RNI No. DEL/1998/4626

ISSN 0973-3507

Universities' Journal of Phytochemistry and Ayurvedic Heights

Vol. II No. 21 December 2016

Potentilla fulgens : Vajradanti

Hibiscus rosa sinensis : Gurhal

Anthocephalus cadamba : Cadamba

Bombax ceiba : Semal

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UJPAH | **Vol. II** | **No. 21** | **DECEMBER 2016**

Abstracted & Indexed by Indian Science Abstracts

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From Chief Editor's Desk

Why Pharma Herbal Research

India having reasonably good institutions for Pharma Industry, thus India exports to almost 200 centuries including US being the largest importer from India. But India has been importing more than double of its drugs from China and the import has reached worth billions of dollar. More than 80% of raw materials for manufacturing drugs by Indian Companies are now being imported from China, for example:

At present India is dependent on China for imports of ingredients of several essential drugs:

- Painkillers – Aspirin, paracetamol
- Anti-diabetics – Metformin
- Stomach ulcers – Ranitidine
- Anti-inflammatories – Ibuprofen
- Antibiotics – Amoxicillin, ciproxfloxacin, cefixime, ofloxacin, ampicillin, metronidazole

One of the main reasons for imports from China is the price competitiveness of these products. In order to reduce the dependence on pharma imports from China, the government is mulling to expand the API/bulk drug manufacturing facilities in India. As a part of the expansion plan, the government is willing to invest Rs. 600 crore in its three newly established bulk-drug parks.

So we lack behind in skills research and if targeting the real things though Nature has blessed us with wisdom, resources and the skill needed for the cultivation of herbs. We have to use our talent to fill this gap. The quantity of certain herbs which are available in abundance in different climates and different altitude-mountains of UK. is best and now the governments of South India are going to trading the same fix diabetes drugs are in top 10 selling lists of India. The sale estimated at 8500 crores is substantially increasing since 2013. The companies like NOVO, Morcha, USV, Pfizer, Wochard, GSK, Sanoz, Aristo, Himalaya, MSD etc. are in top 10 countries.

My sincere thanks to all those who have contributed for bringing out this issue and to all the Board Members of UJPAH, contributors of research papers, participants, all staff members, eminent scientists. May Almighty protect us all from the fury of winter.

Dr. S. Farooq
Chief Editor

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Preliminary Physicochemical and Phytochemical Evaluation of *Diploknema butyracea* (Roxb.) H.J. Lam kernels and Seed Skin

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Abstract . *Diploknema butyracea* (Roxb.) H.J. Lam is a large tree and belongs to family: Sapotaceae. Commonly, it is known as Indian butter tree or Cheura and native to Nepal. It is distributed from Garhwal Himalayas (ranging from 600-1850 m) to Sikkim and up to Bhutan. In Uttarakhand, it occurs abundantly in Pithoragarh district and adjoining areas of Almora, Bageshwar and Nainital district. *It is a multipurpose tree species with several uses as source of oil, fodder, fuel, wood, timber and medicine. It is also used as worm killer and applied over lawns and golf green for destroying earthworms.* The defatted seed flour of *D. butyracea* contains carbohydrates (45.7%), protein (27.4%) and saponins (10.4%) as major constituent. The saponins present in the defatted seed flour possess anti-inflammatory and spermicidal activities. The fatty acid composition of *D. butyracea* seed oil indicates the potential source of palmitic acid which is in great demand in the pharmaceutical industry. This article briefly assesses the preliminary phytochemical evaluation of *D. butyracea* (Roxb.) H.J. Lam. The study scientifically validates the use of plant in traditional medicine.

Keywords: *Diploknema butyracea*, Sapotaceae, Seed oil, Fatty acid composition, Palmitic acid, Phytochemical evaluation

Introduction

Diploknema butyracea (Roxb.) H.J.Lam is a fast growing species belonging to family Sapotaceae. It is also known as Indian butter tree or Cheura. In India, it is distributed from Garhwal Himalayas (ranging from 600-1850 m) to Sikkim and up to Bhutan (*Kurrel et.al. 2008).* In Uttarakhand, it occurs abundantly in Pithoragarh and adjoining areas of Almora, Bageshwar, Champavat and Nainital district *(Negi, et.al., 1988).* It is a large deciduous tree, 40-70 feet high with several uses as source of oil, fodder, fuel, wood, timber and medicine. The plant flowers during winter season and the fruits ripen in June–July. Fruits are ellipsoid (0.8-1.8 cm) and each encloses 1-3 black seeds (0.8 g in weight) and almond shaped kernels (70 % of the weight of seeds) contain 60-70 % oil (Anon, 1952). Oil is used as external ointment to ease rheumatism, paralysis and sprains. The oil cake contains saponins and act as fertilizers, fish intoxicant, pesticides and detergents. The flowers are rich source of sugars and utilized for preparation of gur like products. The bark of the tree is used in the treatment of rheumatism, ulcers, itching and hemorrhage, inflammation of the tonsils, leprosy and diabetes (Sofowora 1993). It is also used as worm killer and applied over lawns and golf green for destroying earthworms. Its seeds are the source of commercial Phulwara fat (60-67 % on kernel basis) which is used in soaps and cosmetics. It is also used by local community for cooking purpose. Phulwara butter may be a potential source of palmitic acid for the pharmaceutical industry. The defatted seed flour of *D. butyracea* (Roxb.) H.J. Lam contains carbohydrates (45.7%), protein (27.4%) and saponins (10.4%) as major constituent. *Madhuca butyracea* (Roxb.) syn. *Diploknema butyracea* has been reported to contain fat, flavonoids tritrrpenoids and saponins which possess anti-inflammatory and spermicidal activities. The bark contains 17% tannins used in tanning, dyeing and as a fish poisoning (Isolation and properties of saponins from Madhuca butyracea seeds (Thaire

et.al., 1987). Looking to the versatile traditional uses of *Diploknema butyracea* (Roxb.) H.J. Lam and modern pharmacological reports, the present study was undertaken to investigate the fundamental scientific bases for the use of this plant by defining and briefly assessing the preliminary phytochemical evaluation.

Material and Methods

Collection of plant material

Seeds of *D. butyracea* (Roxb.) H.J.Lam (Indian Butter tree) was collected at two stages mainly unripe and at mature stage (figure-1). The unripe fruits were collected from Pithoragarh and its adjoining areas in the month of May while the mature fruits were collected from the same place in the Last week of June. The collected seeds were identified and authenticated by Systematic Branch, Botany Division, Forest Research Institute (FRI), Dehradun and a voucher specimen was deposited in the herbarium of the same Division, FRI.

Preparation of the sample for extraction

The unripe covering of fruits was cut into small pieces, dried and chopped for extraction. Ripped kernels and seed coat were also separated manually and coarsely powdered (figure-2).

(a) (b) (c)

Figure-1 (a) Unripe fruits of *D.butyrcea* (b) Skin of unripe fruits (c) Unripe seeds

(a) (b) (c)

Figure-2 (a) seeds (b) seed skin (c) kernels of *D. butyracea*

Preparation of extractives using solvents of elutropic series

The unripe covering of fruits was cut into small pieces, dried and chopped for extraction. Cold extraction of unripe covering of fruits was carried out with the solvents of elutropic series *viz* chloroform and methanol solvent. The ripped seeds were air dried and decorticated. Kernels and seed coat were separated manually and coarsely powdered. Coarsely powdered kernels were extracted with the solvents of elutropic series viz. petroleum ether, chloroform, and methanol by using soxhlet apparatus. After exhaustive extraction, the solvent was removed under reduced pressure and vacuum on rotaory evaporator. The yield of different extractives was determined on moisture free basis (table-1). The process was repeated four times to isolate the different extractives in sufficient quantity. Thin layer chromatography of different extractives *viz.* Petroleum ether, chloroform, methanol and distilled water were carried out in different solvent system. Qualitative and quantitative determination of different extracts was also carried out (Trease *et.al.*1989). The Physico-Chemical properties of fatty oil was evaluated by following AOAC methods (1984) (table-4).

Qualitative phytochemical analysis of kernels and seed skin

The coarsely powdered kernels of *D.butyracea* (Roxb.) H.J. Lam was subjected to qualitative evaluations for the presence and/or absence of different categories of chemical constituents (Patil *et al.,* 2001). The kernel was extracted with methanol and tested qualitatively with special reagents that produce characteristic color change with different categories of chemical constituents. All the qualitative tests were replicated three times (table-5 and 6).

Test for Alkaloids

1ml extract of the seed was treated with Dragendroff's Reagent. Development of orange red color under the influence of the reagent suggested the presence of alkaloids (Danial, 1991).

Test for Flavonoids

To the 1ml methanolic extract of the seed few drops of ferric chloride solution was added. Formation of blue to greenish violet coloration indicated the presence of flavonoids (Feigl, 1966).

Test for Phenolics and Tannins

50mg of the extract was dissolved in 5ml of distilled water. To this, few drops of 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

Test for Steroids

To the 1ml methanolic extract taken in a test tube few drops of Liebermann Burchard Reagent was added. Development of greenish color indicated the presence of steroids (Shriner *et al.,* 1964).

Test for Saponins

2g of seed powder was vigorously shakeed with 10 ml of water. Formation of soapy lather indicated the presence of saponin. The soapy liquid thus obtained failed to reduce Fehling's solution but on hydrolysis with 2N HCl it reduced Fehling's solution confirming it to be saponin (Brain and Turner, 1975).

Test for Carbohydrates

2g of powdered samples was extracted with 5ml of distilled water. To the aqueous extracts of officinal parts, 1ml of Fehling's solution was added and the content was heated up to boiling. Formation of red color confirmed the presence of carbohydrate (Shriner *et al.,* 1964).

Test for Glycosides

Extract was stirred with 1ml glacial acetic acid, after cooling, added few drops of ferric chloride solution, contents were transferred to test tube containing 1ml of concentrated sulphuric acid. After standing reddish-brown layer acquires bluish green colour indicating the presence of glycosides.

Test for Fixed Oils and Fats

A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oils.

Test for Proteins

To the aqueous extract of seed, ethyl alcohol was added and the precipitate was separated by filtration. The precipitate so obtained was washed with ethanol (95%) till it shows no positive test of amino acids with ninhydrine. The aqueous solutions of precipitates produce deep blue colouration with ninhydrine (Shriner *et al.,* 1964).

Isolation of fatty oil from kernels of *D.butyracea* (Roxb.) H.J. Lam

The shade dried, dehisced and crushed seeds (700g) were extracted with petroleum ether (60-80^{0}C) (700ml) for 4hrs in a soxhlet apparatus. The extract was dried over anhydrous sodium sulphate for half an hour. It was then filtered and freed from the solvent by using rotatory evaporator under vacuum and pressure. Transparent yellowish colored fatty oil (400g) was obtained. The yield of the oil was found to be 59.70% on moisture free basis.

Separation of mixed fatty acids and unsaponifiable matter of the oil

Fatty oil (5.0 g) was saponified by refluxing it with 50ml of N/2 alcoholic potassium hydroxide for about 2 hrs. The alcohol was distilled off completely. The soap (solid mass) so formed was diluted with 200 ml of water and the unsaponifiable matter extracted with diethyl ether (3 x 100ml) in a separating funnel. The etheral extract was washed with water to neutral and dried over anhydrous sodium sulphate. Removal of the solvent on a gently heated water bath yielded the unsaponifiable matter (0.894 g, 1.78%). The aqueous soap solution was acidified with dilute hydrochloric acid and warmed on water bath to regenerate the fatty acids. The solution was cooled and liberated acids were extracted with diethyl ether (3x100). The etheral extract was washed with water and dried over anhydrous sodium sulphate. Removal of the solvent gave a mixture of fatty acids (3.89g).

Preparation of methyl esters

The mixed fatty acids (3.89g) were esterified with methanol (30ml) in presence of concentrated sulphuric acid (0.6ml) for four hours. The methyl esters so formed were extracted with diethyl ether and the etheral layer washed three times with 25ml portions of potassium carbonate solution (5%) and then with distilled water. After dehydrating with anhydrous sodium sulphate and filtration, the solvent was removed completely to yield a mixture of methyl esters of fatty acids (1.059g).

Analysis of methyl esters by Gas Liquid Chromatography (GLC)

The analysis of fatty acid methyl esters was carried out on Chemito Gas liquid chromatography fitted with FID. The Silar column was used carrying Nitrogen gas as carrier gas (with a flow rate of 40 ml/min.) The oven temperature of injector was programmed from 160^{o}C-220^{o}C with increase in temperature 3^{o}C /min followed by a final hold up of 15 min. Methyl esters were identified by comparing the retention time of standard fatty acid methyl esters and also by their co-injection. The percentages were considered as weight percentage.

Gas Liquid Chromatography Conditions

Column: Silar; Detector: FID (240^{0}C); Column Temperature: 160-220 ^{0}C (with rise in temperature 3^{0}C/min.); Carrier Gas : Nitrogen; Flow Rate: 40ml/min. Methyl esters were identified by comparing with the retention times of standard fatty acid methyl esters and also by their co-injection. The percentages were considered as weight percentages. The results obtained are given in table-5.

Fatty oil analysis by GC-MS

To confirm the results obtained by GLC, the methyl esters were also subjected to GC-MS analysis (table-6). The conditions are as follows.

GC-MS Instrument: Agilent technologies, Column- 60.0m x 250µm, Initial temperature: 100^{0}C for 10 min., ramp: 3^{0}C/min. to 240^{0}C, hold 10min. Injector temperature: 270^0 C, Source temperature: 180^{0}C, Scan: 50 to 5000Da, Split ratio: 50:1; Split Flow: 50 ml/min., Carrier gas: Helium.

Results and Discussion

Fatty oil (59.70%) isolated from kernels of *D.butyrcea* was analyzed by GLC and GC-MS instrument. The physico-chemical characteristics viz. specific gravity (0.858), refractive index (η_D^{20}) (1.324), acid value (13.57) and saponification value (190.74) of the fatty oil were determined. GLC analysis of a mixture of methyl esters of the fatty acids prepared from the fatty oil showed the presence of four major fatty acids and were characterized as palmitic acid, stearic acid, oleic acid and linolenic acid (table-5). The identified fatty acids constituted almost 99.50% of the mixture of fatty acids obtained from the fatty oil. The fatty acid composition indicated that palmitic acid (52.64%) and oleic acid (36.80%) is the major constituent of the oil. While stearic acid) (8.52%) and linolenic acid (5.03%) are the minor constituents. The fatty oil has a consistency like ghee. It is liquid at temperature 30^{0}C. It may be due to higher content of oleic acid (36.80%).

It is reported that monounsaturated fatty acid (viz. oleic acid) is as effective as polyunsaturated fatty acid (viz. linoleic acid) in lowering plasma total and low density lipoprotein (LDL) cholesterol thereby increasing its nutritional properties. The qualitative analysis of the extracts from the seed sample of *Diploknema butyracea* (Roxb.) H.J.Lam showed the presence of phytochemical constituents such as *alkaloid, tannin,* glycosides, fixed oils, fats, flavonoids, sterols, *phenol,* terpenoids and saponins (table-2 and 3). The presence of flavonoids in the plant indicates that it may have anti- oxidant properties and free-radical scavenging (Sharma, 2006). Steroidal compounds are of importance in pharmacy as they act as sex hormones. Saponins are linked to antibacterial activity and glycosides are associated in lowering blood pressure (Winkel-Shirley, 2002). Table-3 summarizes the quantitative determination of the phytochemical constituents of the plant showing the presence of higher contents of terpenoids and flavonoids. Due to the presence of these bioactive constituents, the plant *Diploknema butyracea* (Roxb.) H.J. Lam is considered as an important medicinal plant and has been utilized in a number of ways in pharmaceutical sector. The phytochemical studies provide valuable information which may help in authenticating the genuine specimen along with the nature of phytoconstituents present in it.

Table-1 Yield (%) of *D.butyracea* (Roxb.) H.J.Lam kernels and seed skin

Solvent	Yield (%)	
	Kernels	Seed skin
Petroleum ether	59.70	4.14
Chloroform	2.98	0.91
Methanol	4.47	5.17
Aqueous	3.53	2.41

Table-2 Qualitative analysis of kernels of *D. butyracea* (Roxb.) H.J. Lam

Sr. No.	Plant Constituents	Chloroform Extract	Methanol Extract	Aqueous Extract
1.	Alkaloids	-	—	—
2.	Carbohydrates	-	+	+
3.	Glycosides	—	+	—
4.	Saponins	-	+	+
5.	Fixed oils and fats	+	-	—
6.	Proteins an amino acids	—	—	—
7.	Phenolic compounds and tannins	—	—	—
8.	Flavonoids	-	+	+
9.	Sterols	+	+	—
10.	Terpenoids	+	+	+

Table-3 Qualitative analysis of seed skin of *D. butyracea*(Roxb.) H.J.Lam

Sr. No.	Plant Constituents	Chloroform Extract	Methanol Extract	Aqueous Extract
1.	Alkaloids	—	—	—
2.	Carbohydrates	-	+	+
3.	Glycosides	—	-	—
4.	Saponins	-	+	+
5.	Fixed oils and fats	+	-	—
6.	Proteins an amino acids	—	—	—
7.	Phenolic compounds and tannins	—	—	—
8.	Flavonoids	-	+	+
9.	Sterols	+	+	—
10.	Terpenoids	+	+	+

Table-4 Physico-chemical properties of *D.butyracea* (Roxb.) H.J.Lam fatty oil

Sr. No.	Properties	Value
1.	Specific gravity	0.858
2.	Refractive index	1.324
3.	Acid Value	13.57
4.	Saponification value	190.74
5.	Iodine value	97
6.	Protein content	7.731
7.	Nitrogen content	1.237

Table-5 Characterization of fatty oil by GLC

Sr. No.	Methyl Esters of Fatty Acid	Composition (wt %)
1.	Palmitic acid (16:0)	52.64
2.	Stearic acid (18:0)	8.52
3.	Oleic acid (18:1)	36.80
4.	Linolenic acid (18:3)	5.03

Table-6 GC-MS analysis of fatty oil of kernels of *D.butyracea* (Roxb.) H.J.Lam

Sr. No.	Chemical Constituent	RT	Composition Area (%)
1.	Hexadecnoic acid	44.372	63.592
2.	9-Octadecnoic acid	49.541	16.975
3.	Octadecnoic acid	50.275	2.897
4.	6-Octadecnoic acid	54.335	0.951
5.	Unidentified	56.144	0.793
6.	Unidentified	58.095	1.457

Conclusion

Diploknema butyracea (Roxb.) H.J. Lam is found to contain flavonoids, *alkaloids*, tannins, glycosides, fixed oils fats, sterols, *phenols*, terpenoids and saponins. In plants, flavonoids can function as attractants to pollinators and seed dispersers, as antioxidants to protect plants against UV radiation, as insect feeding attractants in host species recognition, as signal molecules to facilitate nitrogen fixation, in inducible defense against bacterial and fungal attack. Tannins are widely distributed in almost all plant foods and are found effective in protecting the kidneys and show potential antiviral, antibacterial and anti-parasitic effects (Akiyma et. al., 2001; Kolodziej et. al., 2005; Seyoum et. al., 2006; Lu et. al., 2004).

Further research on this plant may help in the value addition of potent compounds which can finally be subjected to pharmacological activities and clinical trials, thus leading to opening up a new path in the use of this pant as natural product for therapeutic applications.

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Antimutagenic Potential of *Laurus nobilis* L. in *Salmonella typhimurium* Test System

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Abstract - Leaf extract of *laurus nobilis* L. was prepared in methanol and concentrated to dryness. Different concentration of the extract was tested for their antimutagenic activity using *Salmonella typhimurium* test assay. The tested concentrations of the extract showed no mutagenicity compared to standard mutagens tested (Sodium azide, NA, and Methyl methane sulphonate, MMS). The extract exhibited concentration dependent antimutagenicity. At the highest tested concentration (100 µg/plate), the methanolic extract exhibited the percent inhibition of His+ revertants from 54.22 to 75.76 against sodium azide induced mutagenicity in one or other test strains. Similarly, percent inhibition of mutagenicity (60.87 to 77.49) was also recorded against methyl methane sulphonate (MMS) at 100 µg/plate dose in Salmonella tester strains, TA 97a, TA 100, TA 102 and TA 104. However, at lower concentrations (12.5, 25 and 50 µg/plate) of the plant extract, a decrease in antimutagenic activity was recorded. The data obtained clearly indicated the presence of antimutagenic compounds in the crude extract and need further investigation on isolation of active compounds.

Keywords: *Laurus nobilis*, leaf extract, Antimutagenic activity, Mutation, *Salmonella typhimurium.*

Introduction

Research in the past has indicated that somatic cell mutations play a key role in initiation and development of various ailments including genetic disorders and cancers (Sarac et al., 2015). Numerous synthetic and natural entities are known for their DNA damaging properties which may lead to mutations and are known as mutagens. These mutagens act by modification of DNA, which can occur at single base (point mutation) or as large deletions or rearrangements of DNA (Mortelmansand Zeiger, 2000). Mechanisms involve in mutagenesis is complex, however, many mutagens and carcinogens may act through generation of reactive oxygen species (ROS) (Słoczyńska et al., 2014). Effect of these mutagenic agents can be counterbalance by variety of secondary metabolites present in plants (Horn and Vergas, 2003). These protective agents, also known as antimutagens, are capable of decreasing the frequency of chemically induced mutations and strengthen cell defenses against environmental mutagens (Kaur et al., 2010).Antimutagens of plant origin belong to various phytochemical structural groups such as flavonoids, tannins and other phenolic secondary metabolites (Musarrat et al., 2006). From cancer prevention point of view, various natural products and pure compounds have been screened for their antimutagenicity. Various biological assays are developed and used to screen antimutagenic properties of natural and chemical substances, including plant derived natural products. These assays include different test system like bacterial system (Ames test), yeast assay and also include plant and animal cell model (Musarrat et al., 2006; Słoczyńska et al., 2014). Plant derived antimutagens are considered as relatively nontoxic and also possess complementary biological activities (Aqil et al., 2008). Therefore, continuous effort in search of novel and broad spectrum antimutagen from food and medicinal plants is needed.

Laurusnobilis L. is a medicinal plant, commonly known as 'bay laurel' belonging to the *Lauraceae* family. Leaves of *L. nobilis* (Bay leaf, Tejpatta)

and fruit are widely used as traditional medicine and flavor enhancers in foods, also used in cosmetics and pharmaceutical industries (Dadalioglu and Evrendilek, 2004, Shokoohinia et al., 2014). The leaves are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, digestion and flatulence (Matsuda et al., 2002, Dall'Acqua et al., 2009).Its leaves and extracts are also used in management of high blood sugar, microbial infections and eructation problem. Various biological properties of this plant have been documented such as anti-inflammatory, anticonvulsive, antiepileptic and antioxidant properties (Speroni et al., 2011; Dias et al., 2014).Major bioactive compounds reported from bay leaf include flavonoids such as quercetin, luteolin, apigenin and myrcetin derivatives as well as flavonols (Lu et al., 2011; Dias et al., 2014). Along with 10 major flavonoid glycosides, catechin and cinnamtannin B1 were isolated from the leaves infusion of *L. nobilis (*Dall'Acqua et al., 2009). The major sesquiterpene lactones in the leaves, costunolide, and its α-methylene-γ-butyrolactone moiety were reported to be responsible for treatment of digestive disorders and also posses anti proliferative activities (Barla et al., 2007). We have investigated the antioxidant potential of leaves of *L. nobilis* by four different assays and found broad spectrum antioxidant activity (Zahin et al., 2016). However, antimutagenic activity of the plant is poorly explored.Therefore, in this study we determined the antimutagenic activity of leaf extract against direct acting mutagens in Ames *Salmonella* test assay.

Material and Methods

Bacterial strains and chemicals

Salmonella typhimurium strains TA 97a, TA 100, TA 102 and TA 104 were originally provided by Prof. B N Ames, University of California, Berkeley, USA, and maintained in our laboratory. Sodium azide (SA) was purchased from Hi-Media lab. Ltd, Mumbai, India. Methyl methane sulphonate (MMS) was purchased from Sisco Research laboratories Pvt. Ltd Mumbai. All other chemicals and media used were of analytical grade.

Plant material and preparation of extract

Authentic sample of *L. nobilis* leaves was procured from local market and further identified at department of Botany, Aligarh Muslim University. Voucher specimen have been deposited in department of Agricultural Microbiology, Aligarh Muslim University. Methanolic extract of leaves of *L. nobilis* was prepared as described earlier (Ahmad and Aqil, 2007). Briefly, 100g of powdered leaves samples were soaked in 500 ml of methanol for 5 days with intermittent shaking. At the end of the extraction, material was passed through Whatman filter No. 1 and was concentrated to dryness under vacuum on rotatory evaporator at 40°C. Known concentration of extract was reconstituted in DMSO and stored at 4C for further use.

Mutagenicity testing

The Ames Salmonella mutagenicity test (Maron and Ames, 1983) was performed to evaluate the possible mutagenicity of the extracts with some modifications (Aqil and Ahmad, 2007). Hundred µl of extract was mixed with 100 µl of bacterial culture and poured on to minimal glucose agar prepared according to procedure of Maron and Ames (1983). Histidine independent (His+) revertants colonies from minimal glucose agar plate were scored after the incubation for 48 h at 37°C. The tested concentration of plant extract for assessment of toxicity and mutagenic potential were 12.5, 25.0, 50.0 and 100.0 µg/plate dissolve in 100 µl DMSO and 100 µl DMSO as a control vehicle. Nontoxic concentration was categorized as those in which there was well developed lawn, almost similar size of colonies and no statistical difference in number of spontaneous revertants in test and control plates. Triplicate plates were taken for each concentration and whole experiment was repeated twice.

Antimutagenicity assay

The salmonella histidine point mutation assay developed by Maron and Ames (1983) was used to test antimutagenicity of extract with some modification as described and adopted earlier (Aqil et al., 2008).

The preincubation (20 min) procedure was adopted for antimutagenicity testing with the addition of 100 µl of a bacteria strain grown overnight ($1–2\times10^9$ cells/ml), different non-toxic doses of plant extract (12.5, 25.0, 50.0 and 100.0 µg/plate) to be tested and the mutagenic agent in triplicate plates. After plating in a minimal medium and incubation for 48 h at 37°C, the numbers of revertants per plate were counted.

The inhibition rate for mutagenic activity was calculated according to the formula: inhibition rate (%) = $(A – B)/A\times100$, where A is revertants in the positive control and B is revertants in the infusion sample, having subtracted the spontaneous revertants.

Statistical analysis

The results are presented as the average and standard error of three experiments with triplicate plates/ dose/ experiment. The significant differences ($P \leqslant 0.05$) between the means of revertants per plate of the samples in relation to the mutagens were calculated using the Tuckey test.

Results and Discussion

No mutagenic effect of was observed in the leaves extract of *L. nobilis* when tested with *S. typhimurium* tester strains TA 97a, TA 100, TA 102 and TA104 by preincubation method. There was no over toxicity observed and number of spontaneous revertants was identical to that of DMSO vehicle control. *Laurusnobilis*leaves extract at highest tested concentration (100.0 µg/plate) showed significant decrease in percent mutagenicity ranging from 54.22% to 75.76% against Sodium azide induced mutagenicity as observed in different strains of *Salmonella typhimurium*. Similar antimutagenic response was also observed when tested against MMS, where percent decrease of mutagenicity was ranged from 60.87% to 77.49% at 100.0 µg/plate. At concentrations below 100.0 µg/plate, decrease in antimutagenic activity was recorded against the both direct acting mutagens. Antimutagenic activity of the extract was found to be concentration dependent. Percent Inhibition rate of mutagenicity for different concentrations of the extract against direct acting mutagens is presented in Table-1.

Our findings demonstrated that the test plant is a potential source of antimutagenic compounds. We have previously demonstrated antioxidant activity of this plant and also estimated the polyphenolic content in the methanolic extracts (Zahin et al., 2016). Polyphenolic compounds are commonly associated with number of biological activities including antimutagenic activity (Horn and Vergas, 2003; Aqil et al., 2008). Presence of different sequesterene lactone, tannins, glycosides, flavonoids and other polyphenolics in the leaves of the *L.nobilis* have been reported by various workers.(Marino et al., 2004; Fang et al., 2005;Barla et al., 2007; Emam et al., 2010, Dias et al., 2014). Kaempferyl-Coumarate, a commonly occurring flavonol in acylated form was identified as animutagenic principal from *L. nobilis* leaves (Samejima et al., 1998). Further, these constituents are also known for their antioxidant activity which further complement the antimutagenic property of bay leaf (Emam et al., 2010). Mutagen deactivation and direct DNA protection by these phytochemicals may be considered as possible mechanism in inhibition of mutagenic rate, nevertheless, inhibition mechanism is often more complex and involves multiple pathways (Aqil et al., 2008).

The antimutagenic activity recorded in this plant highlights its potential role in protection against direct acting mutagens. Further, *in vitro* and animal model studies are required using S9 activation against both direct and indirect mutagens. Isolation and identification of antimutagenic principals and their efficacy needs to be evaluated.

Table-1 Effect of methanolic extract of *L. nobilis* leaves on the mutagen induced mutagenicity in *Salmonella typhimurium* tester strains

Treatment (Pre-incubation)	Dose (µg/plate)	% inhibition of His+ revertants [values are mean of 3 experiments]			
		TA 97a	**TA 100**	**TA 102**	**TA 104**
Extract+Mutagen (SA)	12.5	14.85	11.82	13.11	7.69
	25.0	30.97	31.88	29.16	27.17
	50.0	56.60	45.47	53.19	41.46
	100.0	75.29	59.75	75.76	54.22
Extract+Mutagen (MMS)	12.5	11.17	0.00	21.69	28.57
	25.0	31.89	21.14	43.33	54.76
	50.0	51.08	48.79	58.01	71.49
	100.0	61.73	60.87	73.43	77.49

Negative control (Plant extract) showed no sign of mutagenicity at the above tested concentrations. Mutagens used are Sodium azide (SA) (1.5 µg/plate) and Methyl methane sulphonate (MMS) (1.0 µg/plate).

Acknowledgement

Financial support from UGC, New Delhi through AMU, Aligarh to M.S. khan in the form of Fellowship is thankfully acknowledged.

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Isolation and Characterization of Flavonoidal Glycoside from Floral extract of *Bombex ceiba*

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A**bstract** - From the ethanolic extract of flowers of *Bombex ceiba*, a new flavonoidal glycoside (3, 6, 7 tri - hydroxy 3', 4' di methoxy flavone 5-O-β-D – rhamnopyranoside) has been isolated together with three known compounds, β-sitosterol, hydroxy anthraquinone, and salicylic acid. The structure of isolated compound was confirmed with the help of chemical and spectral analysis.

Keywords – *Bombex ceiba, Bombacaceae,* flavonoidal glycoside

Introduction

The plant *Bombex ceiba* belongs to the family *ceiba.* It is an deciduous tree, abundant found throughout India as avenue tree or wild in China and Malaysia. Most of the plants of this genus are medicinal and economically important. The developing bud of *Bombex ceiba* is an important vegetable of Garhwal hills. Decoction of dried flowers is given in fever, particularly in malaria. Gum collected from the stem of *Bombex ceiba* are commonly used in abdominal pains, aphrodisiac and digestive disorders .The fibers of the seeds (Kapok) are commercially used for stuffing cushions and pillows. Wood is used for boat and matchsticks.

Material and Methods

Mps. Uncorrected, Column chromatography was carried out on silica gel (60-180) mesh., Merck, eluting solvent ($CHCl_3$: MeOH). U.V. was taken in MeOH. ^1H – NMR spectra were taken using TMS as internal standard and $CDCl_3$ and CD_3OD as solvents, all the signals are expressed as values downfield from TMS.

Collection of plant material

The flowers of *Bombex ceiba* was collected from Badkot Chauras, District Tehri Garhwal, (UK.). The identity of the plant was confirmed by Taxonomists, Department of Botany, H.N.B. Garhwal University, Srinagar Garhwal (U.K.) and the voucher specimen is available in the herbarium of Plant Identification Laboratory of Botany department.

Extraction and Isolation

The air-dried and coarsely powered flowers of the plant were defatted with light petroleum in a soxhlet. The defatted mass was exhaustively extracted repeatedly with 90% aqueous EtOH until the extractive became colorless. All the extracts were mixed and concentrated under reduced pressure using vacuum evaporator.

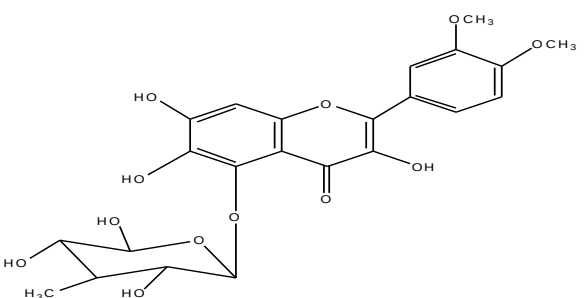
The concentrated extract was adsorbed on silica gel and fractionated through column chromatography using the solvent system, chloroform: methanol (97: 3). The polarity of solvent was gradually increased by addition of methanol. Repeated column chromatography afforded compounds 1 together with β-sitosterol, hydroxy anthraquinone, and salicylic acid (confirmed with TLC of compounds with authentic samples and m.m.p.).

Results and Discussion

Compound 1

It was obtained as pale yellow amorphous powder from chloroform-methanol. Compound 1 gave positive test for flavonoids. UV absorption maximum of compound 1 at 258 and 381 nm were typical of 3 hydroxy substituted flavones. It showed shift of bands with sodium methoxide suggesting the presence of free hydroxyl group in molecule. A shift of bands with sodium acetate solution indicated the location of free hydroxyl group at C-7. Absence of any shifts of band with $AlCl_3$

supported the existence of 4' methoxy group. IR spectrum of compound 1 exhibited characteristic absorption band of hydroxyl groups (3432, 3314 and 3127 cm^{-1}) and carbonyl group at 1670 cm^{-1}. APCIMS spectrum of compound 1 showed a molecular ion peak at m/z 492$[M]^+$ consistent with molecular formula $C_{23}H_{24}O_{12}$, confirmed by mass fragmentation peak at m/z 495$[M+3H]^+$. The generation of important ion peak at m/z 349 $[M+3H-146]^+$ arose by loss of hexose sugar from the molecule. The other prominent ion peak arose at m/z 517$[M+2H+Na]^+$, 533 $[M+2H+K]^+$. The ^1H NMR spectrum of compound showed two one-proton doublets at δ 7.26 (J=9.0 Hz) and δ 7.14 (J=2.4 Hz) assigned to meta coupled H-2' and ortho coupled H-5' respectively. A one proton doublet at δ 6.86 (J=1.4) was accounted to ortho- meta coupled H-6', ABX spin system of ring B. A one proton broad singlet at δ 6.81 ascribe to aromatic proton H-8. The two methoxyl groups resonated as a broad signal of six protons at δ 3.18. A one proton doublet at δ 5.3 (J=9.8 Hz) was attributed to anomeric proton H-1". ^{13}C NMR spectrum of compound showed important signal for carbonyl carbon at 203.2 (C-4) supporting the flavonol type frame work of the molecule. Oxygenated aromatic carbon appeared at 147.5 (C-3'), 142.5 (C-4'), 149.6 (C-5'), 149.0 (C-6), 166.7 (C-9), 135.4 (C-3). The sugar carbon resonated between 106.8 and 72.1. The ^1H NMR ^{13}C data was compared with the flavonol molecule. Acidic hydrolysis of compound 1 yielded on an aglycon identified as 3,5,6,7 tetrahydroxy 3', 4' dimethoxy flavone and sugar as rhamnose (by direct comparision of TLC with authentic sample). Thus on the basis of spectral data and chemical analysis compound 1 was identified as **3, 6, 7 trihydroxy 3', 4' dimethoxy flavone 5-O-β-D – rhamnopyranoside**



Compound -1

Table -1

Positions	δ_C	δ_H	Positions	δ_C	δ_H
2	148.3		1"	106.8	5.96(1H,d,J=9.8)
3	135.4		2"	76.9	5.78(1H,d,J=4.9)
4	203.2		3"	86.1	5.4(1H,d,J=2.0)
5	149.6		4"	77.4	3.90(1H,d,J=9.0)
6	149.0		5"	72.1	
7	195.9		5"a		3.88(1H,d,J=1.2)
8	101.4	6.81(1H,d,J=2.4)	5"b		3.93(1H,d,J=1.6)
9	166.7		OCH_3	56.3	3.18(brs, 6H)
10	103.2		OCH_3	54.4	
1'	119.7		CH_3	20.5	1.82(s, 3H, rhm)
2'	122.4	7.26(1H,d,J=9.0)			
3'	147.5				
4'	142.5				
5'	115.1	7.14(1H,d,J=2.4)			
6'	111.4	6.86(1H,d,J=1.4)			

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A Carminative Herb 'As' Essential Oil Found Stronger Inhibition Effect than Nitrofurantoin, Methicillin and Cefixime Antibiotics

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Abstract - The aim of the study was to assess the antibacterial effect of essential oil of *'AS'* and their synergistic antibiotic drugs against *Escherichia coli* and *Staphylococcus aureus*. The essential oil was prepared using Clevenger apparatus. The antibacterial activities of this oil was evaluated using the Disk diffusion method. The inhibitory zones were recorded in millimeters. The minimal inhibitory concentration (MIC) of the essential oil against *E. coli*, *Staph. aureus* and *Pseudomonas aeruginosa* were assessed using microdilution method. The synergistic effect between plants and extraction of antibiotics drugs was assessed using Disc diffusion method. Our results indicate the possibility of using 'AS' essential oil in the treatment of bacterial infections, and the results of this study was encouraging despite the need for clinical studies to determine of the real effectiveness and potential toxic effects *in vivo*. These results revealed the importance of plant essential oil when associated with antibiotic drugs in control of bacteria.

Keywords: Essential oil, Antimicrobial activity, Minimum inhibitory concentration

Introduction

Plants produce a diverse range of bioactives molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semisynthetic derivatives of natural products and used in traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products.

In the present time, drug resistance in microbes is a very serious problem. Hence, the plant origin herbal medicines are considered as safe alternatives of synthetic drugs. Currently, Ayurveda considered as a vital system of medicine and earned the worldwide recognition having non-toxic substances.

However, newly discovered non-antibiotic substances such as certain essential oils (Sonboli *et al*., 2006) and their constituent chemicals (Chavan *et al*., 2006) have shown good fighting potential against drug resistant pathogens (Cowan, 1999; Ahmad and Beg, 2001).

Essential oils are aromatic oily liquids which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots by steam distillation. Scientifically these oils have been proved highly potent antimicrobial agents in comparison to antibiotics. These plant essential oils are rich source of scents and used in food preservation and aromatherapy.

These possess multiple antimicrobial i.e., antibacterial (Ozcan *et al*., 2006), antifungal (Cafarchia *et al*., 2002), anticancer, antiviral and antioxidant properties (Salehi *et al*., 2005; Vardar-Unlu *et al*., 2003), against viruses, bacteria and fungi (Kalemba and Kunicka, 2003).

Pimenta racemosa (Mill.) J. W. Moore. (syn. *Pimenta acris* Kostel,) is cultivated for the production of essential oil exploited in industry. The oil is commonly called "bay oil" or "Myrcia oil". This spice is used to aromatize the food (Leung et Foster, 1996)**.** The culinary uses are the same as those of the "bay-tree sauce" (*Laurus nobilis*). A decoction of bark taken out of infusion, is used against hypertension. The essential oil extracted from the leaves presents the disinfectants and astringent properties; it is moderately toxic by oral way because of its high percentage of phenol, but as a matter of principle it does not give place to allergic reactions at the man (Opdyke, 1973). It is used in the manufacturing of

creams, lotions, detergents, or in the shampoos. It is also used in perfumery (Opdyke, 1973) like febrifuge (Ayedoun *et al.*, 1996). It possesses anti-inflammatory and analgesics properties (Duke, 1986; Robineau, 1991). Essential oil intervenes in the patented formula of a capillary cosmetic composition (Orenga, 2003). The most asserted biological properties are the antimicrobial effects which are explained by the high percentage of phenols. In the present study, antimicrobial potential of essential oil was screened against pathogenic bacterial strains.

For antimicrobial susceptibility of essential oil, MIC values and growth inhibition zone diameters were determined.

Material and Methods

Extraction of essential oils: Essential oils used in this study were extracted from the AS'. These were separately grounded and powdered in domestic Mixi and hydrodistilled in a Clevenger's apparatus by the technique of Guenther (1948) to obtain essential oils. Before application, solubility of essential oil was tested. Dilution was made by adding fresh solvents and stored at 5ºC till used.

Bacterial cultures: Cultures of of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), were maintained in SCD Broth (2% w/v) at 37ºC in the laboratory. For inoculation, a portion (100: l) of overnight culture of each bacterial strain was mixed in 15 ml of media for each test and control separately. For activity testing, bacterial cultures were stored at 4ºC and sub cultured after every 8th day in solid agar plates.

Screening of antibacterial activity: Antimicrobial activity of essential oils on bacterial growth was assessed. For this purpose, essential oils were diluted by using serial micro dilution method with Broth culture medium at a final concentration range from 32 to 0.0078: l/mL. In each test, AS essential oils were added to fresh suspension after making serial dilution. The 'AS' essential oil was assayed for antibacterial activity in triplicate. Before conducting experiments all the conditions were standardized to determine MIC and MBC values *in vitro.*

Filter paper Disc diffusion assay: Agar disc diffusion method was used for screening of antimicrobial activity of 'AS' essential oil. For antimicrobial activity testing essential oil was diluted by adding equal volume of solvent. From this a known volume of 'AS' essential oil was coated on separate sterile filter paper discs (Whatman No. 1) measuring 6 mm in size. These oil-impregnated discs were made dry under laminar flow cabinet. Bacterial inoculum was spread evenly on to the surface of each agar plate with sterile rubber pad spreader and essential oil coated discs were positioned in the centre of inoculated agar plate. The essential oil was assayed in triplicate. Sterile distilled water was used as negative control, while broad-spectrum antibiotics i.e. Nitrofurantoin, Methicillin and Cefixime were used as positive control for obtaining comparative results. All treated and untreated plates were incubated for 24 h at 37 ºC and size of inhibition zone diameters surrounding filter paper disc was measured. For determination of Minimum Bactericidal Concentration (MBC), growth inhibitory assays were performed. For this purpose, inoculum size was adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml) in sterile agar plates. Both test and control cultures were kept at 37ºC for 24 h. For comparison, both negative and positive controls were set and bacterial colony number was counted. The least concentration at which no visible growth was obtained in agar plates was considered as MBC. For evaluation of inhibition two parallel controls were set and bacterial growth was obtained in presence and absence of various quantities of essential oils.

Results and Discussion

Determination of MIC and MBC values: The MIC value of AS essential oils are presented in

table-1. The essential oil of the leaves of AS almost has an antimicrobial activity very interesting against *Staphylococcus aureus* ATCC 25923. MIC = (0.30 ± 0.02) mg/mL and *E.coli* and *Pseudomonas aeruginosa*(2.24 ± 0.13) mg/mL respectively, an average activity against the three others microbial stocks.

Inhibition zone diameter: In the present study,effectiveness of 'AS' essential oil was also confirmed by filter paper disc diffusion assay and growth inhibitions zone diameters was measured. Results are presented in table-2. AS essential oil have shown larger growth inhibition zone diameters in comparison to synthetic antibiotics (Plate-1) against *Staphylococcus aureus*. The essential oil has shown 33 mm inhibition zone diameter against *Staph aureus followed by E.coli and Pseudomonas aeruginosa*.

Present study reveals the antimicrobial susceptibility of essential oils against *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*.. It is proved by low MIC and MBC values obtained in essential oils when used against each bacterial culture. Lowest MIC values (0.30 mg/mL) against *Staphylococcus aureus.*

Further, effectiveness of essential oil was determined by agar disc diffusion method and inhibition zone diameters were measured (Plate-1). Based on growth inhibition zone diameters obtained bacterial strains were divided in to three categories i.e. resistant (>7 mm), intermediate (>12 mm), and susceptible (>18 mm). As in the present study, inhibition zone diameters were obtained more than 18mm in size, which are significantly much larger than the antibiotic drugs and proves.

Susceptibility of essential oil: In the present study, essential oil have shown strong antimicrobial effects on gram positive than gram-negative bacteria in suspension culture. However, inhibition zone diameters obtained in filter paper disc diffusion assays have shown better effectiveness of essential oil against Gram-positive bacteria. It may be due to volatile action of essential oil and absence of lipo-polysaccharide layer in Gram positive bacteria that might function as an effective barrier against any incoming bio-molecule (Inouye *et al*., 2001; Delaquis *et al*., 2002).

Table-1 MIC of 'AS' essential oil

S.No.	Microbial cultures	Minimum Inhibitory Concentration (MIC) (mg/mL)
1.0.	*Escherichia coli* ATCC 25922	2.24 ± 0.13
2.0.	*Staphylococcus aureus* ATCC 25923	0.30 ± 0.02
3.0.	*Pseudomonas aeruginosa*	2.24 ± 0.13

Table-2 Antimicrobial activity of 'AS' essential oil against test organisms

S.No.	Microbial cultures	Diameter of zone of inhibition(mm) AS essential oil	Diameter of zone of inhibition(mm) Antibiotics)		
			Nitrofurantoin	Methicillin	Cefixime
1.0.	*Escherichia coli* ATCC 25922	28	20	18	20
2.0.	*Staphylococcus aureus* ATCC 25923	33	29	28	19
3.0.	*Pseudomonas aeruginosa*	23	19	16	20

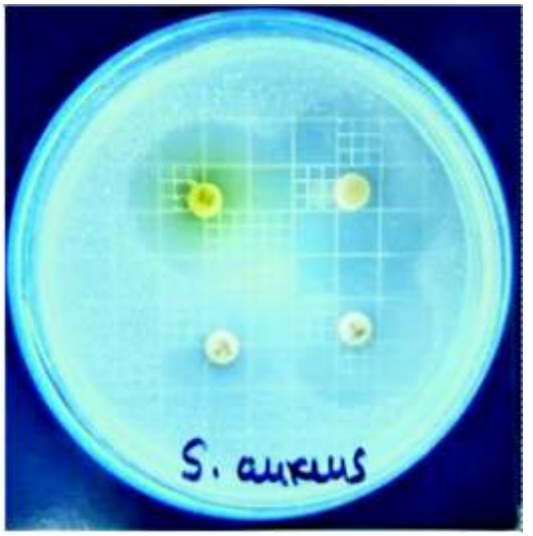

Plate-1 Antimicrobial activity of "AS" essential oil Against *Staph. aureus*

Conclusion

This study emphasizes antimicrobial properties of AS essential oil against human pathogenic bacteria. It has been observed that the essential oil possess both bacterio-static and bactericidal activity much higher than that of synthetic antibiotics when tested *in vitro*. Hence, AS essential oil can be recommended for therapeutic purposes and be used as an alternative medicine after toxicolological analysis and clinical studies.

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Antibacterial and Phytochemical Investigation of Ethanolic Extract of *Cymbopogon citratus*

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Abstract- The present study was carried out to evaluate the antibacterial and phytochemical activity of ethanolic extract of *Cymbopogon citratus* against the bacteria isolated from the nasal and throat samples which were collected from the patients suffering from respiratory diseases. The bacterial species which were isolated are *E-coli, Salmonella typhi, Bacillus cereus* and *Staphylococcus aureus*. These bacterial species were identified on the basis of morphological and bio-chemical characteristics of recovered isolates. The antibacterial activity of *Cymbopogon citratus* was performed by the Agar well diffusion method with two Gram +ve and four Gram –ve bacterial species. From the present study, it has been found that ethanolic extract of *Cymbopogon citratus* showed a broad spectrum of antibacterial activities against Gram-negative (*E-coli and Salmonella typhi*) bacterial species in comparison to the Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) bacterial species.

Introduction

Plants have been known to synthesize a variety of chemical substances, such as phenolic compounds, terpenes, steroids, alkaloids, glycosides, fats and others. They also synthesize secondary metabolites, which are of no apparent importance to the plant's life. However, these have been found to have profound effects on animal systems with therapeutic properties[1]. With current advanced technology, plants are being analyzed and their therapeutic abilities investigated more intensely. Today these methods have been used to isolate an ever increasing number of medicinal substances from plant sources. These medicinal plants are the sources of important drugs of the modern world. *Cymbopogon citratus* (lemongrass) belonging to the family *Gramineae*[2] is a perennial tall grass with rhizomes and densely tufted fibrous roots. The fresh stalks and leaves have green colour and a lemonlike odour. *C. citratus* is used in different parts of the world in the treatment of digestive disorders, fevers, menstrual disorder, rheumatism and other joint pains[2]**.** The plant is a native herb of lndia and is cultivated in other tropical and subtropical countries[3]**.** It is used in the manufacture of perfumes; coloured soaps and synthesis of vitamin A. In Folk medicines in certain parts of Nigeria essential oil is used as an insect repellant. In certain medications, it is used for mental illness[4]. It is an antifungal, antioxidant and deodorizing agent[5]. In combination with other herbs, it is largely used as cure for Malaria[6]. In the present study, an attempt has been made to study the Antibacterial and Phytochemical activities of *Cymbopogon citratus* leaves.

Material and Methods

Collection and Extraction of plant material

The plant material used was the dried leaves of *Cymbopogon citratus* collected from the Herbal garden of Shoolini University, Solan, H.P. and identified by Botanical Survey of India, Dehradun. The dried and powdered leaves of *Cymbopogon citratus* were extracted with ethanolic by Soxhlet apparatus. By removing the solvents in Rotary Evaporator (Butchi Type) at 70-80^0 C, the crude extract was obtained.

Antibacterial activity

Preparation of test microorganisms

The nasal and throat samples were collected from the patients suffering from respiratory diseases. All samples were collected in sterile containers. The bacterial isolates were recovered from urine samples by enrichment culture technique. Portion of sample measuring 1 ml was added in 5 ml of nutrient broth on the very same day of collection and incubated at 37^0 C in incubator for 24 hours. Enriched samples were then streaked on 5 different media, MacConkey agar, Mannitiol Salt agar, Vogal Johnson agar Base, Blood agar, Nutrient agar. The various morphological characteristics of recovered isolates viz., colony morphological (colour, shape, arrangement and gram staining) properties were studied. The various biochemical tests were carried out for identification of isolates[7]. Using a straight wire touch, 5-10 well isolated colonies of particular microorganisms against which antimicrobial activity was to be tested were inoculated in Nutrient broth medium and incubated at 37^0 for 4-6 hrs until its turbidity matched with 0.5 McFarland std.

Determination of antibacterial activity

The Agar well diffusion method was used. 0.5 ml of inoculum of test organism was mixed with Muller Hinton Agar media. It was shaken and poured in sterilized Petri dishes. 5 wells of 6mm diameters were punched with the help of cork borer and 50 µl of tested material (100mg/ml) were poured in wells. Then incubated the plates for 24 hrs at 37^0C. The plates were observed for zone of inhibition in mm and the zone were measured by using scale.

Phytochemical investigation of *Cymbopogon citratus*

The Qualitative analysis was carried out to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure.

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added[7]. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent[8]. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer[8].

Terpenoid and steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoids and green bluish color for steroids[8].

Flavonoids

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones[9].

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic, tannins and green black for catecholic tannins[10].

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added hot and observed for brick red precipitate.

Results and Discussion

Generally, plant extracts are usually more active against gram positive bacteria than gram negative bacteria[11]. According to Abu-Shanab et al. (2004)[12], gram negative bacteria are more resistant to plants extract as compared to gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism. In the present study, the *in-vitro* antibacterial activity of ethanolic extract of *Cymbopogon citratus* against seven strains of pathogenic bacteria isolated from the patients suffering from respiratory diseases were examined by Agar well diffusion method and their potency was assessed by the presence or absence of inhibition zones and zone diameters against Gram+ve and Gram-ve bacterial species (**table-1 and table-2**). The working concentration of the extracts was 100mg/ml each.

Table-1 Zone of inhibition of ethanolic extract of *Cymbopogon citratus* against Gram+ve bacterial species.

Bacterial species	Ethanolic extract (mg/ml)
Bacillus cereus	16 mm
Staphylococcus aureus	18 mm

It has been found that in case of Gram+ve bacteria, ethanolic extract of *Cymbopogon citratus* showed maximum antibacterial activity against *Staphylococcus aureus* (18 mm) and then against *Bacillus cereus* (16 mm).

Table-2 Zone of inhibition of ethanolic extract of *Cymbopogon citratus* against Gram-ve bacterial species.

Bacterial species	Ethanolic extract (mg/ml)
E-coli	18 mm
Salmonella typhi	24 mm

On the other hand, in case of Gram-ve bacteria, ethanolic extract of *Cymbopogon citratus* showed maximum antibacterial activity against *Salmonella typhi* (24 mm) and then against *E-coli* (18 mm). From the present study, it has been found that ethanolic extract of *Cymbopogon citratus* showed a broad spectrum of antibacterial activities against Gram-negative (*E-coli and Salmonella typhi*) bacterial species in comparison to the Gram-positive (*Bacillus – cereus* and *Staphylococcus aureus*) bacterial species.

Phytochemical Activity

Qualitative analysis carried out on each plant extract showed the presence of phytochemical constituents and the results are summarized in table-3.

Qualitative analysis of leaf extract showed the presence of alkaloids, glycosides, terpenoids and steroids, flavonoids, tannins and reducing sugars. In the present study, ethanolic extract of *Cymbopogon citratus* leaves tested positive for

Table - 3

Plant extract	Alkaloids	Glycosides	Terpenoids & Steroids	Flavonoids	Tannins	Reducing Sugars
Ethanolic extract of *Cymbopogon citratus*	+	+	+	+	–	+

terpenoid and glycoside. Many phytochemicals are present in the plants as glycosides (with a sugar moiety attached) Generally, Cardiac glycosides serve as defense mechanism against cardiovascular diseases as reported[13]. This may, therefore, explain its therapeutic effect against cardiovascular and digestive problems. The presence of terpenoid and phenol also explains some of the pharmacological action of the leaves extract. Phenolic compounds are important components in vegetable foods, infusions and teas for their beneficial effects in human health[14].

Glycosides were found to be present in *Cymbopogon citratus*, a compound that has been shown to aid in treatment for congestive heart failure and cardiac arrhythmia. This is another reason why this plant is widely used in traditional medicine. Glycosides work by inhibiting the Na^{-}/K^{-} pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle, improves cardiac output and reduces distention of the heart. This research work has revealed further potentials of this plant in the area of pharmacology as potential source of useful drugs. The study therefore, has provided some biochemical basis for ethno pharmacological uses of these plants in the treatment and prevention of various diseases and disorders.

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Phytochemical Investigation and Effect of Ethanolic Extract of *Potentilla fulgens* on oral Microflora

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Abstract - Almost everyone faces a dental problem at some point in their lives. Dental health encompasses the likelihood of making healthy choices in relation to diet, smoking, tobacco, oral hygiene and utilization of dental health services. Various lifestyle products are available in market to improve oral health in which toothpastes are most common and useful one. But toothpaste contains so many chemicals which on prolonged use may severely affect oral health. Now a days much attention is laid on herbal replacement of toothpaste which are superior in biocompatibility and medicinally more beneficial to the existing one. Neem, miswak, clove, bajradanti etc are among most preferred herbs for this purpose. In the present study, phytochemical status and antimicrobial action of *Potentilla fulgens* against oral microflora isolated from teeth of dental patient was studied and it was observed that the ethanolic extract of *P. fulgens* was most effective in inhibiting growth of such bacteria's.

Keywords: Organic toothpaste, Antimicrobial action, Microflora, *Potentilla fulgens*

Introduction

Oral diseases refer to the diseases of mouth such as dental plaque, dental caries and cavities, calculus or tartar, periodontal (gum) disease etc. The mouth is an important organ with many different functions. It is also prone to a variety of medical and dental disorder. Modern life style is too busy that people do not have time to look after their oral health and neglect a regular visit to the dentist. Modern lifestyle includes the unhealthy diet pattern which is more common in children. Most of the food items from fast food establishment are high in sugar and other carbs which are the enemy of teeth.

The various lifestyle-products are available to maintain the oral health such as mouth rinse or mouthwash, Listerine, toothpicks, chewing sticks, tooth brushes, toothpaste etc.Toothpaste is the most widely used amongst them. Now a days, traditional system of dental care i.e., the use of herbs is back to the trend. Various plant extracts like neem, miswak, xanthoxylum and withenia somnifer are used in toothpastes.[1-3]

Potentilla fulgens Wall. ex Lehm. (Rosaceae) is an important medicinal plant of higher Himalaya, known for its therapeutic and commercial importance. It is an important medicinal plant with various therapeutic and commercial importance such as its root stock and whole herb is utilized as astringent and tonic for curing gums and tooth ailments. More than three hundred species of genus *Potentilla* Linn. are used in Ayurvedic, Unani, Siddha, Chinese and Tibetan systems of medicine[4-7] due to high content of polyphenols present in their aerial and underground parts. These polyphenols form stable complexes with metal ions, proteins and polysaccharides and help healing of wounds, burns and inflammations, hinder gut secretions and protect underlying mucosa from toxins and irritants, control dental caries and ameliorate degenerative diseases[8].

In Uttarakhand, the species grow in higher Himalaya up to an altitude of 1800-4350 m. In medieval ages *Potentilla* extracts (water, milk, honey and alcoholic) were used for curing toothache, throat inflammations and other dental disorders. Twigs and leaves are used as tooth brush by Bhotias in Uttarakhand, India. The species are utilized commercially for the

manufacture of Vicco Vajradanti tooth powder and paste[9-12].

Being inspired by such a great significance of *Potentilla fulgens* in treating oral disorders, we directed our efforts to the study of phytoconstituents present in the plant and to evaluate their effects on oral microflora.

Material and Methods

The full grown plant of *Potentilla fulgens* were collected from village Balan, District Chamoli, Uttrakhand and were authenticated by Dr. Sandeep Dhyani, H.O.D Dept. of Biotechnology G.R.D PG IMT, Rajpur, Dehradun and Systemic Botany Division, F.R.I, Dehradun, Uttrakhand. The plant parts were dried at room temperature (25-35℃) for 5-6 days and subjected to further processing.

Preparation of Various Extracts

Different solvents were used for the extraction purpose in a specific sequence based on increasing polarity viz., petroleum ether, chloroform, ethanol and distilled water. Each time before extraction with the next solvent, the powdered material was dried in hot air oven below 50℃. The individual fractions obtained were concentrated under reduced pressure in water bath and weighed.

Phytochemical Constituents

The individual extract was subjected to qualitative phytochemical screening for the presence of some chemical constituents such as steroids, phenolics, fixed oil, alkaloids, glycosides, saponins, flavonoids, tannins and carbohydrates.[13]

Antimicrobial Activity of Extracts

Preparation of Plates

Samples were randomly collected from people before brushing teeth in morning by using sterile cotton swabs. The samples were immediately transferred to 3ml of Brain heart infusion broth (HiMedia) and were vigorously shaken; later samples were placed in incubation chamber at 37 ^{0}C for 24 hours. Dilution (10^{-5}) of inoculated samples was prepared and placed on the Nutrient agar plates.

Nutrient agar plates were placed in incubation chamber for incubation. After 24 hours, the cultured plates were carefully and systematically viewed for any visible growth. Special attention was given to colony presentation which includes, colony growth pattern, size and shape, concentration and changes made by the growing organism. Suitable colony was picked, purified and stocked for further studies. After observing the slides under the microscope the isolated species were found to be Streptococcus species and Bacillus species.

Antimicrobial Sensitivity Tests

Antimicrobial sensitivity test was conducted according to the method specified by NCCLS (Now CLSI, Clinical Laboratory Standard Institute) and described by Collins *et al.*, 1995. The discs (HiMedia) for antimicrobial activity were prepared by dipping them in the extract and DMSO solution(50%). Sensitivity of isolates of *E.coil, S.aureus, B. cereus, Streptococcus sps* and *Bacillus sps* were tested for *Potentilla fulgens*(30µg). Discs (HiMedia) containing different extracts were placed on the surface of the agar using a pair of sterilised forceps. The discs were incubated at 37℃ for 24 hrs. Each plate was observed for the zone of inhibition around disc, which was measured in millimetres.

Results and Discussion

500gm of dried raw material of *Potentilla fulgens* was extracted using various solvent (pet ether, chloroform, ethanol, water) in soxhlet extraction assembly. Highest percentage yield was obtained for ethanolic extract which was 3.0%. The various extracts of *P.fulgens* were then subjected to qualitative chemical test and the results of the study are shown in table-1.

(+) Presence, (++) Good presence, (-) Absence

Results of phytoconstituents study show that the ethanolic extract of *Potentilla fulgens* was rich in

Table-1 Qualitative phytochemical examination of extracts from *Potentilla fulgens.*

Test performed	Pet. Ether extract	Chloroform extract	Ethanol extract	Water extract
Test for Alkaloids				
Mayer's test	-	+	++	+
Dragendroff test	-	+	+	+
Hager's test	-	+	++	+
Wagner's test	-	-	+	+
Test for carbohydrates				
Fehling test	-	+	-	++
Molish test	-	+	-	++
Test for phenolic compounds and Tannins				
Dil. $FeCl_3$ test	-	-	++	+
Test for sterols				
Salkowaski test	+	+	-	-
Libermann test	+	+	-	-
Test for Proteins and Amino acids				
Milon's test	-	-	+	+
Ninhydrin test	-	-	++	+
Biuret test	-	-	+	+
Test for saponins	++			
Test for glycosides and flavonoids	-			

alkaloids, phenols, tannins, saponins and amino acids where as carbohydrates and sterols were absent, whereas water extract also shows same significant presence and absence of these compounds. The petroleum ether and chloroform extracts were, however, rich in steroidal content while alkaloids and carbohydrates were also present in chloroform extract.

The antibacterial activity of all the extracts of *P. fulgens* showed highest activity against all the above bacterial strains isolated and cultured from infected teeth. The ethanolic extract was found to exhibit maximum antibacterial activity against *S aureus* where as it showed minimum antibacterial activity against *E coli*. The results of study are summarized in table-2.

Table-2 Antimicrobial activity of extracts of *Potentilla fulgens*

Test Organism	*Potentilla fulgens* extracts				Ciprofloxacin (30µg)
	Pet. ether	Chloroform	Ethanol	water	
E. coli	1	3	7	0.5	21
B. cereus	0	5	8	0.5	20
S. aureus	1	1.5	18	1	26
Streptococcus sps	1.5	2	20	1	25
Bacillus sps	1	3	8	0	24

Conclusion

Dental health of a person depends on his dietary habits, oral hygiene and life style. For the maintenance of oral hygiene, there are various products available in markets which contain various extracts of medicinal plants. *Potentilla fulgens* is an important medicinal plant as its roots stock and whole herb is utilized as tonic and for

curing gums and tooth ailments. Its ethanolic extract has maximum anti-microbial activity as compared to acetone, chloroform and water extracts on the oral bacteria collected from the mouth of dental patient (table-2). Result of phytochemical study shows that the ethanolic extract is rich in tannins, proteins, alkaloids, and terpenoids. The major terpenoids reported from the plant are protentene-A, protentene-B, epicatechine, and rutine. Similarly, the plant bears biflavonoid (potifulgen), proanthacinadines.[14-16] The constituents either alone or in combination might be responsible in reduction of microbial concentration of oral pathogens. However, exact mode of their antimicrobial action is a matter of further research. Thus, we can say that toothpaste containing ethanolic extract of *P. fulgens* can serve better to treat various dental disorders.

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Diabetes Control by Massage Oil/ Probiotic Substitute of Ancient Wheat

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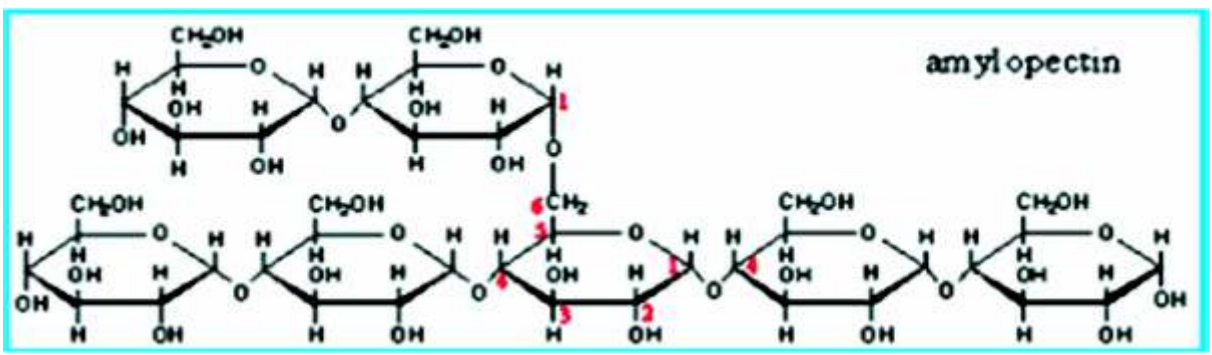
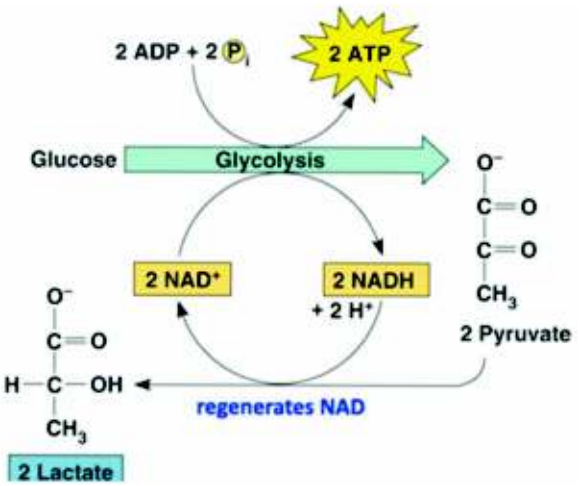
Abstract - Modern wheat is different from wheat of ancient time. Modern wheat has very high amylopectin which is making people diabetic because molecules of amylopectin contain up to three million glucose molecules, which are easily digested and absorbed in intestine to cause prediabates and diabetes. Since people can not cut wheat totally from their daily food intake, therefore attempts were made to convert glucose of amylopectin into non carbohydrate component called lactic acid to decrease the glycaemic index (Glucose count) of wheat flour by the application of probiotic bacteria in the wheat flour. When roti's and other foods made of this wheat flour are eaten and herbal diabetes oil was massaged into the body, the high's of blood sugar was found to be controlled within 24 hours in diabetes patients.

Keywords: Modern wheat, Diabetes, Amylopectin, Lactic acid, Glycaemic index.

Introduction

Wheat today is not same as it was a thousand,one hundred or even 60 years ago. Modern wheat is different from the wheat of ancient time. Norman Borlaug's[1] discovery doubled the amount of wheat produced by India between 1965 and 1970. He was awarded Nobel prize in 1970. Today's wheat is genetically modified and biologically different.Modern wheat has been hybridized which means it has been cross -bred with other species to grow larger grain having more amylopectin. It has a higher yield and contains more carbohydrates. The hybridization of wheat has caused changes in the starch.Amylopectin is a soluble polysaccharide and highly branched polymer of glucose. Molecules of amylopectin[2] contain up to three million glucose molecules. It is one of the two component of starch, the other being amylose. Amylose molecules consist of a single mostly unbranched chains with 500--20,000 glucose units. Unlike amylopectin, amylose is not soluble in cold water. Starches that are high in amylopectin are digested and absorbed more quickly in intestine than starches with a high amylose content and produce larger post prandial glucose and insulin responses. Almost all of wheat eaten today is high yield dwarf wheat. Dwarf wheat has shorter stems and a much greater yield. Therefore, it is much cheaper than other varieties





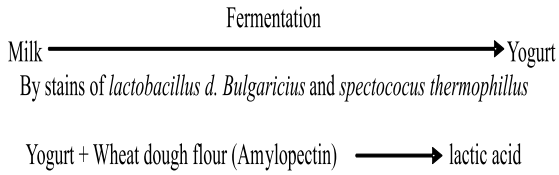
and more economically feasible. Modern wheat plant were cross-bred to absorb more nitrogen from the soil and grow larger grain bearing tip part of the stem. However,these large tip parts were too heavy for the plants long stem,causing to bend. This made them more stable to support the larger grain bearing tip part of the stem.

According to a growing number of dieticians and nutritionists,wheat in any form could be one of the most injurious food for our health. Giving up wheat in all its forms is tougher than giving up smoking because the grain is so inextricably a part of our daily diet, from parathas to pasta, samosa to sandwiches. People can not cut it totally from their daily food intake. Therefore, in the present study we have attempted to convert maximum glucose of wheat flour into lactic acid; a non carbohydrate by contaminating wheat dough with yogurt.

Lactic acid exists naturally in two optical isomers d(-) lactic acid and l(+) lactic acid. Yogurt and other cultures milk products which contain lactic acid bacteria,can enhance gastro intestinal system function. In fact the potential benefits of yogurt and other food containing living organisms are so numerous that they form a special sub-group of functional foods:probiotic food.

Material and Methods

We have added one tea spoon yogurt[3] into the wheat flour dough to convert most of the amylopectin into lactic acid[4, 5] by the help of probiotic bacteria. For the preparation of yogurt, the direct fermentation of milk was done by adding two specific strains of bacteria called *lactobacillus Bulgaricus* and *spectrococous thermophilis*. Two different wheat flour dough (A) and (B) had been prepared by adding 60 ml of tap water into 100 gm of wheat flour separately. Only sample A was mixed with one tea spoon yogurt. After waiting for four hours, pH of A and B was measured by pH meter.



Yogurt is same as curd with the difference that in its preparation, the direct fermentation of milk is done by adding two specific strains of bacteria called *lactobacillus.d. Bulgaricus and spectrococus thermophilis*. These micro organisms which are also known as probiotics convert glucose into lactic acid which is able to decrease the glycaemic index i.e., glucose count of the wheat which is mainly responsible for pre diabetes and diabetes (post prandial blood glucose).

Discussion

Lactobacillus is a gram positive,anaerobic or micro aerophilic, rod-shaped, non-spore forming bacteria. They are a major part of the lactic acid bacteria group. Its members convert lactose and other sugars to lactic acid. Some *lactobacillus* species are used as starter cultures in industry for controlled fermentation in the production of yogurt, cheese and other fermented foods. The bacteria metabolize sugars into lactic acid which lowers the pH of their environment, creating sourness. *Lactobacillus Bulgaricus* is commonly used alongside with *streptococcus thermophillus* as a starter for making yogurt. The two species work in synergy, with *L.d. bulgaricus* producing amino acids from milk proteins, which are then

used by *S. thermophilus*. Both species produce lactic acid which gives yogurt its tart flavour and acts as a preservative. The resulting decrease in pH also partially coagulates the milk proteins such as casein, resulting in yogurt's thickness. While fermenting milk, *L.d. bulgaricus* produces acetaldehyde, one of the main yogurt aroma components.Some strains of *L.d. Bulgaricus* GLB44 also produce bacteriocins which have been shown to kill undesired bacteria in vitro. Wheat fermented with lactic acid bacteria inhibits the growth of proteolyptic because of the low pH produced by the fermentation of amylopectin. *Lactobacillus Bulgaricus* can also be taken in tablet form although the best way to benefit from its qualities is through yogurt consumption.

Result

pH of (A) which is considered as control, was found to be greater than 7 which reveals that sample (A) was basic in nature. pH of all the samples of (B) were found to be lower[6,7] than 7, hence they were acidic due to degradation of amylopectin into glucose and then conversion of glucose into the lactic acid. The value of post prandial glucose reading from patient one to the last was found much higher than the post prandial glucose reading after massage of the oil[8] and eating roti's made of probiotic wheat flour. The result from the table reveals that the synergized effect of herbal massage oil and probiotic wheat flour was highly effective in overcoming the high's in sugar level produced due to the resistance developed by the body of patient against oral drugs and insulin.

Conclusion

The data from the table supports the conversion of amylopectin of wheat flour into non carbohydrate, lactic acid. Thus the glycaemic index[9,10] of wheat flour was decreased, as a result there was better control in blood glucose level of diabetes patients.

Table- Probiotic Wheat Flour/Herbal Oil Controlling Post Plandial Blood Sugar

S.No.	Date	Before Massage					After Massage and Eating Bread	
		No. of Drops Massaged	No. of Oral Allopathic Tablets taken	Units of Insulin taken	Post prandial Plasma Glucose mg/dl	P^{II}	Post prandial Plasma Glucose mg/dl	P^{II}
1.	3-4-2011	50	3	x	334	>7	225	<7
2.	6-5-2011	50	3	x	X	>7	X	<7
3.	3-4-2011	50	1	40	387	>7	191	<7
4.	6-5-2011	50	1	40	377	>7	190	<7
5.	22-6-2012	50	2	X	X	>7	X	<7
6.	4-7-2012	50	2	X	260	>7	120	<7
7.	14-7-2012	50	2	5	240	>7	144	<7
8.	10-3-2012	50	1	18	567	>7	338	<7
9.	10-6-2012	50	1	15	X	>7	X	<7
10	22-6-2012	50	2	X	X	>7	X	<7
11.	12-7-2012	50	2	X	280	>7	120	<7
12.	1-5-2011	50	2	40	310	>7	290	<7
13.	1-6-2011	50	1	25	290	>7	213	<7
14.	1-7-2011	50	1	25	213	>7	202	<7
15	1-8-2011	50	1	25	202	>7	152	<7
16.	1-9-2011	50	1	25	X	>7	X	<7
17.	20-2-2012	50	2	X	228	>7	107	<7
18.	21-3-2012	50	2	X	X	>7	X	<7
19.	16-7-2012	50	2	X	X	>7	X	<7
20.	24-7-2012	50	2	X	300	>7	155	<7

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Anti-Inflammatory Activity of Different Extracts of *Aloe vera*

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Abstract- Herbs have been used in clinical medicine for thousands of years. However, it is only in the recent times that we have been able to employ scientific methods to prove the efficacy of many of these herbs and to give us a better understanding of their mechanism of action. *Aloe vera* (Liliaceae) known as "medicinal aloe" is commonly used in diabetes, skin burn, hyperlipidemia but it's anti-inflammatory effect is not well established. This article focuses on the use of *Aloe vera* as an anti-inflammatory drug. Different extracts of leaves of *Aloe vera* were studied for their anti-inflammatory activity in rodents. The leaves of the plant were extracted with Pet. ether, chloroform, acetone, methanol and water successively in increasing polarity of solvents. Preliminary phytochemical investigation was carried out to identify various phytochemical constituents present in the various extracts. T.L.C. was also done to separate different components. The anti-inflammatory activity was studied on carageenan induced rat paw oedema acute inflammatory model. Different extracts showed the presence of alkaloids, terpenes, steroids, saponins and carbohydrate. Aqueous and chloroform extracts showed significant degree of anti-inflammatory activity. The results demonstrated that extracts of *Aloe vera* gel have very good anti-inflammatory activity.

Keywords: *Aloe vera*, Anti-inflammatory activity, Plethysmograph.

Introduction

Aloe vera (*Aloe barbadensis Miller*) is a shrubby or arborescent, perennial, xerophytic, succulent, pea- green coloured plant which belongs to *Aloaceae* (Liliaceae) family; grows mainly in the dry regions of Africa, Asia, Europe and America. In India, it is found in Rajasthan, Andhra Pradesh, Gujarat, Maharashtra and Tamil Nadu. The plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. Evidence supporting the early use of *Aloe* was discovered on a Mesopotamian clay tablet dating from 2100 BC (Surushe *et al*., 2008; Acharya and Shrivastav, 2008).

Aloe vera has marvelous medicinal properties. Scientists have discovered over 150 nutritional ingredients in *Aloe vera.* (Balasubramanian J, Narayanan N, 2013). There seems to be no single magic ingredient. They all work together in a synergistic way to create healing and health giving benefits. The ten main chemical constituents of *Aloe vera* include: amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosacharides, salicylic acid, saponins and sterols (Barcroft, 1999). Located in the sap of the leaves are twelve anthraquinones, a phenolic compound that has stimulating effects on bowels and antibiotic properties. In small amounts, the anthraquinones do not have a purgative effect. They help with the absorption from the gastro intestinal tract and have anti-microbial and pain killing effects. The most important anthraquinone are aloin and emodin. They are antibacterial, anti-viral and analgesic (Atherton, 2002).

Material and Methods

Preparation of extracts

Fresh *Aloe vera* leaves were collected from Forest Research Institute, Dehradun, (India). The plant was identified in the herbarium of the Department of Botany, Forest Research Institute, Dehradun. The extracts were prepared using Soxhlet extractor from green leaves, minced, homogenized and dried at 35° C. 90 g of dried

leaves were extracted by using different solvents in the order of increasing polarity viz. Pet ether, Chloroform, Acetone, Methanol and water. The test samples were prepared in 1% Tween 80 and administered to the respective groups in a dose of 100 mg/kg body weight (Combest L., 2000).

Anti-inflammatory Activity

Swiss albino rats of either sex weighing 150-300 g were procured from the Institute's animal house. They were housed in groups of 5 animals in polypropylene cage under controlled conditions for one week before experiment and fed on a standard diet. Animals were fasted overnight with free access of water prior to the experiments. Five groups were treated as test groups and one as standard and the other one as control.

In order to identify a substance as anti-inflammatory, it is necessary to test it's pharmacological activity in animal models. Test on animals are necessary to understand how a drug will work in the context of metabolic and homeostatic mechanism that are active in vivo. To identify unexpected adverse effects and to estimate the doses that are pharmacologically active without producing unwanted effects, in vivo studies must be conducted. The anti-inflammatory activity was studied by the rat paw edema method.

Inflammation is a response of the tissue to infection, irritation and foreign substance. A variety of chemical agents are used to induce edema in the feet of rodents. Anti-inflammatory agents may then be detected by their ability to diminish/prevent the edema. The anti-inflammatory activity of different extracts of *Aloe vera* was evaluated by carageenan induced paw edema in rats. **Carageenan** is a mucopolysacchride derived from Irish Sea moss (*Chondrus cirspus)*. It is found to be more useful agent of choice over the other like Brewer's yeast, formalin, dextran, egg albumin etc. A 0.1 ml of 1% (w/v) soln. of carageenan was injected subcutaneously in the right hind paw of the animal. **Tween-80** was used as a control. 1% w/v **Tween-80** solution was used as a vehicle and was given orally in the dose form as 10 ml/kg body weight. A suspension of various extracts in Tween 80 was given to the animals in the dose of 100mg/Kg. body weight. **Diclofenac Sodium (**Sodium-2[2-6-dichlorophenylamino] Phenylacetate) **was used as a** standard anti-inflammatory drug for comparison. It was given orally as a suspension in the dose of 10 mg/Kg body weight.

For statistical studies, 7 groups of 5 animals each were taken. Initial reading of each group was taken and then Tween 80 was given orally to the first group which served as the control.. Diclofenac-Sodium was given to the second group and petroleum ether, chloroform, acetone, methanol and water extracts were given to groups 3 to7 respectively. After 30 minutes of drug administration, 0.1 ml of 1% suspension of carageenan was injected in the sub plantar region of the right hind paw of each rat. The paw volume was measured after an interval of 3 hours. The change from the initial paw volume in ml after administration of carageenan was noted in each animal with the help of Plethysmograph.

Phytochemical and Thin Layer Chromatography Analysis

Phytochemical tests were performed on various extracts of *Aloe vera* as per standard techniques. Thin Layer Chromatography of the extracts was performed on Silica Gel 60 with ethyl acetate/methanol/water 100:16.5:13.5 (v/v) or benzene/methanol 4:1(v/v). The chromatograms were observed under an Ultraviolet lamp at 254-300 nm.

Results and Discussion

After performing different Phytochemical tests, the various phytoconstituents that have been identified in different extracts are summarized in Table-1. As can be seen from the data that pet ether extract contained only steroids, the chloroform extract contained steroids and phenols, the acetone extract contained only steroids. Both methanol and water extracts contained

carbohydrates whereas the water extracts contained phenols and saponins also.

The anti-inflammatory activity was measured on albino rats as mentioned above. The results of various groups are summarized in Table-2. As can be seen from the data that the water extract showed the best anti-inflammatory activity. To confirm the significance of data, one way Anova test was carried out. It was observed that the value of F was highest for Diclofenac Sod. (174.298) and the value of F for water extract was comparable (114.3) to Diclofenac Sod. at a degree of freedom equal to nine. This value of F is more than the Table value at this degree of freedom. Hence showing that our results are quite significant. Finally, a comparison chart of the activity of control, standard and all the extracts is shown in Figure-3.

Conclusion

The study showed that the aqueous and chloroform extracts of the Aloe vera contain compounds with a potential to reduce carageenan induced edema. We found that at standard doses used, *Aloe vera* aqueous extract showed inhibition which is produced by the well established anti-inflammatory agent (Diclofenac Sodium). We have demonstrated that aqueous and chloroform extracts showed anti-inflammatory effect on carrageenan-induced edema. Based on the results of this study, we have come to the conclusion that *Aloe vera* has potential Anti-inflammatory compounds, which thus provides a scientific proof for the utilization of the plant for treatment of inflammatory processes. There are many studies going on to find out the compounds and pathways responsible for the anti-inflammatory effects described here.

Table-1 Table showing the results of Phytochemical analysis on various extracts of *Aloe vera*

Category Test performed	Pet-ether	Chloroform	Acetone	Methanol	Water
Alkaloids					
1. Mayer's test	-	-	-	-	-
2.Wanger's test	-	-	-	-	-
3. Hager's test	-	-	-	-	-
Carbohydrates					
1. Fehling's test	-	-	-	+	+
2. Molisch test	-	-	-	+	+
3. Benedict's test	-	-	-	+	+
Steroid					
1. Salkowaski test	+	+	+	-	-
2. Libermann's test	+	+	+	-	-
Amino acid					
1. Ninhydrin test	-	-	-	-	-
2. Biuret test	-	-	-	-	-
Saponins					
1. Foam test	-	–	–	–	+
Phenolics and flavonoids					
1. Ferric chloride test	–	+	–	–	+
2. Vaniline test	–	+	-	–	+
3. Zinc HCl test	–	+	–	–	+
4. Lead acetate test	–	+		–	+

Table-2 Table showing a comparison of the anti-inflammatory activity of the control, standard and all the experimental extracts

Drug	Dose	Mean Inflammation ±S.D.	Percentage Inhibition	F values
Tween 80 (control)	10 ml/kg	0.18±0.002	0%	_____
Diclofenac sodium (standard)	10 mg/kg	0.052±0.0087	70%	174.298
Petroleum ether Extract	100 mg/kg	0.168±0.0083	11.3%	0.043
Chloroform extract	100 mg/kg	0.104±0.0005	53%	67.163
Acetone extract	100 mg/kg	0.108± 0.0008	38.6%	55.149
Methanol extract	100 mg/kg	0.182±0.0008	4.5%	0.043
Water extract	100 mg/kg	0.082±0.0004	57.0%	114.333

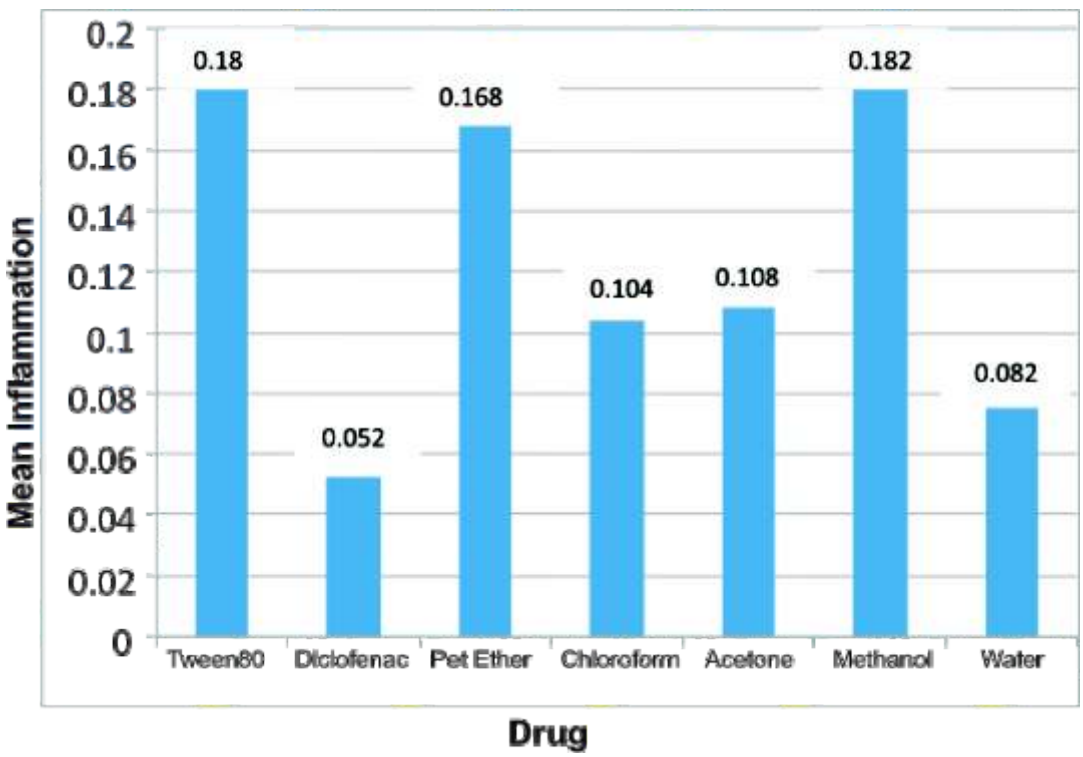

Figure-1 Figure showing the mean inflammation of the control, standard and various extracts

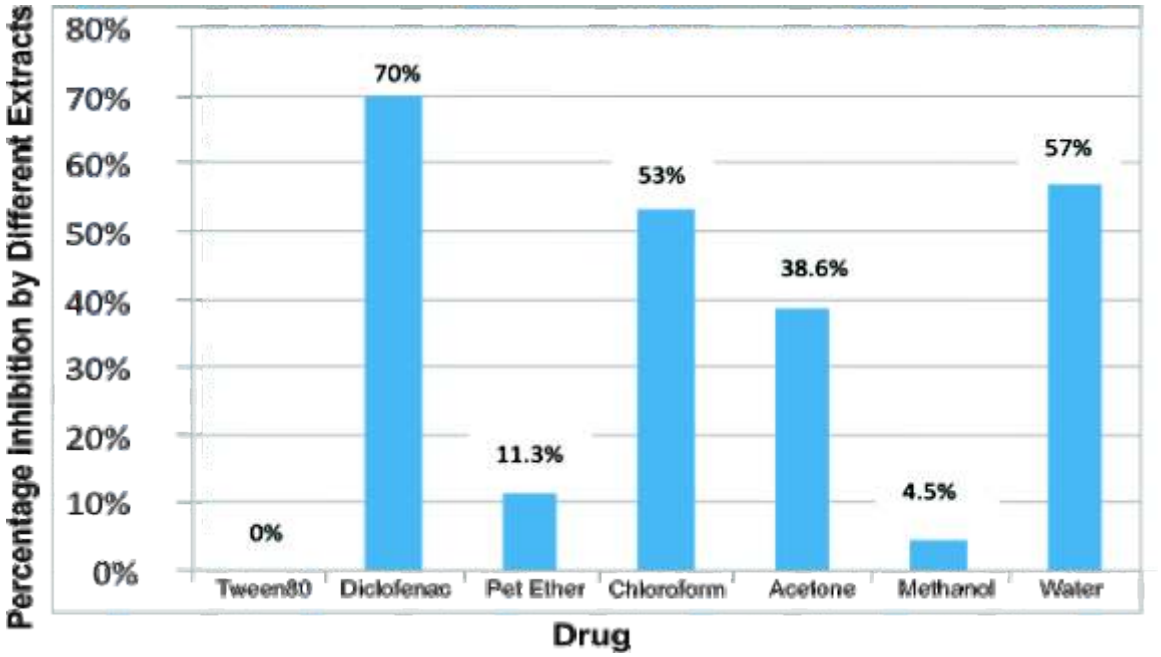

Figure-2 Figure showing the Anti-inflammatory activity of control, standard and various extracts

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Phytochemical Investigation and Evaluation of Anti-Oxidant and Anti-microbial Potential of *Calotropis procera* Leaves Extract

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Abstract- The present study has been designed to analyse the phytoconstituents of various extracts of *Calotropis procera* leaves. Total ethanolic extract was prepared and fractionated by using chloroform, ethyl acetate and butanol. These extracts were then subjected to qualitative chemical tests for the identification of various phytoconstituents viz., alkaloids, carbohydrates, glycosides, proteins, tannins, sterols, saponins, amino acids etc. The present study demonstrates that total ethanolic extract of leaves was rich in carbohydrate, sterols, phenolic compounds and amino acids. Total ethanolic extract and all fractions were screened for antioxidant and antimicrobial potential. From antioxidant studies, it was observed that the total ethanolic extract showed % inhibition (DPPH) 54.28 as compared to standard 93, while antimicrobial potential was observed in ethylacetate fraction against the organism *P. aeruginosa.*

Keywords: *Calotropis procera, Ocimum gratissimum*(Linn.), Phytoconstituents, Extraction

Introduction

The traditional knowledge on the use of medicinal plants has been widely acknowledged across the world. According to the WHO, 80% of the world's population in developing countries uses traditional medicines[1]. Exploration of medicinal plants and their industralization give an assurance of the safety, quality and efficacy, thus herbal products and herbal remedies are widely been used to treat health disorders[2,3].

Himalayas covering 18% of the Indian subcontinent accounts for more than 50% of India's forest and contains 30% of India's economic species. Roughly 40% of plants provide active ingredients for modern drugs, and because of their use in traditional medicines, they cause interest researchers. Furthermore, the traditional knowledge with its holistic and systematic approach supported by experimental base can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines.

Calotropis procera Linn. a wild growing plant of family Asclepiadoidae is an Ayurvedic shrub having excellent medicinal properties[4]. It is a small, erect and compact shrub which is found in waste lands and occurs as milkweed in cultivated areas. Each part of this plant bears excellent medicinal properties and used for curing different human ailments. The decoction of the plant is used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism, asthma and as an expectorant and purgative[4].

Each part of *Calotropis procera* (CP) contains several phytoconstituents belonging to different classes of natural products. The roots of the plant exhibit hepatoprotective activity, anti-inflammatory, antipyretic, analgesic and antimicrobial effect and larvicidal activity. There are reports on hepatoprotective effect of ethanolic extract (70%) against CCl_4 induced hepato-oxidative stress in Albino rats[5,6]. Similarly, an *in vitro* antimicrobial activity of C*alotropis procera* etract were done on certain groups of pathogenic microorganisms and their findings support the traditional use of this plant in the treatment of different infections[7]. While the methanolic and aqueos extract of C*alotropis procera* Linn leaves exhibited strong antioxidant and antibacterial activities[8].

The present study has been designed to analyse the phytoconstituents of total ethanolic extract and its fractions and also to screen them for antioxidant and antimicrobial potential.

Material and Methods

Plant material

Leaves of *calotropis procera* were collected from FRI, Dehradun during September, October . The leaves were dried in shade at room temperature and grinded to a coarse powder and perculated with ethanol for 24 hrs.

Chemicals and reagents

The solvents and chemicals were of analytical grade. Chloroform, ethanol, ethyl acetate were purchased from Merck India Ltd. Mumbai. Benedicts reagent, Fehling reagent, Mayer's reagent, Dragendroff reagent, Hager reagent, Wagner reagent, Molish reagent, Millon reagent, Ninhydrin reagent, NaOH pellets, $FeCl_3$ were purchased from Ranbaxy Fine Chemicals Ltd, New Delhi. Picric acid and hydrochloric acid was purchased from S.D. Fine Chemicals Ltd. Mumbai. α-naphthol, H_2SO_4 was purchased from Merck India Ltd. Mumbai. Mueller Hinton Hi Veg Agar, Peptone, Beep extract and DPPH were obtained from Himedia.

Preparation of extracts

The fresh leaves of C*alotropis procera* were dried in shade at room temperature for two days followed by tray drying [40-50°C] for 3-4hrs and powdered to obtain coarse powder and then perculated with ethanol for 24 hrs. The extract was filtered and concentrated under reduced pressure. To the residue so obtained was added equal amount of water fractionated with chloroform, ethyl acetate and n-butanol successively. Respective fractions were concentrated under reduced pressure to obtain successive concentrates. Total ethanolic extracts and their fractions were subjected to phytochemical investigations and we're also screened for antimicrobial and antioxidant potential.

Qualitative Phytochemical Analysis

The total ethanolic extract and its fractions of *calotropis procera* leaves were subjected to qualitative chemical tests for identification of various phytoconstituents viz., alkaloids, carbohydrates, glycosides, proteins, tannins, sterols, saponins, amino acids etc. These phytoconstituents are solely responsible for biological activity of these plants.

Antioxidant Activity

DPPH radical Scavenging Activity

This is most widely reported method for screening of antioxidant activity of many plant drugs. For measuring radical scavenging ability, free radical species like 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical was used[8]. This radical can accept an electron on hydrogen radical from an antioxidant and be measured by spectrophotometric method at 517 nm. The solutions of DPPH (100µM), Standard solution Ascorbic acid (100mg/ml) and test sample solution of total ethanolic extract and its fractions were prepared in methanol. To the methanolic solution of DPPH, test sample solution and the standard were added in different concentrations. Equal amount of methanol was added to control. After 30 minutes, absorbance was measured in triplicate. The percentage of scavenging was calculated by comparing the control and test samples with the following equation.

Inhibition (%) = (Control Absorbance–Test Absorbance) X 100/Control Absorbance

Antimicrobial Activity

The antimicrobial activities of ethanolic extract and its fractions (chloroform, ethyl acetate and butanol) were determined by filter paper Disc method.

Paper Disc Technique

Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40°C for 30 minutes. The prepared Nutrient agar plates were seeded with each of the test bacteria and the

filter paper discs were placed on each plate. The plates were incubated at 37°C for 48 hours. The zones of inhibition were measured and recorded. The bacterial strains used were *Escherichia Coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Streptococcus gordonii and Salmonella enteric*. The cultures were obtained from the standard cultures maintained in the Department of Microbiology, SBSPGI, Balawala, Dehradun, (India).

Results and Discussion

Qualitative phytochemical constituents of *Calotropis procera leaves*

Ethanolic extract and its fractions (chloroform, ethyl acetate and butanol) were screened for phytochemical constituents. Results showed the presence of carbohydrates, sterols and amino acids in total ethanolic extract. Chloroform fraction showed the presence of carbohydrates, flavanoids, sterols, ethyl acetate fraction showed the presence of flavanoids and phenolics while n-butanol fraction was rich in flavanoids, alkaloids, carbohydrate, and amino acids. (Table-1). From antimicrobial activity (Table-2) it was observed that only ethyl acetate fraction of total ethanolic extract of *Calotropis procera* leaves showed zone of inhibition of 12mm against P. *aeruginosa*. From antioxidant studies, it was observed that total ethanolic extract showed DPPH % inhibition 54.28 as compared to standard ascorbic acid as 93. Also among all other fractions, maximum inhibition was found to be in butanol fraction 58.9% while ethyl acetate and chloroform exhibited 52.5 and 56.1% inhibition respectively.

Table-1 Results of Phytoconstituents present in ethanolic extract and its fractions (chloroform, ethyl acetate and butanol) of *Calotropis procera* leaves

Test performed	Total ethanolic extract	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction
Test for Flavanoids				
Shinoda's test	-	+	-	+
Lead Acetate Test	-	+	+	+
Test for Alkaloids				
Mayer's test	-	-	-	++
Dragendroff's test	-	-	+	-
Hager's test	-	-	-	++
Wagner's test	-	-	-	++
Test for Carbohydrates				
Fehling test	+	+	-	++
Molish test	+	+	-	++
Benedict test	+	+	-	++
Test for phenolic compounds and Tannins				
Dil. $FeCl_3$-test	+	-	+	-
Test for sterols / Triterpenoids				
Salkowaski test	++	+	-	-
Liberman-Burchard's test	++	+	-	-
Test for Proteins and Amino acids				
Ninhydrin test	-	-	+	++
Biuret test	+	-	-	+

Table-2 Results of Antimicrobial activity in total ethanolic extract and its fractions (chloroform, ethyl acetate and butanol) of *Calotropis procera* leaves against different bacterial strains

Organisms used	Total ethanolic extract (30 µl)	Chloroform fraction (30 µl)	Ethyl acetate fraction (30 µl)	n-butanol fraction (30 µl)
Escherichia Coli,	8	-	-	9
Bacillus cereus,	-	-	-	-
Pseudomonas aeruginosa	8	7	12	6
Staphylococcus saprophyticus	-	-	-	-
Streptococcus gordonii	-	-	-	-
Salmonella enteric	-	-	-	-

Table-3 % Inhibition of DPPH radical of total ethanolic extract and its fractions (chloroform, ethyl acetate and butanol) of Calotropis procera

S.No.	Extracts (in 100 mg/ml conc.)	Absorbance in triplicate			Concordance absorbance	% Inhibition
1.	Control	1.066	1.090	1.018	1.061	-
2.	Standard	0.055	0.094	0.073	0.072	93.0
3.	Ethanol	0.453	0.458	0.519	0.485	54.28
4.	Ethyl acetate	0.456	0.493	0.530	0.493	52.5
5.	n-butanol	0.466	0.499	0.535	0.5	58.9
6.	Chloroform	0.437	0.465	0.493	0.465	56.1

Conclusion

From the above studies, it could be concluded that C*alotropis procera* leaves are rich in carbohydrates, amino acids and flavanoids. N-butanol fraction of its total ethanolic extract exhibited maximum % inhibition (DPPH) 58.9 as compared to standard ascorbic acid in the dose of 100 mg/ml. Thus further purification of n-butanol fraction may result in isolation of anti-oxidant compounds with enhanced activity. No significant activity was observed against microbial strains except against *P. aeruginosa*.

Acknowledgement

The authors gratefully acknowledge to Director and management of SBSPGI, Balawala, Dehradun, India for providing the necessary facilities during this experimental study.

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Antibacterial Activity and HPTLC Analysis of Flowers of *Anthocephelus cadamba* and *Hibiscus rosa sinensis*

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Abstract- The antibiotic resistance has become prevalent with the over administration of antibiotics to treat common infection. However, there is a great problem that capacity of most of the microbial species which have developed resistance to all different classes of antibiotics. The different parts of the plants have been used to treat infectious diseases caused by bacteria. Till now infectious diseases are still difficult to be eradicated so there is needs to search potential antibacterial medicines with less side effects. Thus, the natural compounds capable of inhibiting the growth of pathogenic bacteria are the need of an hour. Keeping these points in view, the objective of the study is designed to investigate the antibacterial effect of flowers of *Anthocephalus cadamba and Hibiscus rosa-sinensis* against *S. aureus and E.coli.* The methanolic extract of *A. cadamba* was found to be most effective against the selected microbial strains. HPTLC of the methanolic extract of both the plants was recorded for the purpose of standardization.

Keyword: Antibacterial activity, HPTLC, *A.cadamba, Hibiscus rosa-sinensis*

Introduction

Resistance towards revealing antibiotics have become widespread among bacteria and fungi hence new class of antimicrobial substances are urgently required. Natural products which have tendency to kill the bacteria and give health and hygiene are called natural antibiotic i.e. ayurvedic antibiotics. In today's era there is a strong need of natural products which could acts as an effective antibiotic without any side effects and without harming the immune system of a person. There are several studies which reveal the presence of such compounds with antimicrobial properties in various parts of plant. The petals have some protective mechanism against microbial attack in most of the plants[1]. The flower petals of a large number of plant species of *Hibiscus rosa-sinensis gr*owing in the vicinity of our environment were screened for their antibacterial activity.

Anthocephalus cadamba (*Rubiaceae*) is widely distributed throughout the greater part of India and is used as a folk medicine in the treatment of fever, anaemia, uterine complaints, blood diseases, skin diseases, leprosy, dysentery, and for improvement of semen quality. The leaves are recommended as a gargle in cases of stomatitis[2]. Some scientific studies have been carried out to reveal its antimalarial[3] and antihepatotoxic activities[4]. The major constituents of bark are triterpenes, tripernoid glycosides, saponins, indole alkaloids cadambine, 3 a-dihydrocadambine, cadamine, isocadamine and isodihydrocadambine[5-8]. In recent years, many possible sources of natural antibiotics are used for several infectious diseases, mostly bacterial and fungal infections. Phytochemistry of *A. cadamba* and its application in the treatment of various ailments like diabetes mellitus, diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds, ulcers, debility and antimicrobial activity.

Hibiscus rosa-sinensis belongs to the family *Malvaceae*. With attractive and colourful flowers, plants of Hibiscus are widely planted as ornamentals and are used in traditional medicine. Hibiscus species have been used as a folk remedy for the treatment of skin diseases, as an anti fertility agent, antiseptic and carminative. *Hibiscus Rosa sinensis* possesses many biological activities such as antipyretic, analgesic and anti-inflammatory activities[9, 10]. It has also been reported that flowers of this possess anti-spermatogenic, androgenic, anti-tumor and anticonvulsant properties. In addition, the leaves and flowers have been found to be aid in the healing of ulcers. Infusion of the petals is given as refrigerant and demulcent. Many chemical compounds like Cyandin, Quercetin, Hentriacontane, Calcium oxalate, Thiamine, Riboflavin, niacin and ascorbic acids have been isolated[11, 12].

Thus, after reviewing the economic significance of these two plants, we decided to explore the antibacterial efficacy of these plants. The present study reveals that the evaluation of antibacterial activity in flower extract of *Anthocephalus cadamba* and *Hibiscus rosa-sinensis* against human pathogens viz. *Staphylococcus aureus and Escherichia coli.*

Material and Methods

Collection of Plant Materials

Flowers of *A.cadamba* and *Hibiscus rosa sinensis* were collected from the garden of The Himalaya Drug Company, Dehradun (Uttaranchal). The plant material was washed and dried. After drying it was grinded into a fine powder.The powdered plant material was then taken for extraction procedure.

Methanolic Extraction

Air-dried powder of flowers of *A.cadamba* and *Hibiscus rosa sinensis* (50 g) were thoroughly mixed with 200 ml methanol. The mixture was placed at room temperature for 24 hrs. Solution was then filtered through muslin cloth and then re-filtered through Whatmann filter paper no. 41. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of both methanolic crude extracts were stored at 4°C in sterilized bottles for further use.

Antibacterial Activity

The bacterial strain *Staphylococcus aureus and E.coli were used for the*e antibacterial activity. These bacterial culture were maintained on Nutrient agar slants at first being incubated at 37 °C for about 18-24 hours and then stored at 4 °C as stock for antibacterial activity. Fresh culture was obtained by transferring a loop full of cultures into Nutrient broth for dilution and then diluted Nutrient broth was transferred to Nutrient agar which was then incubated at 37 °C over night. To test antibacterial activity, the well diffusion method was used.

Culture Media Preparation

The microbiological media preparation was done as per standard instruction provided by the Himedia Laboratries, Mumbai. The media used for antibacterial activity were Nutrient agar and Nutrient broth. They were prepared and sterilized at 121 °C at 15 PSI for 20 mins in autoclave.

Plate Preparation: 25 ml of pre autoclaved Nutrient agar was poured into 90 mm diameter pre sterilized petri-plates. These petri-plates were allowed to solidify at room temperature.

Well Diffusion Method

In vitro antibacterial activities of methanolic extracts of flowers of *A.cadamba* and *Hibiscus rosa sinensis* were determined by standard agar well diffusion assay[13]. After the plates solidified, the freshly prepared microbial broth culture suspension (about 20 µL) was spread over the Nutrient agar media using l shaped sterilized glass spreader separately under the aseptic condition using Laminar Air Flow. Well were made in each plate with the help of borer of 6 mm diameter. In these well 50 ml of methanolic extract were individually loaded. The plates were then incubated in the upright position at 37º C for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously, addition of the respective solvents instead of extracts was carried out as negative controls and the standard was used for positive control. After incubation the diameters of the results and growth inhibition zones were measured.

High Performance Thin Layer Chromatography

The high performance thin layer chromatography (HPTLC) studies of *A.cadamba* and *Hibiscus roas sinensis* were carried out on a pre-coated silica gel plate (0.2 mm, Merck 60 F 254, Germany) as the stationary phase. Chloroform: methanol (85:15) was used as the mobile phase for *A.cadamba* and chloroform: methanol (90:10) for *Hibiscus rosa sinensis*. The extract was spotted as a band using a Camag Linomat 5 sample applicator (CAMAG, Switzerland).

The plates were observed in UV 254nm, UV 366nm and were scanned on a CAMAG TLC scanner 3 using the Wincats software.

Results and Discussion

The results of HPTLC of *Anthocephalus cadamba and Hibiscus rosa sinensis* shows the presence of wide range of phytochemicals in their extracts which were absorbed at different Rf values in the form of bands when put in twin through chamber (mobile phase). These bands were visualized when silica plate was photographed in a reprostar and TLC scanner which gave the graphical representation of it.

The antibacterial activities of *A.Cadamba* and *Hibiscus rosa-sinensis* flowers were carried out. The flower extract shows an antibacterial activity against the human pathogens *S.aureus* and *E. coli (Table-1 and Table-2)*. The inhibition of bacterial growth in-vitro by the extracts of flower could be due to the presence of some active compounds in the extracts. These active compounds may act alone or in combination to inhibit bacterial growth. There are several reports published on antibacterial activity of different herbal extracts.

The flower extracts of *A.cadamba* and *Hibiscus rosa sinensis* were screened for antibacterial activity against human pathogenic bacterial strains. *S. Aureus* and E. *coli* are a common member of the normal flora of large intestine[14]. It is leading facultative organism in the gastrointestinal tract and colonizes the tract. It is responsible for causing diarrhea. Thus the flower extracts can be used as an important antibiotic to cure disorders caused by the different strains of bacteria.The results of antibacterial activity of these two herbs showed their zone of inhibition against *Staphylococcus aureus and E.coli* . It was quite clear by the results that *Anthocephalus cadamba* showed excellent activity against both grams positive as well as

Table-1 Results of Antibacterial activity against *Staphylococcus aureus*

Sr. No.	**Plant**	Zone Of Inhibition
1	*Anthocephalus cadamba*	30mm
2	*Hibiscus rosa sinemsis*	17mm
	Standard (Positive control)	
1	Nitrofurantoin	23mm
2	Methicillin	20mm
3	Cefixime	11mm

Table-2 Results of Antimicrobial activity against *E.coli*

Sr. No.	**Plant**	**Zone Of Inhibition**
1	*Anthocephalus cadamba*	20mm
2	*Hibiscus rosa sinemsis*	12mm
	Standard (Positive control)	
1	*Ciprofloxacin*	22mm

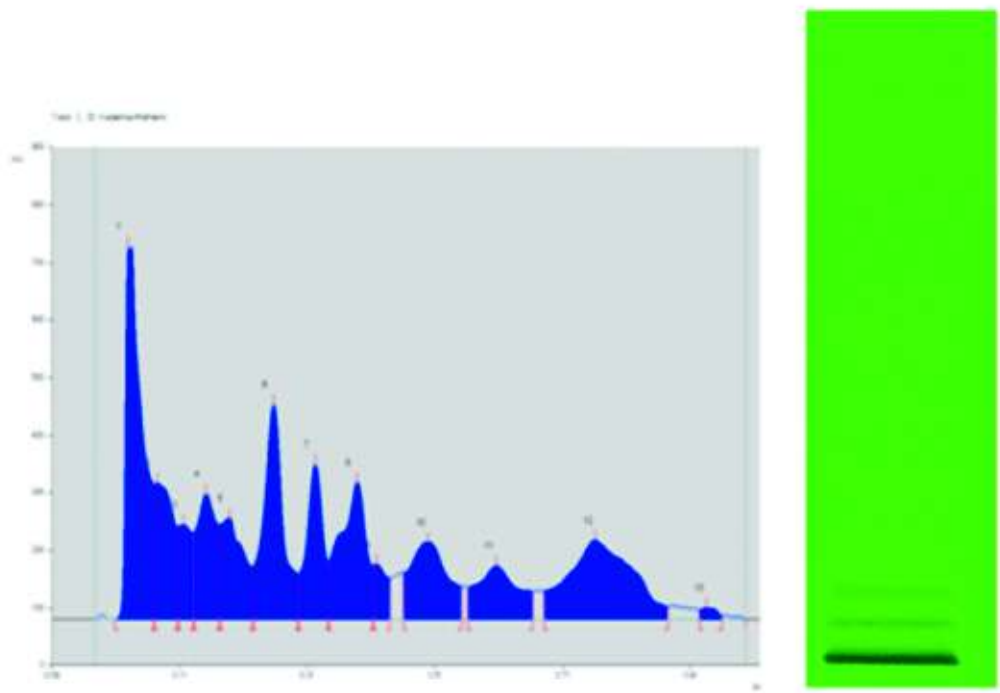

Figure-1 General HPTLC fingerprint profile of *A.cadamba at 254nm*, Mobile phase: chloroform:methanol (85:15).

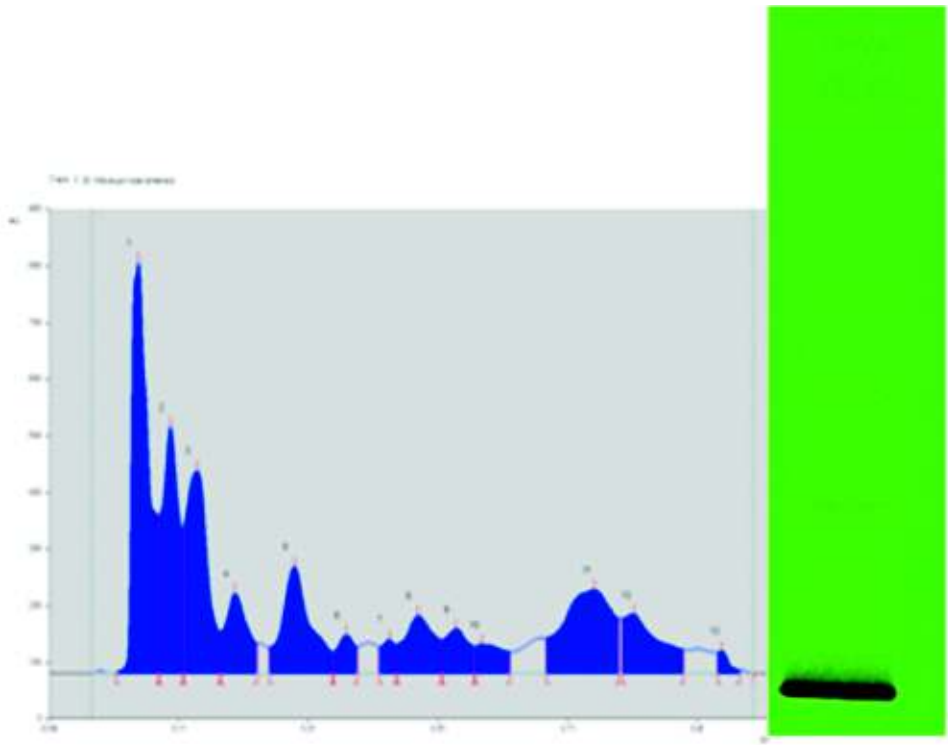

Figure-2 General HPTLC fingerprint profile of Hibiscus rosa-sinensis *at 254nm*, Mobile phase: chloroform:methanol (90:10).

gram negative bacteria, hence it can be seen as future antibiotic.

The significant antibacterial effect of *A.cadamba* and *hibiscus rosa sinensis* against two pathogens confirmed that the compounds present in the crude extract are responsible for the effective antibacterial activity. Thin layer chromatography studies indicated the presence of more than ten different compounds (Fig. 1 & Fig. 2), confirming the synergistic action.

Conclusion

The antbacterial efficacy of flowers of *A.cadamba and Hibiscus rosa sinensis* show that these plants possess promising antibacterial properties. The plants are widespread in India, they can be suggested as a readily available and renewal source of antimicrobial agents instead of artificially synthesized chemicals. In comparison to *Hibiscus rosa sinensis*, *Anthocephalus cadamba* showed excellent activity against both gram positive as well as gram negative bacteria, hence it can be seen as future antibiotic. The present studies direct is to conclude that these extract could inhibit human pathogens growth. The results are good. The most active extract can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation.

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About Flowers

Potentilla fulgens: Vajradanti

Hibiscus rosa sinensis : Gurhal

Anthocephalus cadamba: Cadamba

Bombax ceiba:Semal

Botanical Name : *Potentilla fulgens*

Hindi name : Vajradanti

Family : Rosaceae

Medicinal uses: *Potentilla fulgens* is an important medicinal plant of higher Himalaya known for its therapeutic and commercial importance. It shows various pharmacological effects such as antimicrobial, anthelminthic, anti-fertility, antioxidant, anti-diabetic, anti-arthritic, hepato-protective, diuretic, cytoprotective, antidiarrhoeal, analgesic, antileukemic, anti-inflammatory and hypoglycemic properties. Vajradanti contain iridoid glycosides, barlerin and varbascoside. Two iridoid glycosides, barlerin and acetyl barlerin.

Common name : *Gurhal*

Botanical name : *Hibiscus rosa sinensis*

Family : Malvaceae

Hibiscus rosa sinensis is a perennial ornamental shrub available throughout India. Various parts of this plant like flowers, leaves and roots have been known to possess medicinal properties like oral contraceptive, laxative, aphrodisiac, menorrhagic etc. Flowers of the plant are used in diabetes, epilepsy,bronchial catarrh and leprosy. The chemical constituents of flowers are Thiamine, Riboflavin, Niacin and Ascorbic acid, Apigenidin,citric acid, fructose, glucose, oxalic acid, pelargonidin, quercetin.

Name : Cadamba

Biological name : *Anthocephalus cadamba*

Family : Rubiaceae

Anthocephalus cadamba is widely distributed throughout the greater part of India, especially at low levels in wet places. The roots, fruits, leaves, bark skin is used from medicinal purposes. *Anthocephalus cadamba* has been reported to possess wound healing, antioxidant, antimalarial and *Antimicrobial* activity. The chemical constituents presents are the alkaloid cadambine and 3 dihydrocadambine, A glycosidic alkaloid –3 isodihydro cadambine, A new triterpenic acid cadambagenic acid – and along with quinovic acid.

Common name : *Semal*

Botanical name : *Bombax ceiba*

Family : Bombacaceae

Bombax ceiba is commonly known as silk cotton tree. *Bombax ceiba* is an important medicinal plant of tropical and subtropical India. Bombax ceiba flowers have been shown to contain the β-Dglucoside of β-sitosterol, free β-sitosterol, hentriacontane, hentriacontanol, traces of an essential oil, kaempferol, and quercetin. Shamimin, a newly discovered flavonol C-glycoside has been isolated as a pale yellow powder from the ethanolic extract of fresh, undried leaves of *B. ceiba*.

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Chemical and Biological Approaches to Therapeutically Relevant Natural Products and Bioactive Compounds, July 29-30, 2017, Andover, NH; http://naturalproducts.pharmaceuticalconferences.com/

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Council Initiative for promotion of reverse pharmacology in Ayurvedic drug development

Uttarakhand State Council for Science & Technology (UCOST) was established in the last quarter of 2005 in Dehradun. Since its inception council has encouraged the research and development activities in the state and has funded projects in various disciplines of Science & Technology. Council provides financial assistance in R&D, International Travel supports, Entrepreneurship Development Program (EDP), Seminar/Symposium/ Conference/Workshop grants etc.In innovation promotion program the grassroots level for application/ invention catering local needs and all individuals with demonstrable talent are being promoted. The council aims to forge partnership between Central and State Governments, NGOs, R & D institutions, academia and industry, Council will act as hub, maximizing collaboration between various organizations and promote science in multidisciplinary mode. As an initiative, Coordination Cell of the Council are being set up at various institutions.The council has established a state-of-art Regional Science Centre in Uttarakhand sponsored by NCSM, Kolkata, catering to needs of the people of state especially school going children. **The regional science Centre will also have an innovation lab sponsored by National Innovation Council.**

As far as medical science is concerned, UCOST **has initiated an ambitious "Drug Development" program to promote drug development in Ayurveda** within the ambit of reverse pharmacology and the guidelines laid down by WHO for the development of natural products. We have recently reviewed promising therapeutic effects of Herbo-mineral Formulations for prophylaxis of Chronic Pancreatitis and migraine, *Faltrikadi kwath* for prophylaxis of Hepatitis B and started to facilitate advanced R&D following reverse pharmacology. We are intended to work on drug development for some tropical diseases in near future under **Drug Development program provided that the aspiring Vaidya or Ayurvedic traditional healers have maintained meticulous record of their clinical work.**

I extended my best wishes to Universities Journal of Photochemistry and Ayurvedic Height for their endeavor in Herbal research.

Dr. Rajendra Dobhal
Director General