helps to increase body mass while old honey produces constipation and decreases body mass. Honey should not be heated or consumed warm as it causes toxic effect. Cold honey should always be preferred.

In ayurveda, honey is know by many names. The names differ from one region to the other. However, the most common names are madhu, makshika, madwikam, kshaudram, saradham, vantham, varadi, bringavantham and pushparasolbhavam.

Honey is hygroscopic in nature, with a pH of 3.24.5. It prevents colonization and bacterial growth in tissues due to this acidic nature. Most microorganisms do not grow in pure honey because of its low water activity (aw) of 0.6. Honey also has antibacterial properties. The presence of hydrogen peroxide and a high osmotic pressure also contribute to the antibacterial effect of honey.

The contents of Honey are Sugars like fructose, glucose, sucrose, maltose, lactose and other disaccharides and trisaccharides, Proteins, fats, vitamins, minerals, enzymes and amino acids,, Volatile aromatic substances.Ashes and water etc.

Various ingredients of honey have helped it to become not only a sweet liquid but also a natural product with high nutritional and medicinal value.

The medicinal quality, taste, texture, color, aroma of honey differs according to the geographical area and the species of plants from which it has been collected.

Nectar is the sweet fluid that many dicotyledenous plants use to attract insect pollinators. The composition of nectar varies from plant species to plant species,

Keeping in view of the richness of naturally occurring chemicals in plant nector and honey, their purity and therapeutic importance the present study is undertaken to compare the honey with natural plant sweeteners through phytochemical analysis.

2.0. Material and Methods:

Four samples of of honey were procured from the market in order to determine their purity and comparison with nector (natural sweetener).

1.0. Test for purity of Honey

1.1. Pure honey pours down in the shape of a serpent whereas the impure honey falls flat on

the plate. Apply a little honey on a stick and light it on fire, if it starts burning consider it to be pure.

1.2. Pure honey is transparent.
A dog will not lick it. Pure honey has a good smell. It melts in heat and frosts in cold season.

1.3.The dissolving test.
Get a glass of water and a tablespoon of honey are all you need for the first test.

Empty the honey into the water. If the honey is impure, it will dissolve in the water - the most common additive to honey is syrup of jaggery, which dissolves. If it is pure, the honey will stick together and sink as a solid lump to the bottom of the glass.

This test can also be completed by mixing equal parts honey and methylated spirits (denatured alcohol). Pure honey will settle to the bottom. Impure honey is more likely to remain dissolved and make the solution milky.

1.4.The flame test.
Get a lighter and a candle with a cotton wick. This test is better if you don't have as much honey to spare.

Dip the cotton wick of the candle into a bit of the honey and shake off the excess.

Attempt to light the wick. If it burns, then it is completely pure honey. If it refuses to burn, then the presence of water is not allowing the wick to burn. (If there is only a very small amount of honey on the wick, though, it might still burn. It will produce a crackling sound, and it would be best to blow out the wick and try it again this time using more honey.)

1.5.The absorption test
Pour a few drops of honey on blotting paper and observe whether or not it is absorbed. If it's absorbed, the honey's not pure. If you don't have blotting paper, pour a little bit of honey on a white cloth, then wash the cloth. If there is any stain left by the honey, it is probably not pure.

2.3. Phytochemical analysis

2.3.1. Chromatographic analysis (HPTLC ANALYSIS)

2.3.2. Sample Preparation
Honey samples are homogenized and approx.1g is weighted in a measuring flask and water is added at 10 ml.

2.3.3. Sample application
Bandwise with TLC sampler4 (ATS4), track distance 7.5 mm, distance from lower edge 8mm,application volume 1-12µl,24 tracks.

2.3.4. Chromatography
Automatic developing chamber with 10 ml ethyl acetate, migration distance 50 mm, drying time 5 min.

3.0. Results and Discussion
The present study was carried out so as to test purity of honey using prescribed scientific procedures as well as the comparison of honey with nector (natural sweetner).

All the samples of honey used in this study was found pure and complies all the test for purity and a natural sweetner is also procured from

the local supplier.

The pure honey samples were compared with natural sweetner through HPTLC, a band was found common in all the five test samples revealed the presence of phytochemical present in the honey as well as in the honey as shown in (Figure-1).

In HPTLC densitogramthe HMF zones (hRf 82) were clearly separated from the various matrix compounds of the honey samples indicating the fresh honey samples.

Matching of the spectra of honey with corresponding fraction of natural sweetner at Rf=0.82 and =200 nm,to ensure that they are the same substances may be HMF (Hydroxy methyl furfural) as shown in (Figure-2)

Honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. The definition of honey depends upon who defines it. Most people think of honey as excellent food, but some others consider it an elixir, and still others as medicine. Essentially, honey is an invert sugar (a mixture of glucose and fructose) dissolved in 14 to 20% water with minor amounts of organic acids, along with traces of minerals and vitamins.

Honey is derived from the nectar of flowering plants which the honey bee collects. Nectar consists primarily of 10 to 50% sucrose, glucose, and fructose and 50 to 90% water. The source of honey determines many of the attributes of honey such as aroma, flavor, color and composition.

4.0. References

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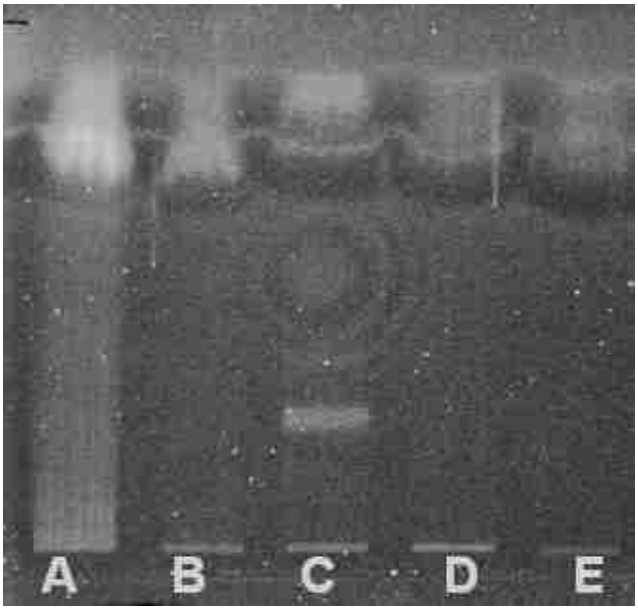
FIGURE 1



@ 366nm

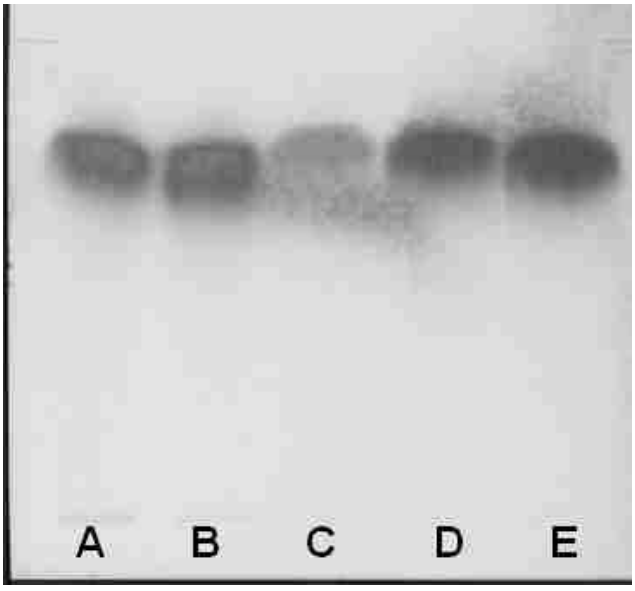
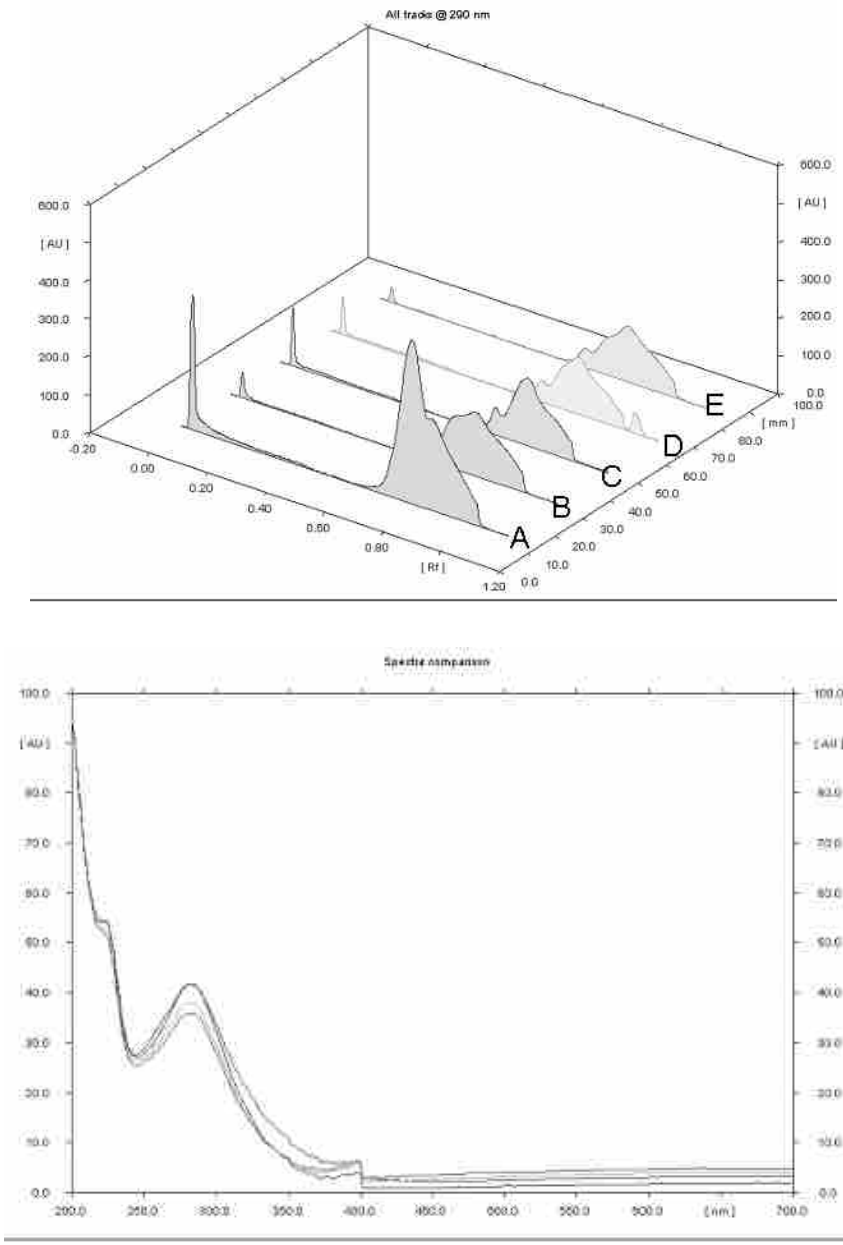


Figure-1- After Derivatization

A HONEY SAMPLE-1
B HONEY SAMPLE-2
C NATURAL SWEETNER
D HONEY SAMPLE-3
E HONEY SAMPLE-4

FIGURE 2



Matching the spectra of HONEY with the corresponding fraction of NATURAL SWEETNER at Rf=0.82 and =200nm, to ensure that they are the same substances may be HMF (5-hydroxymethylfurfural).

Antibacterial Activity of Acetone Extract of *Helicteres isora.L* (fruit)

Dinesh kumar, Dehradun, Uttrakhand, India

Abstract

Helicteres isora L. (Family : Sterculiaceae) distributed widely in forests and hills throughout india and commonly known as East Indian Screw tree, is a medicinally important sub-deciduous shrub or a small tree. Almost all parts of the plant are used in traditional medicinal system for curing various diseases. Water extract of the fruits show anti-HIV activity and reported to possess anti-spasmodic activity. The roots showed significant hypolipidaemic and hypoglycemic activity while bark has shown significant hypoglycemic activity. Hence, the present study was undertaken to investigate the ability of acetone extract of shade dried fruit of H.isora to fight against drug resistant bacteria . The Hexane : actone (92:8,v/v) fraction of the column chromatography exhibited the optimum plasmid curing activity and hence was taken for further studies.

Key words: Antibacterial activity-antibiotic resistance- *Helicteres isora L* fruit-Drug resistant

Introduction:

Helicteres isora L. (Family : Sterculiaceae) distributed widely in forests and hills throughout India and commonly known as East Indian Screw tree, is a medicinally important sub-deciduous shrub or a small tree. Almost all parts of the plant are used in traditional medicinal system for curing various diseases. Water extract of the fruits show anti-HIV activity and reported to possess anti-spasmodic activity. The roots showed significant hypolipidaemic and hypoglycemic activity while bark has shown significant hypoglycemic activity.

The emergence of multiple drug resistant (MDR) bacteria to commonly used antibiotics is a severe health problem and major challenge to the global drug discovery programmes.(Alanis.AJ;2005).The problem of explosive escalation of antimicrobial resistance has only been worsened by steady decrease of the number of new antibiotics introduced in the last 10 to 15 years (Coates A & Bax; 2002).After the discovery of quinolones, only one class of antibiotics, the oxazolidinones has been introduced to the market(Clemett D;2000).All the other antibacterial agents entered the market during this period were structural modification of existing molecule. The need for new antibiotics is more pressing than ever (Shriram, et al;2008). Most of the genetic determinants that confer resistance to antibiotics are located on plasmids. These extra chromosomal DNA sequences are often transferable to other bacteria in the environment and can be responsible for the emergence of multiple resistances to antibiotics (Schelz Z;2006).The present study was planned

to investigate the antibacterial activity of acetone extract of the fruits of H.isora against the bacterial strains S.aureus and E.coli, Enterococcus.faecalis and Bacillus cereus.

Material and method

Plant material :

The fruits of H.isora were collected from forests in Himalayan region, uttrakhand state; India and sample were authenticated by botanical survey of India, Dehradun

Plant Extraction:

Shade dried fruits of H. Israel were finely powdered with auto mix blender .250g dry powder of fruits was soaked in 1 litre acetone. The crude acetone extract was prepared by cold percolation for 12h at room temp (26 C).The filtrate was concentrated in vacuo at 40 C. The recovered solvent was reused for further extraction. This process was repeated three times to get total acetone extract. Last traces of the solvent of the total acetone extract were removed under vacuum to get the crude extract as a dark green coloured semi solid (gel like) residue of 10.65g (1.06%).

Bacterial strains:

Bacterial strains S.aureus and E.coli were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Determination of resistance to antibiotics:

Antibiotic resistance was determined by disc diffusion method.(Bauer AW at al;1966). : The antibiotic disc were placed on brain heart infusion agar plate on which test bacterial cultures were spread .The plates were incubated at 6*C for 2h to allow pre diffusion of antibiotics and then at 37*C for 24h after which the zone of inhibition around the antibiotic disc was measured. The cultures were then assessed as resistant, intermediate sensitive according to the interpretation table provided by the manufacturer (Don Whitely Scientific Equipments/ Hi media, Mumbai, India).

Determination of minimal inhibitory concentration (MIC) and sub-inhibitory concentration (SIC);

The MICs was determined by agar dilution method brain heart infusion medium was supplemented with specified concentration of antibiotic. Test bacterial cultures were inoculated (105 cells per spot) on these plates and incubated at 37 C for 24h. The lowest concentration of antibiotic that inhibited the growth was termed the MIC. The highest concentration of antibiotics that allowed the growth of bacteria was considered as SIC.

Table II. Curing of antibiotics resistance by *H. isora*

Bacterial strain	MIC (µg/ml)	SIC (µg/ml)	% Curing efficiency Mean ± SE (n=3)	AB.cured
Enterococcus faecalis (VRE)	>800	800	14 ± 1.0	Vancomycin, Ampicillin
Staphylococcus aureus (VRSA)	800	400	ND	--
Escherichia coli	>800	800	26 ± 1.5	Gentamycin
Bacillus cereus	>800	800	22 ± 0.9	Kanamycin
Salmonella Typhi	800	400	ND	--
Shigella sonei	>800	800	ND	--
Pseudomonas aeruginosa	>800	800	ND	--
Bacillus subtilis (pUB110)	>800	800	ND	-
Escherichia coli (pUC18)	800	400	ND	--
Escherichia coli (RP4)	800	400	2 ± 0.4	Ampicillin, Kanamycin, Tertracyclin
Escherichia coli (R751)	>800	800	ND	--

None of the 100 colonies tested showed phenotypic loss of antibiotic resistance.
MIC, minimal inhibitory concentration; SIC, sub inhibitory concentration; AB, resistant to particular antibiotics

Results & Discussions

The crude acetone extract showed antibacterial and antiplasmid activity. the same fraction could cure plasmids from Enterococcus faecalis, Escherichia coli, Bacillus cereus and E. coli (RP4) at curing efficiencies of 14, 26, 22 and 2 per cent respectively. The active fraction mediated plasmid curing resulted in the subsequent loss of antibiotic resistance encoded in the plasmids as revealed by antibiotic resistance profile of cured strains. The physical loss of plasmid was also confirmed by agarose gel electrophoresis.

The comparison of antibiotic resistance profile of original hosts and their cured derivatives revealed that E. coli isolates originally resistant to ciprofloxacin, cefoperazone, ceftazidime and roxithromycin became sensitive to each of these antibiotics as a result of plasmid curing. Similarly, resistance to tobramycin, ampicillin, cloxacillin and vancomycin was eliminated in the cured derivatives of E. faecalis as a result of plasmid curing. Resistance to gentamicin, tobramycin and roxithromycin was eliminated subsequent to plasmid curing in S. typhi.

Majority of the plasmid curing agents reported earlier such as acridine dyes, ethidium bromide and sodium dodecyl sulphate are unsuitable for therapeutic application due to their toxicity or mutagenic nature. Also each of the known curing agents is effective against only a limited number of plasmids in a limited number of hosts. Thus, there is a constant need of identifying novel curing agents that are more effective and non toxic. The present results have offered organic extracts of H. isora as a new and safe plasmid curing agent. These finding resulted in the possibility of a new type of combination between antibiotics and potential drugs effective against plasmid encoded multiple antibiotic resistance.

The frequency of spontaneous loss for such plasmids has been known to be less than one in

106cells. In comparison, the antibiotic resistance curing efficiencies observed in present study were extremely high (106 times higher). The antibiotic resistance may occur due to mutations. Mutagenic activity of the compound can be harmful especially in clinical applications. It is necessary to ensure that antibiotic resistance curing was due to loss of plasmid-encoded genes and not due to mutations, which was confirmed by the physical loss of plasmid observed in agarose gel electrophoresis.

Interpretation & conclusions:

The active fraction of acetone extract of H. isora fruits cured R-plasmids from Gram-positive and Gram-negative clinical isolates as well as reference strains. Such plasmid loss reversed the multiple antibiotic resistance in cured derivatives making them sensitive to low concentrations of antibiotics. Acetone fractions of H. isora may be a source to develop antiplasmid agents of natural origin to contain the development and spread of plasmid borne multiple antibiotic resistance.

Acknowledgment

I am indebted to Dr.S.Farooq, President , The Himalaya Drug Company, Dehradun, Uttrakhand for providing all necessary facilities to conduct this work.

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About Flower on Cover Page

Coleus forskohli (Makandi)

Hindi Name: Makandi, Pather Chure
Ayurvedic Name: Makandi
Botanical Name: Coleus forskohlii

Habit & Habitat
Native to India, Coleus grows on the dry slops of Indian plains and in the foot hills of the Himalaya. It also found in Nepal and Sri Lanka.

Coleus is strongly aromatic and the leaves have distinctive camphor like scent.

Key Constiruents
- Volatile oils
- Diterpenes (Forskolin)

Key Actions
Coleus (Makandi) herb is reported first time as Anti-HIV herb by NIPER (National Institute of Pharmaceutical & Research, India.) in the year 2009. The drug shot to fame in western medical circles when one of its constituents Forskolin, was first isolated in the 1970s. Now it is cultivated on a large scale in Gujarat and approximate 1000 tons is harvested each year.

The drug is used as a heart tonic, digestive, anti-inflammatory, anti-spasmodic and as a hypotensive. It is also used in Glaucoma and Bronchial Asthma.

Dr. Maya Ram

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March 20, 2012
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International Conference on Bioinformatics and Biomedical Science- ICBBS 2012- Bangkok, Thailand

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March 11, 2013
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Notes

Notes

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COUNCIL INITIATIVES FOR PROMOTING HERBAL RESEARCH IN UTTARAKHAND

Uttarakhand State Council for Science and Technology (UCOST) was established in last quarter of the 2005 in Dehradun. Since its inception Council has encouraged the Research and Development activation in the State and has funded projects in various disciplines of Science and Technology. Council has provided financial assistance to peer reviewed projects and provide encouragement for researchers, scientists and for the development of trained manpower. Today the work initiated in 2005 has resulted in publications in reputed journals. I take pride in writing the references of the papers in herbal research that I have received from few of principal investigator of UCOST funded projects.

Aggarwal P et.al. (2009). Int. J. Chem. Sci., 7 (3): 1850; Univ. J. of Phyto-Chemistry and Ayurvedic Heights, 2:13-16; Mishra J et.al. (2010). Int. J. of Intgr. Biol., 9 (1) : 26; Singh R. D. et.al. (2010). India, Int. J. of Biol. and Biochem., 6(6):901 910; Sharma V et.al. (2010). Asian J. of Microsoft Biotech Env. Sc., 12(3):677-680; Srivastava N et.al. (2010). J. of Plant Sci., 5(4):414-419; Int. J. of Green Pharm., 4(4):220-228; Singh P et.al. (2010). Biol. T. Elem. Res., 138:293-299; Negi J. S. et.al. (2009). Biol. T. Elem. Res., 133:350-356; 135;27-282; Pharm. Rev., 4(8):215-220; (2010). Biol. T. Elem. Res., 136:364-371; 138:300-306: Nat. Prod. Comm., 5(6):907-910; (2011). J. of Med. Pl. Res., 5(10):1900-1904; Joshi S et.al. (2009). J. of Pl. Chromat., 22(6):1-3.

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