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Mangifera indica (Mango)

Syzygium cumini (Jamun)

Cinnamomum zeylanicum (Dalchini)

Cinnamomum tamala (Tejpatta)

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Editorial

Dear Readers,

Innovation should be our endeavour. The Spirit of Innovation fuels us to aggressively grow. Idea dates back to Charak **born** in 300 BC. He was one of the principal contributors to the ancient art and science of Ayurveda, a system of medicine and lifestyle developed in Ancient India. Acharya **Charak** has been crowned as the Father of Medicine. His renowned work, the "**Charak** Samhita" considered as an encyclopedia of Ayurveda, was written on whatever had been the way of giving the findings that time, but today is the time to either strengthen these discoveries by scientific documentation or find out newer miracles from the herbs using best technologies available today. Morphine and quinine are the herbal products which are still in use. Morphine was first extracted from opium in pure form in the early nineteenth century. It was used widely as a painkiller during the American Civil War, and many soldiers became addicted. **Quinine**, as a component of the bark of the cinchona (quina-quina) tree, was used to treat malaria since as early as the 1600s, when it was referred to as the "esuits' bark", "cardinal's bark" or "sacred bark". Now, these phytochemicals are scientifically studied using advanced technologies and documented.

One of the most pressing aspects is that the pharma industry of India is growing. The Indian pharmaceutical market is the next biggest concerning quantity and the thirteenth largest concerning value, according to a report by Equity Master. India is the biggest provider of generic medications internationally using all the Indian generics accounting for 20 percent of global exports concerning volume. Naturally, consolidation is now a significant feature of the Indian pharmaceutical marketplace as the business is extremely fragmented. India enjoys a significant position in the worldwide pharmaceuticals sector. The nation also has a huge pool of engineers and scientists having the capability to steer the business forward to a much greater degree. Currently, over 80 percent of these antiretroviral drugs used worldwide to fight AIDS (Acquired Immuno Deficiency Syndrome) are provided by Indian pharmaceutical companies.

We also cannot ignore biodiversity of India and richness in herbs of each state. Uttarakhand is richest in its topography and so in variety of herbs. There is rich biodiversity with 65% of area under forest. The Uttarakhand Himalaya has its unique setting within the Western Himalayan region. India is one of the 12 mega biodiversity centers representing about 3800-4000 species of flowering trees of the world.

The rich floral diversity of the state comprises 5096 species of Angiosperms and Gymnosperms. Uttarakhand is a home for many species of birds, mammals and reptiles. A total of 4907 faunal species including mammals, birds, reptiles, etc have been reported from the state of Uttarakhand.

Lastly, My sincere thanks are due to all those scientists, research scholars, students and teachers who contributed their research papers for bringing out this issue and also I express my sincere gratitude to all Board members, guests, media, Staff of the UCOST and all those who will release this issue. Last but not the least, my heartfelt gratitude to the Director General, UCOST Dr. Rajendra Dobhal who himself have always taken keen interest in the UJPAH Publication and release of its 24th issue as he has been doing in the past.

Dr. S. Farooq
Chief Editor

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Phytochemical and Chromatographic Evaluation (HPTLC/HPLC) of Tulsi (*Ocimum sanctum; Ocimum basilicum* and *Ocimum gratissimum) Part-I*

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Abstract-*Ocimum sanctum* Linn also known as Tulsi or Holy basil is an aromatic plant. It is widely used in Ayurveda and Siddha system of medicine to cure various ailments. It is one of the holiest and sacred herbs grown in India. This plant is known to possess antiseptic, analgesic, anti-inflammatory, antimicrobial, antistress, Immuno modulatory, hypoglycemic, hypotensive and antioxidant properties. Phytochemicals are secondary metabolites, which are produced by medicinal plant. The major aim of present study was to investigate the phytochemical screening and chromatographic studies of extracts of *Ocimum sanctum* Leaves.

The leaves have shown the presence of all the phytoconstituents/ phytochemicals like alkaloids, glycosides, phenolic compounds tannins, and flavanoids etc. The methanolic extract of *Ocimmum sanctum* leaves were found to have accountable number of phytoconstituents. The results of the above studies support the use of this plant as therapeutic agent for human and animal diseases and the importance of the ethno-botanical approach as a potential source of bioactive substances.

Keywords: Tulsi, Phytoconstituents, Phtochemicals, Bioactive substance, Chromatographic studies

Introduction

Medicinal plants are of great importance to the health of individual and communities. The medicinal value lies in chemical active substances that produce specific physiological action on human body. Medicinal plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources (Atal and Kapoor, 1989). Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries (Siddiqui, 1993).

Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (WHO, 1993).

Ocimum sanctum commonly known as tulsi, belongs to family Lamiaceae, and it is native of India and distributed wide spread in the world. It is called Holy Basil in English. *Ocimum sanctum L.* is an erect, much branched sub-shrub 25-55 cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5 cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close whorls (Kritikar and Basu,1965). Tulsi is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is cultivated for religious and medicinal purposes and for its essential oil. In traditional system of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum Linn*. Have been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The plant has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions (Sen, 1993 and Sirkar,1989).

Material and Methods

Collection and Identification

Fresh leaves of Tulsi (*Ocimmum sanctum*) collected from The Himalaya Drug Company Campus, Dehradun, Uk. in the month of March, 2018 and taxonomic identification of the plant was done from Department of Pharmacognosy, the Himalaya Drug Company, Dehradun, Uk.

Preparation of Powder

Fresh plant leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of Plant Material

Aqueous extraction

20 gm of *Ocimum sanctum* leaves powder was taken and mixed with sufficient quantity of distilled water. The obtained liquid extract was subjected to rotary evaporator and subsequently concentrated and stored in refrigerator at 4°C (Trease and Evans, 2002).

Ethanol extraction

20 gm of *Ocimum sanctum* leaves powder was taken and mixed with sufficient quantity of methanol. The obtained liquid extract was subjected to rotary evaporator and subsequently concentrated and stored in refrigerator at 4°C (Trease and Evans, 2002).

Phytochemical studies (Khandelwal, 2011)

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, phenolic compound, terpenoid and steroids, flavonoids, reducing sugar and tannin by the following procedure:

Test for Alkaloids for detection of alkaloids

500 mg of extract was dissolved in 20 ml of HCl (1%), then filtered and the following test was performed.

To 2 ml of filtrate, 1 ml of Mayer's reagent was added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

To 2 ml filtrate, 2 ml of Wagner's reagent was added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

2 ml of filtrate and 1-2 ml of Hager's reagent were added. A prominent yellow precipitate indicated the test as positive.

Test for glycosides

For detection of glycoside, 500 mg of extract was dissolved in 20 ml of concentrated HCl, then filtered and the following test was performed.

To 2 ml of filtrate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonium solution was added to it. Pink color indicated the presence of glycosides.

2 ml of filtrate was mixed with each of the 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled on ice and carefully concentrated H_2SO_4 was added. A color change from violet to blue to green indicated the presence of steroidal nucleus.

Extract was mixed with 1 ml of glacial acetic acid containing 2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 1m l of concentrated

Test for Phenolic compound and Tannins

To 2 ml of filtrate, 1-2 drops of 5% $FeCl_3$ solution was added. A dark green color indicated the presence of phenolic compound.

To 2ml of filtrate, 0.5 ml of lead acetate solution was added. A bulky white precipitate indicated the presence of Phenolic compounds.

Test for Flavanoids

Extract were treated with 3 ml of 2% NaOH solution. Formation of intense yellow color which becomes colorless on addition of dilute acid (H_2SO_4), indicates the presence of flavonoids. The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Saponins

The extracts (aqueous and methanolic) were diluted with 20 ml of distilled water separately and further shaken for 15 mins in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

Chromatographic studies

HPTLC (High Performance Thin Layer Chromatography)

Extraction

Around 500 mg of air dried leaves of kapoor tulsi were refluxed with different solvents viz. Methanol, Ethanol, Hexane, Chloroform and Acetone for 30 min at 80 °C. The resulting solution was filtered. This solution was used for HPTLC analysis.

Chromatogram Layer

TLC plates silica get 60 F254, 20 X 10cm (Merck)

Chemicals

Methanol, Ethanol, Hexane, Chloroform, Acetone, Toluene and Ethyl Acetate.

Standard solution

Dissolved 10 mg of reference compound Oleanolic acid in 10 ml of Methanol

Applied 10 µl of all extract, volatile oil and oleanolic acid in TLC plate.

Chromatography

In the Twin Trough Chamber pre-saturated (For 15 min, using wetted filter paper) with Toluene: Ethyl Acetate (93 : 07 v/v) used as a mobile phase and migration distance, 85 mm.

Measurement was made at 254 nm and 366 nm using reprostar 3. For visualization of volatile and Oleanolic acid, the plate was manually dipped in vanillin–sulphuric acid reagent followed by heating at 105 °C for 5 min in oven.

HPLC (High Performance Liquid Chromatography)

Made the test substance into fine powder and weighed it accurately about 1 g in 250 ml flat bottom flask. Added 50 ml of methanol and extracted it by refluxing on a water bath at 80^0C for 30 minutes. Allowed the residue to settle and decanted the dissolved extract into 100 ml volumetric flask. Repeated the same process with each 20 ml solvent until the methanol extract became colourless. Made the volume up to the mark with methanol. Filtered the extract through 0.45µ filter paper.

HPLC Condition

Column	:	C_{18} Luna (250 × 4.6 mm 5 µ), Make: Phenomenex
Flow rate	:	0.8 ml/minute
Mobile phase	:	Methanol: sodium phosphate buffer (89:11)
Wavelength	:	210 nm
Approximate retention time	:	15-20 Minutes
Run time	:	Approximately 30 minutes

Calculation of the Peak Area Calculate the area of ursolic acid peak in the standard chromatogram in case, if pure ursolic acid is used as standards (or), the total area of two major adjacent peaks out of which the first peak corresponds to olienolic acid and the second peak corresponds to ursolic acid. Similarly, calculate the area of both the corresponding peaks in the sample chromatogram.

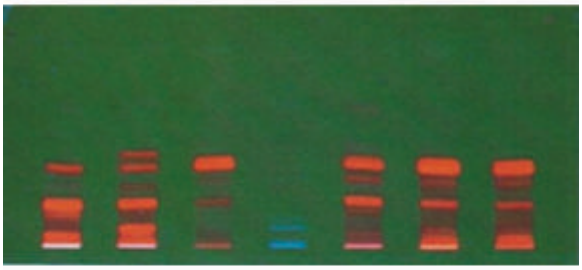
Results and Discussion

The secondary metabolites contribute much in the direction of the therapeutic activities of medicinal plants for bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and antibacterial activity etc (Kritikar and Basu,1987; Batta and Santhakumari,1971). Several

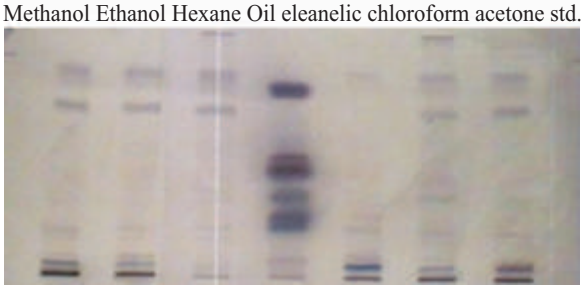
Table- Phytochemical evaluation of Tulsi leaves extracts

S.No.	Phytochemical constituents	Methanolic extract	Aqueous extract
1.0.	Alkaloids	+	+
2.0.	Glycosides	-	+
3.0.	Phenolic compounds	-	+
4.0.	Tannins	+	-
5.0.	Flavonoids	+	-
6.0.	Saponins	+	-

Meth etha Hexane Oil eleanelic Chloroform acetone std.



Methanol Ethanol Hexane Oil eleanelic chloroform acetone std.



Methanol Ethanol Hexane Oil eleanelic chloroform acetone acid.

Figure-1: HPTLC of Fresh Tulsi Leaves in different extracts

therapeutic effects of Ocimum sanctum was established in Indian system of medicine. Various phytochemicals that are present in leaves of *Ocimum sanctum* are responsible for this therapeutic effect. Presence of phytochemicals were analyzed by the qualitative tests which are shown in the **Table.**

In the table, methanolic extract of *Ocimmum sanctum* recorded the presence of alkaloids, glycoside, flavanoids and phenolic compounds. Aqueous extract also shows the presence of alkaloids, carbohydrates, tannins and saponins. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects (Pandey and Anita,1990; Gurib-Fakim, 2006). *Ocimum sanctum* leaves possessed good antibacterial activity confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. The alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants (Cragg et al.,1999; Khanna and Bhatia, 2003) these bioactive principles are actually the defensive mechanisms of the plants against pathogen.

Chromatographic evaluation (HPTLC and HPLC)

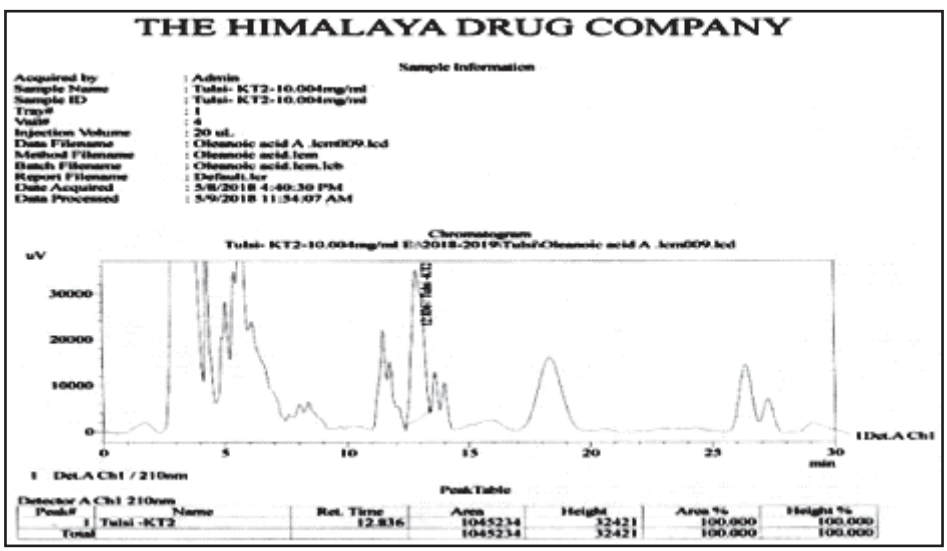


Figure-2:HPLC of fresh Tulsi leaves

were shown in Figure-1 and 2 which indicates the presence of bioactive marker Oleanolic acid.

Conclusion

This study provides evidence that both the leave extracts of *Ocimmum sanctum* are rich source of active phytoconstituents as confirmed by phytochemical evaluation and chromatographic analysis. More detailed study of tulsi plant will help to identify more and new active principles and this can be exploited as a source of developing new drugs which may be used for modern drug discoveries.

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Phytochemical Analysis and In-vitro Antifungal Activity of *Murraya koenigii* Leaf Extracts

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Abstract- The present study deals with the phytochemical screening and in-vitro antifungal assay of different extracts of *Murraya koenigii* leaves. Phytochemical screening revealed the presence of diverse group of phytochemicals in different extracts of the plant. Methanol extract recorded the presence of maximum number of chemical constituents including alkaloids, terpenoids, phytosterols, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrates, proteins, and amino acids. Total phenolics, flavonoids and saponins were detected in all the extracts except petroleum ether extract whereas alkaloid, phytosterol and terpenes were absent in aqueous extract. Terpene was detected in petroleum ether, ethylacetate and methanol extract and tannin in ethylacetate and methanol extracts. In-vitro antifungal assay of extracts against *Alternaria alternata*, *Aspergillus flavus*, *A. niger, Candida albicans, Cryptococcus neoformans* and *Fusarium moniliforme* revealed that the methanol extract of *Murraya koenigii* leaves was highly active against all the tested fungi as compared to other extracts, which was then followed by acetone and ethylacetate extracts. The minimum inhibitory concentration (MIC) values of all extracts for the six test fungi were found low falling in the rage of 8.5–12.25 mg/ml. The study, therefore, validated the traditional use of *M. koenigii* leaves as antimicrobial and showed its promise in development of effective and safe biofungicide.

Keywords: *Murraya koenigii,* Leaf extracts, Phytochemical analysis, Antifungal activity

Introduction

On account of the residual toxic effects of synthetic fungicides on environment and living biota, there is demand for eco-friendly and safe antifungal agents of biological origin. In this context, using plant extracts/formulations as antifungal agent in plant protection, food preservation and diseases control is considered as a practicable and environmentally benign option (Srivastava and Tripathi, 2003). Also, it can be a sustainable mode of agriculture in the organic farming system (Hazarika et al., 2013). Therefore, exploration of plant resources for their antifungal activity against various pathogens is inevitable for management of fungal pathogens. In line with the notion, a number of plants have been investigated for their antifungal efficacy against various harmful fungal species (Mishra et al., 2016; Pal et al., 2016; Tarannum et al., 2017; Tripathi et al., 2015, 2017).

Murraya koenigii (L.) *Spreng* commonly known as Curry leaf tree and belonging to the family *Rutaceae* is an evergreen shrub or a small tree native of India. The plant is highly valued for its characteristic aroma and bioactive compounds. It is widely used in Indian cookery as a flavoring agent for centuries and accredited for a range of therapeutic applications in traditional medicine (Jain, 2012; Dhongade et al., 2013). Different parts of *M. koenigii* are used in folkloric medicine for the treatment of various diseases. It is pharmacologically regarded as anti-emetics, anti-diarrheal, antimicrobial, febrifuge, blood purifier, anti-depressant, anti-ulcer, anti-oxidative, cytotoxic, anti-inflammatory and said to be used

in body aches, vomiting, kidney pain and reducing cholesterol level (Adebajo et al., 2004; Aswatha Ram et al., 2002; Shah and Juvekar, 2006; Rao et al., 2011). It is reported to possess significant wound healing capacity (Anand *et al.,* 2011). Leaves of *M. koenigii* contain essential oil which is believed to represent the very essence of odour and flavour (Sinha, 2012). The plant reported to contain major alkaloids namely mahanine, O- methyl murrayamine A, O- methyl mahanine, koenine, koenigine, koenidine, girinimbiol, bispyrayafoline, girinimibine, isomahanine, koenimbine, and bismahanine (Kumar et al., 2012). These plant secondary compounds or phytochemicals prevent and combat microbial attack (Copping and Menn, 2000). This has prompted intensive research on plants for development of phytofungicides as safe alternatives to synthetic fungicides that could potentially be used in the management of insect-pests. Antimicrobial properties of plant extracts have recently been of great interest in both research and industry owing to their possible use as natural substitute of their synthetic counterparts. Such plant species can play an important role in the development of natural and safe antifungal formulations due to their effectiveness, less side effects and relatively low cost as compared to synthetics. The study was aimed at exploring the phytochemical composition and antifungal properties of *M. koenigii* leaf extracts.

Material and Methods

Plant Material

Fresh leaves of *M. koenigii* were collected from the Botanical Garden of Forest Research Institute (FRI), Dehradun. Uttarakhand, India and were identified and authenticated by Systematic Botany Section of Department of Botany, FRI, Dehradun, India. The collected leaves were cleaned properly under running tap water to make them free from soil and dust and then dried in shade with regular turning. Dried leaves were chopped and ground to coarse powder using an electronic grinder. Powdered leaves were stored in airtight cellophane bags in a cool dry place till further use.

Preparation of Extracts

Dried and powdered leaves (100 g) were taken in a beaker and extracted successively with hexane, chloroform, acetone, methanol and sterile distilled water (500 ml x 3 each) in the order of increasing polarity through stirring with mechanical stirrer for 8 hr. The extracts so obtained were filtered through filter paper (Whatman No.1), concentrated using rotary flash evaporator and finally evaporated to dryness to obtain respective crude extracts. The extracts were finally dried over anhydrous sodium sulphate and stored in sealed glass bottle at 5°C until further analysis.

Phytochemical Analysis

The extracts of *M. koenigii* leaves obtained by exhaustive extraction with hexane, chloroform, ethylacetate, acetone, and methanol were subjected to qualitative phytochemical screening to detect the presence and/or absence of different categories of chemical constituents Such as alkaloids, flavonoids, phenolics, tannins, steroids, saponins, carbohydrates, glycosides, proteins and amino acids, etc. The extracts were tested qualitatively by standard methods using special reagents that produce characteristic colours changes with different categories of chemical constituents (Harborne, 1993; Trease and Evans, 2002; Olawale-Abulude, 2007; Kumar et al., 2009, Tiwari et al., 2011). All the qualitative tests were replicated thrice.

Test Fungi

Antifungal activity of *M. koenigii* leaf extracts were evaluated against some important and frequently occurring pathogenic fungi viz., *Alternaria alternata*, *Aspergillus flavus*, *A. niger, Candida albicans, Cryptococcus neoformans* and *Fusarium moniliforme*. These fungi were isolated from the infected saplings by Standard Blotter Method (ISTA, 1999) and identified based on

growth characteristic, mycelial morphology, spore morphology and other important characters using standard protocol (Barnett and Hunter, 2000; Mukadam, 2006). Pure cultures of each of the selected fungal species were made separately and maintained at on PDA slants. These pure cultures were used for antifungal assay.

Preparation of test solutions

The concentrations of 25 mg/ml of each extract were used for antifungal activity assay of *M. koenigii* leaves. Each of the dried extract was dissolved in Dimethyl sulfoxide (DMSO) to obtain a stock solution of 25 mg/ml. All test solutions were kept in refrigerator at 4^0C till future use.

Preparation of fungal inoculums

For antifungal assay cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 1-5x10^5 cfu/ml.

Preparation of media

The medium was prepared by dissolving Potato dextrose agar (PDA) media (HiMedia) in distilled water and autoclaving at 121^0C for 15 minutes. 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm diameter) and allowed to solidify which were used for antifungal assy.

Antifungal activity assay

Antifungal activity of *M. koenigii* leaf extracts was determined using agar-well diffusion method (OECD, 2001). Spore suspensions (0.2 ml) were applied on the surface of the presterilized and autoclaved PDA petridishes and spread by using a sterile glass spreader. Wells of 6 mm diameter were made in centre of each of the PDA petriplates with the help of sterilized cork borer. The wells were filled with test solutions of leaf extracts as prepared above with three replications for each treatment. Carbendazim (2 mg/ml) and DMSO were served as positive and negative control respectively. All the petridishes including treatments and controls were allowed to diffuse at room temperature for 2 hours and then incubated at room temperature (28 ± 2^0C) for 72 hours. After incubation, the antifungal activity of extracts was determined by measuring the diameter (mm) of inhibition zones.

Determination of MIC

The minimum inhibitory concentration (MIC) was determined through the broth dilution method (Gatsing et al., 2010; Chandur, 2013). Fungi were first grown in the potato dextrose broth for 24 hrs and then the inoculums were diluted for five times (10^{-5}dilution) to control its vigorous growth. Then each test tube was added with 1.8 ml of potato dextrose broth and different concentrations leaf extracts followed by inoculation of 0.2 ml of respective fungi and kept at 28°C for 48 hrs. The tubes were examined for visual turbidity. Lowest concentrations of the extracts showing no turbidity (without microbial growth) were considered as the minimal inhibitory concentration (Prescott et al., 1999).

Results and Discussion

Phytochemical screening

The petroleum ether, chloroform, ethylacetate, acetone, methanol and aqueous (water) extracts of *M. koenigii* leaves were subjected to phytochemical screening for the presence of alkaloids, terpenoids, phytosterols, phenolics, flavonoids, tannins, saponins, glycosides, carbohydrates, protein, amino acids according to standard procedure. The results of phytochemical analysis are presented in the Table-1.

The qualitative phytochemical screening revealed the presence of various phytoconstituents in different extracts of the plant (Table-1). It is evident from the results that the methanol extract recorded the presence of maximum number of chemical constituents including alkaloids, terpenoids, phytosterols, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrates, proteins, and amino acids. Total phenolics, flavonoids and saponins were detected in all the extracts except petroleum ether extract whereas alkaloid, phytosterol and terpenes were absent in

Table-1 Qualitative analysis of *M. koenigii* leaf extracts

Phytochemicals	Extracts					
	Pet. ether	Chloroform	Ethylacetate	Acetone	Methanol	Water
Alkaloids	+	+	+	+	+	–
Terpenoids	+	–	+	–	+	–
Phytosterols	+	+	+	+	+	–
Phenolics	–	+	+	+	+	+
Flavonoids	–	+	+	+	+	+
Tannins	–	–	+	–	+	+
Saponins	–	+	+	+	+	+
Glycosides	–	+	–	+	+	+
Carbohydrates	–	–	–	–	+	+
Protein	–	–	+	+	+	+
Amino acids	–	–	+	+	+	+

aqueous extract. Terpene was detected in petroleum ether, ethylacetate and methanol extracts and tannin in ethylacetate and methanol extracts.

Presence of diverse range of secondary metabolites in *M. koenigii* leaves is indicative of significant therapeutic activity. Presence of steroidal compounds is of importance in pharmaceutical application as these compounds are responsible for several biological functions in the human body. The presence of flavonoids and phenolic constituents, which are considered to be good free-radical scavengers, indicate that the plant may have antioxidant properties. Flavonoids are accountable for biological actions viz., antioxidant, anti-allergic, antiinflammatory, hepatoprotective, anti-carcinogenic, anti-viral and anti-thrombotic activities (Najafi et al., 2010). Tannins are linked to antibacterial (Scalbert, 1991), anti-diarrheal, antihemorrhoidal, and hemostatic activities (Akiyama *et al.*, 2001). Terpenoids are large and diverse class of naturally occurring phytochemicals found in all classes of living organisms displaying analgesic and anticancer (Ali et al., 2008) and antibacterial (Selvan et al., 2012) properties. Terpenoids also play an active role in wound healing, strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply (Krishnaiah et al., 2009). The presence of saponins protects plants from microbial pathogens (Kumar and Upadhyaya, 2010) and is responsible for anti-inflammatory activities. Saponins have the property of coagulating and precipitating red blood cells, thus possesses cholesterol binding and haemolytic activity. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones (Yadav and Agarwala, 2013). However, further work is required to investigate the relationship between phytochemical profile and various pharmacological activities. Presence of different types of phytochemicals in ***M. koenigii***, provide scientific explanation to the medicinal value of the plant which may contribute to potential use of the plant in pharma industries.

Antifungal activity assay

The antifungal activity of petroleum ether, chloroform, ethylacetate, acetone, methanol and aqueous (water) extracts of *M. koenigii* leaves determined against altogether six pathogenic fungi by the agar-well diffusion method. The growth inhibitory activities of all the extracts against the tested fungi are summarized in Table-2.

The results of antifungal activity assay clearly show that all the extracts have antifungal activity against the all the tested pathogenic fungi. Fungal

Table-2 Antifungal activity of *M. koenigii* leaves extracts against tested fungi

Extracts	Zone of Inhibition (dia. in mm, Mean±SD)					
	Alternaria alternata	*Aspergillus flavus*	*Aspergillus niger*	*Candida albicans,*	*Cryptococcus neoformans*	*Fusarium moniliforme*
Pet. ether	5.33±0.25	5.13±0.55	5.26±0.16	3.26±0.19	5.66±0.43	4.96±0.26
Chloroform	6.63±0.15	5.86±0.21	5.87±0.11	6.25±0.15	6.55±0.25	5.65±0.29
Ethylacetate	10.19±0.13	8.33±0.31	10.79±0.21	11.59±0.21	10.19±0.23	12.45±0.13
Acetone	9.25±0.19	11.85±0.21	11.15±0.31	9.25±0.36	13.15±0.31	11.19±0.33
Methanol	12.23±0.21	15.63±0.56	14.23±0.35	15.43±0.33	16.13±0.23	14.83±0.16
Water	8.55±0.09	9.65±0.15	10.25±0.16	9.25±0.16	11.25±0.25	10.55±0.15
Carbendazim	15.01±0.07	18.63±0.21	17.85±0.15	16.83±0.15	18.83±0.35	17.96±0.13
DMSO	–	–	–	–	–	–

growth inhibition results shown in table-2 clearly indicate that petroleum ether, chloroform, ethylacetate, acetone, methanol and aqueous extracts of *M. koenigii* leaves exhibited varying degrees of antifungal activity against all the six test fungi. From the result, it is also evident that growth inhibition in all the fungi is highest with methanol extract suggesting highest antifungal activity as compared to other extracts tested. Of the different extracts, it is observed that growth inhibitions in all tested fungi with petroleum ether and chloroform are much less than that of positive control. However, acetone extract in case of *Aspergillus flavus, Aspergillus niger,* and *Cryptococcus neoformans* and ethylacetate in case of *Alternaria alternate*, *Candida albicans,* and *Fusarium moniliforme* are considerably effective on growth inhibition of respective test fungi as depicted in Figure-1. Methanol extract showed growth inhibition nearly at par with synthetic fungicide Carbendazim (positive control). The mean radial growth inhibition in the tested fungi, *Alternaria alternata*, *Aspergillus flavus*, *A. niger, Candida albicans, Cryptococcus neoformans* and *Fusarium moniliforme* with different extracts of *M. koenigii* leaves ranged between 5.33-12.23, 5.13-15.63, 5.26-14.23, 3.26-15.43, 5.66-16.13 and 4.96-14.83 mm respectively (Figure-1).

Minimum inhibitory concentrations (MIC) of *M.*

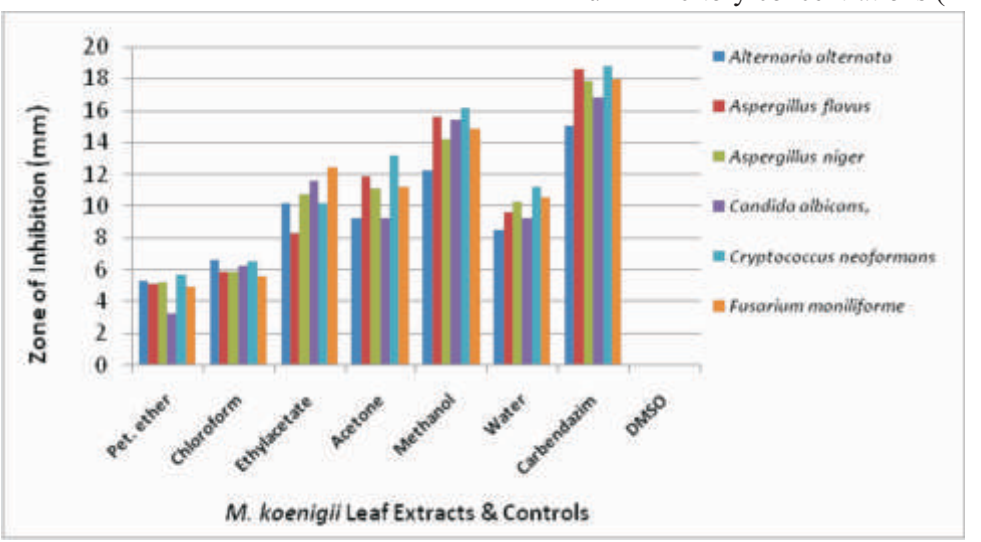

Figure-1 Zone of Inhibition (mm) in test fungi with different extracts of *M. koenigii* leaves

koenigii leaves extracts for all the six test fungi were found in the range of 8.5 – 12.25 mg/ml. It is confirmed by the results that the extracts of *M. koenigii* leaves have low MIC values against all the tested fungi.

The antifungal activity of leaves of *M. koenigii* is due to presence of phytochemical constituents of complex molecular structure and diverse action mechanisms, viz. alkaloids, terpenoids, flavonoids, phenolics, tannins, saponins that are known for their antimicrobial properties. The present investigation also supports the traditional use of the plant as antibacterial and antifungal agent. *In vitro* antifungal activity of extracts against pathogens justifies the folk medicinal use of curry leaf tree for the treatment of diarrhoea, dysentery and skin eruptions.

Conclusion

Synthetic fungicides have been used to control plant diseases for several years. Continual of such synthetic chemicals in plant protection has resulted in environmental contamination and toxicity to living organisms which has necessitated the development of ecofriendly fungicides from biological sources. Plants provide an important source of potentially useful chemical molecules for the development of new biocidal agents. Compared to synthetic fungicides, plant-derived herbal fungicides show relatively low or no toxicity, thus are safe and may serve as essential tools for plant disease management. From the present study, it is concluded that *M. koenigii* leaves exhibiting antifungal activity comparable to commercially known synthetic fungicide can be a promising source of botanical fungicide. Nevertheless, further studies will be required to investigate their specific chemical compounds responsible of the bioefficacy, mode of action, safety, and phytotoxicity in order to develop efficacious and safe fungicidal products from the plant.

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Hypoglycemic Effect of *Pterocarpus marsupium Heart Wood* Extracts on Diabetic Rats

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Abstract- Plants and plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of diabetes in many parts of the world. Polyherbal formulation which is relatively a new concept in the field of natural products is gaining much attention worldwide. These formulations are found very effective in chronic illnesses but plants selected for formulations need thorough investigation. Keeping in view the above facts, the present study focuses on effect of *Pterocarpus marsupium* heart wood extracts on diabetes induced rodent models. Heartwood of *Pterocarpus marsupium* was taken in the present study to expolore its antidiabetic potential. Various extracts of the heart wood were prepared using different solvents in an order of their increasing polarity and all extracts so prepared, were evaluated for their antidiabetic potential. The pharmacological study of current phase showed the excellent anti-hyperglycemic potential of the aqueous extract causing reduction in blood glucose level by 55.56% as compared to the standard glibenclamide 62.22 %. Further, aqueous extract was found to be safe up to a dose level of 2000 mg/kg after performing acute toxicity studies.

Keywords: *Pterocarpus marsupium,* Glibenclamide, Antidiabetic

Introduction

Diabetes mellitus often referred to simply as diabetes, is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from low levels of the hormone insulin with or without abnormal resistance to insulin's effects. The characteristic symptoms are excessive urine production (polyuria), excessive thirst and increased fluid intake (polydipsia), blurred vision, unexplained weight loss and lethargy. These symptoms are likely to be absent if the blood sugar is only mildly elevated[1]. Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of diabetes in many parts of the world. In the present times, available evidence suggests a high prevalence of utilization of alternative medicines for the treatment of diabetes in some regions of the world. Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in the treatment of various human ailments. It would be therefore, worthwhile to search some alternate remedies for diabetes, which would be acceptable to the public[2]. Poly-herbal formulation which is relatively a new concept in the field of natural products is gaining much attention worldwide[3]. These formulations were found very effective in chronic illnesses. In treatment of diabetes many polyherbal formulations are available in the market and are also very effective. With this aim in mind, the present study was attempted to explore anti-diabetic potential of few medicinal plants so that new formulation could be developed. Plant selected for the present study was *Pterocarpus marsupium* (Vijaysar), various extracts of its heart wood was prepared and its anti-diabetic effect was studied.

Material and Methods

All chemicals and reagents used in study were of laboratory grade and purchased from HiMedia and Rankem Pvt Ltd. Magnifying lens, sieve (No. 250), UV-Vis spectrophotometer (UV-250, SYSTRONIC, India), light microscope (Sigma Scientific Instruments, Chennai), muffle furnace, water bath, desiccators (Perfit, India), UV-chamber (Camage UV-Capinet), HPTLC (Camage Linnomate 5), pre-coated TLC (silica gel 60 F450 – Merck), and ash less filter paper (Merck) were used for the study.

Procurement of Plant Material

Heartwood of *Pterocarpus marsupium was procured from* Dehradun, Uk., India and authenticated by NISCAIR, New Delhi having Consult/2010-11/1676/274 (*Pterocarpus marsupium*). The plant material was subjected to their standardization as per the parameters suggested by WHO[4] and results were compiled in order to identify and quantify the details of plant materials.

Preparation of Extracts

The heartwood of *Pterocarpus marsupium* was dried in shade at room temperature for 2 days followed by drying at 40-50°C for 3-4 hrs and powdered to obtain coarse powder. 980 g of powder was extracted with petroleum ether, chloroform, acetone and methanol and water successively to collect extracts of different polarity compounds. The solvents were removed by evaporation under reduced pressure to obtain a semi-solid mass. The extracts were kept in separate desiccators followed by weighing to calculate the percentage yield of each extract

Standardization of Raw Material

It is found that in Indian herbal market in place of genuine crude drug, botanical drugs having similar appearance are being sold. Hence, strict need of serious measures to check the adulteration is very prominent. This can only be achieved by raw drug standardization, domestication and cultivation of medicinal plants to ensure sustained supply of quality plant material to production centres. Standardization of raw drug includes passport data of raw plant drugs in respect to taxonomic identification and authentication study on the material part – root, rhizomes, stem, bark, leaf, flower, fruit, seed, etc.; collection detail – location, stage and development of plant, time, pre-processing storage etc.; organoleptic character of the raw materials – odour, taste, colour, microscopic, macroscopic description, and chemical composition.

Macroscopic and Microscopic Examination

Microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface. However, since these characteristics are judged subjectively, substitutes or adulterants may closely resemble the genuine material.

Determination of Foreign Matter

To determine the foreign matter, content material was spread on a thin layer and the foreign matter was sorted either by visual inspection, using a magnifying lens (6x or 10x), or with the help of a suitable sieve, according to the requirements for the specific plant material. The remainder of the sample is passed through a No. 250 sieve; dust is regarded as mineral admixture. The portion of this sorted foreign matter is weighed to calculate the foreign matters.

Determination of Moisture content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. The most common method of determining moisture content is loss on drying (LOD). LOD is the loss of mass expressed as percent w/w. To estimate the LOD, 5g of the air dried crude drug is accurately weighed in a flat

dried weighing bottle and then dried to constant mass, the final weight is noted and the LOD calculated by formula.

$$LOD = \frac{\text{Final Wt.}}{\text{Initial Wt.}} \times 100$$

Determination of Volatile Oils

In order to determine the volume of volatile oil, the plant material is distilled with water and the distillate is collected in a graduated tube. The aqueous portion separates automatically and is returned to the distillation flask. For this purpose, about 10 g of the plant sample is placed in a round bottom flask already fitted with a Clevenger apparatus and distilled for about 15 hrs. The oil (if any) is collected and weighed. The yield of volatile oil is quantity expressed in mg per 100 g of the plant material.

Total Ash Value

The *total ash* method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

For determination of total ash 2-4 g of the ground air-dried material, accurately weighed, in a previously ignited and tarred crucible (usually of platinum or silica) and spread in an even layer. It is then ignited by gradually increasing the heat to 500- 600°C until it is white, indicating the absence of carbon. The residue is allowed to cool in a suitable desiccators for 30 minutes, and weighed without delay. The content of total ash is calculated in mg per g of air-dried material.

Acid Insoluble Ash

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

For determination of acid insoluble ash 25 ml of hydrochloric acid (~70g/l) is added to crucible containing total ash with a watch-glass and boiled gently for next 5 minutes. The watch-glass is rinsed with 5 ml of hot water and added to the crucible. The insoluble matter is collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter is transferred to the original crucible, dried on a hot-plate and ignites to constant weight. The residue is allowed to cool in a suitable desiccators for 30 minutes, and weighed without delay. The content of acid-insoluble ash is calculated in mg per g of air-dried material.

Total Alcohol Extractive

For the determination of amount of active constituents extracted with ethanol from a given amount of sample by cold maceration method, placed about 4.0 g of coarsely powdered air-dried material, accurately weighed in a glass-stoppered conical flask with 100 ml of the ethanol for 6 hours, shaking frequently, and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent, transferred 25 ml of the filtrate to a flat-bottomed dish and evaporated to dryness on a water bath. Dried at 105°C for 6 hours, cooled in a desiccators for 30 minutes and weighed without delay. Calculated the content of extractable matter in mg per g of air dried material.

Total Water Extractive

For the determination of amount of active constituents extracted with water from a given amount of sample, similar procedure was adopted as described above for total alcohol extractive except replacing ethanol by water. Calculated the content of extractable matter in mg per g of air dried material.

Animal toxicity[5,6]

Acute toxicity study of aqueous extract of *Pterocarpus marsupium,* was carried out on albino mice (According to Knudsen and Curtis). The animals used in the toxicity studies were

approved by the Institutional Animal Ethics committee, approved by CPCSEA. (Reg No. CPCSEA/273) Albino mice of sex and body weight between 25-30 gms, aged 8-10 weeks were taken for the study. After 18 hrs of fasting, a dose of 250 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg and 2000mg/kg of body weight was administered orally to each group, having 6 animals each. The mortality and motor reflux response was observed for a period of 72 hrs.

Antidiabetic Activity[7]

Albino rats of wistar strains of either sex were taken for the study having a body weight of 80 to 200 gms. Animals were housed in polypropylene cages in Institutional Animal House of SBSPGI, Balawala, Dehradun and fed with normal rat pellet diet and water *ad libitum*. Diabetes was induced to the animals by giving an intra peritoneal dose of alloxan 100 mg/kg of body weight to the overnight fasted rats. After 72 hours of alloxan administration, the blood of animals was analyzed for blood glucose level and all those animals which were having a blood glucose level more than 150 mg/dl were considered diabetic. The diabetic rats were then grouped in different groups having 05 animals in each group. All animals in each group were marked for their identification (H = Head, B = Back, T = Tail, P =Plain, HT = Head-Tail). These animals then received different types of treatment. Plant extracts were given at a dose level of 150 mg/Kg body weight. All extracts of heartwood were administered to animals and reduction in blood glucose level was studied.

Results and Discussion

Results of Standardization are summarized in table-1. Various extracts of the plant were prepared and their antidiabetic and related effects were studied. Results are summarized in table-2. From the results, it could be seen that the percentage reduction in blood glucose level was maximum (5.25 ± 2.24) in case of petroleum ether extract followed in increasing order by chloroform (19.72 ± 3.75), acetone (30.85 ± 2.95), methanol (47.81 ± 1.43) extract of the plant *P.*

Table-1 Standardisation Parameters

Parameters	Pterocarpus marsupium	
	Experimental Yield	Reports existing
Loss on Drying (%)	10.50 %	-
Percentage Volatile Oils (%)	Absent	-
Ash Value (%)	5.19%	NMT 2 %
Acid Insoluble Ash (%)	1.396%	NMT 0.5 %
Alcohol Extractives (%)	2.056%	NLT 7 %
Water Extractives (%)	11.62%	NLT 5 %

NMT = Not more than this
NLT = Not less than this

Table-2 Result of Preliminary Antidiabetic Activity

Group	Initial Blood Glucose Level (mg/dl)	Blood Glucose Level After Treatment (mg/dl)	% Reduction in Blood glucose Level
Normal	112.8±14.956	108±10.134	3.09±5.33
Control	224±13.627	217±10.7	3.15±1.21
Standard (Glibenclamide)	223±24.259	84.2±9.418	62.22±1.68
Pet. Ether *P marsupium*	228.8±39.289	216.2±33.116	5.25±2.24
Chloroform *P marsupium*	173±36.297	138.4±26.406	19.72±3.75
Acetone *P marsupium*	199.6±10.164	137.8±2.863	30.85±2.95
Methanol *P marsupium*	183±69.489	95.25±35.169	47.81±1.43
Aqueous *P marsupium*	211.5±41.251	94±23.137	55.56±3.43

marsupium. The aquous extract of the said plant showed the maximum percentage reduction in blood glucose level.

Conclusion

It is concluded that among all the extracts of *P. marsupium,* aquous extract showed the maximum reduction in blood glucose level by 55.56% as compared to the standard drug glibenclamide, 62.22%.

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Screening of i*n- vitro* Antidiabetic Activity of Herbs

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Abstract – *In-vitro* analysis of the anti-diabetic effect of aqueous extracts of the medicinal plants of *Mangifera indica*, *Syzygium cumini, Cinnamomum zeylanicum*, *Cinnamomum tamala* and *Valeriana wallichii* was undertaken in this study. They were tested for inhibition of α-amylase activity by DNSA colour reagent. The aqueous extract of seeds of *Syzygium cumini* showed maximum inhibition of α-amylase activity. Seeds of *Mangifera indica*, bark of *Cinnamomum zeylanicum* and *Cinnamomum tamala* showed both a strong inhibition of α-amylase. *Valeriana wallichii* showed low inhibition of α-amylase activity, *Syzygium cumini* was found to possess maximum anti-diabetic properties. The findings indicate that all the above plants possess antidiabetic properties with varying degrees. They can be used to develop natural drugs which may be used in lieu of commonly used strong allopathic drugs which possess a number of harmful side effects.

Keywords: Antidiabetic activity, Herbs, α-amylase

Introduction

Diabetes is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications. In 2007, diabetes caused 3.5 million deaths globally (Das, 2008). Diabetes affects mainly the developing countries like India. Indeed, India presently has the largest number of diabetic patients in the world and has been infamously dubbed as the 'diabetic capital of the world' (Abate and Chandila, 2007). Diabetes mellitus is epidemic in India as a result of societal influence and changing lifestyles. Diabetes has been known in India for centuries as 'a disease of rich man' but has now spread among all masses (Gupta and Mishra, 2007).

Diabetes Milletus (DM) is characterized by elevated plasma glucose concentrations resulting from insufficient insulin and insulin resistance, or both, along with disturbances in metabolism of lipids, carbohydrates and proteins (Patel et al. 2011; Warjeet, 2011). As the disease advances, it leads to complications like retinopathy, neuropathy, nephropathy, stroke, ischemic heart disease, peripheral vascular disease and a wide array of heterogeneous diseases (WHO, 2006). The incidence of diabetes in developing countries have reached epidemic proportions and International Diabetes Federation (IDF) projects a rise from 382 million people with diabetes to 592 million between 2013 and 2035 (IDF, 2014). The current treatment to combat type-2 diabetes is the usage of oral hypoglycemic drugs such as α-glucosidase inhibitors, sulphonylureas, biguanides, thiazolidenediones and meglitinide analogues besides injectable insulin, which become important when blood glucose levels cannot be controlled by diet, exercise, weight loss and oral medications. Whereas, insulin replacement therapy is the mainstay for patients with type-1 DM (Kumar and Clark, 2002; O'Dea, 1984). However, prominent side-effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have no side-effects. This has led to the growing interest in phytomedicines. Their extracts are studied to know about their efficacy, safety and mechanism of action. The same has been recommended by World Health Organization (WHO), as 80% of the people rely on plant based medication (Mentreddy et al.2005). The effect of these plant extracts can be studied *in-vitro* that includes perfused whole organs, tissues, cells in primary or immortal culture, sub cellular

membranes, purified receptor and enzymes that can establish mechanisms and definite toxicities. Further, biological systems can be studied using live animals which are necessary to study how such mechanisms behave under clinical or pathophysiological conditions (Kruthika et al. 2012). *In-vitro* models are fairly based on a specific process, wherein activity of an enzyme on a metabolic reaction or binding to a receptor within a given cell type can be studied. *In-vitro* studies are of considerable value in identifying the mechanism of action of a test material and are more economical. It provides an alternative to animal testing in many aspects which include investigation of organs or tissues derived from animals that could be used to test many replicates or samples. Immortalized cell lines, although originally derived from animals or humans, do away completely with the repeated need for animal tissue in assays. There is reduced variability in cells from a single cell line and have the advantage of genetic homogeneity. *In-vitro* assay is an ideal way of obtaining active components with defined biological activity during fractionation process and later can be tested *in vivo* to confirm their effects (Amalasoumyanath, 2006).

Material and Methods

All the plants used are of Indian origin and are well known by herbal pharmacologists for their medicinal properties.

Inhibition of carbohydrate digesting enzyme

Pancreatic α-amylase

Pancreatic α-amylase, an important enzyme of digestive system hydrolyzes starch into mixture of smaller oligosaccharides comprising of maltose, maltotriose and oligoglucans which are further degraded by glucosidase into glucose that enters the blood stream upon absorption.

This leads to elevated Post-prandial hypergylcemia (PPHG). Hence, it is important to control these two aspects in the treatment of type 2 diabetes (Eichler, 1984). The inhibition assay is carried out as illustrated by Miller (Miller, 1959)using the chromogenic 3,5- dinitrosalicylic acid (DNSA) method. The assay mixture is prepared using 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 6 mM sodium chloride, 0.04 units of Porcine Pancreatic α-amylase (PPA) solution and test samples with different concentrations were preincubated at 37°C for 10 minutes. To the same, 500 µL of 1% (w/v) starch solution (prepared using above buffer) was added and incubated at 37°C for 15 minutes. DNSA reagent (1.0 mL) is added to stop the reaction and placed in boiling water bath for 5 minutes, cooled, diluted and measured at 540 nm. The control without test sample represents 100% enzyme activity. The absorbance produced by test sample is eliminated by including appropriate sample control without enzyme and starch. Acarbose, a known PPA inhibitor can be used as a positive control.

The % of inhibition for α- amylase is calculated as follows

$$\% \text{ Inhibition} = EC - (ET - TC)/EC \times 100$$

Where, EC is enzyme activity of control, ET is enzyme activity of test and TC is test control.

Table- Inhibition of pancreatic α-amylase by herbs

Concentration µg/ml	Percentage inhibition of Pancreatic α-amylase by different herbs				
	Syzygium cumini	***Mangifera indica***	***Cinnamomum zeylanicum***	***Cinnamomum tamala***	***Valeriana wallichii***
20	23.33±0.5	20.21±0.5	18.23±0.5	16.43±0.5	13.33±0.5
40	34.44±0.8	31.22±0.8	29.34±0.8	24.44±0.8	20.14±0.8
60	56.66±0.2	52.46±0.2	40.61±0.2	36.62±0.2	27.66±0.2
80	71.48±0.6	69.18±0.6	58.48±0.6	41.44±0.6	38.48±0.6
100	84.11±0.2	75.10±0.2	71.11±0.2	65.11±0.2	54.12±0.2

Results and Discussion

Inhibition assay for α-amylase activity (DNSA)
The results of the DNSA study are summarized in Table. All the above five plants showed varying effect on glucose utilization. At all concentrations, Jamun seeds (*Syzygium cumini*) showed maximum inhibition of the enzyme with the highest value of 84% seen at 100 mg/ml concentration of plant extract. *Mangifera indica* seeds showed the next highest value of 75.10% seen at 100 mg/ml concentration of plant extract. *Cinnamomum zeylanicum* and *Cinnamomum tamala* showed the third highest value of 71.11% and 65.11 seen at 100 mg/ml concentration of the plant extracts and *Valeriana wallichii* showed the least inhibition of enzyme with a highest value of only 54.12% seen at 100 mg/ml concentration of plant extract.

Plants serve as an excellent source of various therapeutic agents. One of the major advantages of using plants is that they seldom show the deleterious side effects commonly associated with other allopathic drugs. This study investigated the ability of *Syzygium cumini*, *Mangifera indica* seeds, *Cinnamomum zeylanicum* and *Cinnamomum tamala* to serve as effective anti-diabetic agents.

Syzygium cumini is reported to have hypolipedemic effect; it reduces blood cholesterol, triglycerides and free fatty acids**.**

Conclusion

This study provides scientific evidence of anti-diabetic effect of these five plants- *Syzygium cumini*, *Mangifera indica*, *Cinnamomum zeylanicum, Cinnamomum tamala and Valeriana wallichii*. These plants are very affordable to the common man and can be easily incorporated in their daily diets. They can be further analysed to develop anti-diabetic drugs free from harmful side effects.

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Essential Oil Composition of Industrially Important *Ocimum basilicum* Cultivated by Farmers of Uttarakhand, India

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Abstract- A study on chemical composition of essential of basil cultivated in different areas was carried out. The hydro-distilled essential oil content from leaves of basil (*Ocimum basilicum L.*) ranged from 0.5% to 0.8 % (v/w). The maximum amount was observed in Jaspur and Udam Singh Nagar while the minimum in Khanpur, Haridwar, UK., India. The essential oils consisted of methyl Chevicol as the most abundant component (76.9-70.7%) followed by linalool (20.3-18.3%) and α-biabolene (1.9-1.1%). Cultivated produce of farmers and the essential oil composition in the areas under study are more or less the same in respect of its major constituents.

Keywords: Variation, Hydro distillation GC-MS, Methyl chevicol, Linalool

Introduction

Basil (*Ocimum* spp.), belonging to the Lamiaceae family, is a perennial shrub which grows in several regions all over the world[1-2].The *Ocimum* genus (Lamiaceae) are annual and perennial herbs and scrubs native to the tropical and subtropical regions of Asia, Africa and Central South America[3-4]. It is traditionally used as medicinal herb in the treatment of headaches, coughs, diaorrhea, worms and kidney malfunctions[5]. It also has a long history as culinary herb, its foliage adds a distinctive flavor to many foods. It is also considered to be a source of aroma compounds and essential oils containing biologically active constituents that are insect repellent, nematocidal and possesses antibacterial activity[6-8].

Basil essential oil has been extensively used in the flavouring of confectionery and baked goods condiments, sausages and meat, salad dressing, non alcoholic beverages, ice creams. It has also found wide application in perfumery, as well as in dental and oral products[9]. The essential oils in different basil cultivars are variable.

The prevalent components are monoterpenes and phenylpropanoids[10-11]. Many *Ocimum* species contain primarily monoterpene derivatives such as limonene, camphor, 1, 8-cineole, linalool and geraniol[12-13]. Others contain primarily phenyl derivatives, such as eugenol, methyleugenol, chevicol, estragole, methyl-cinnamate, often combined with various amounts of linalool[14- 15]. There are usually considerable variations in the contents of the major components within this species obtained from different geographical origins[16].

In view of this, there is lot of possibility to variability in cultivated produce by farmers in different regions of Uttarakhand, no study, so far is reported till date in this respect and hence the present investigation on essential oil composition of *O. basilicum* harvested by farmers in different areas of Uttarakhand, India.

Material and Methods

Plant material

Leaves of *Ocimum basilicum* were collected from Vikasnagar, Haridwar, Jaspur and Rajawala regions of Uttarakhand. These leaves were

cultivated by the registered farmers in the month of December, 2009. The species authentification and identification was done by Botanical survey of India, North circle, Dehradun.

Extraction of Essential oils

Shade dried leaves of each plant population were separately hydro-distilled for 4 hours using a Clevenger apparatus[17]. The oil contents [(v/w) (%)] were estimated on a dry weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and stored in sealed vials at 4^0C before analysis.

Gas Chromatography

The gas chromatograph (GC) analysis of the volatile oils were carried out using an Agilent Technology 7890 gas chromatograph equipped with a FID detector and a HP-5 fused silica column (30 m x 0.32 mm x 0.2 µm film thickness). Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210°C and 230°C respectively. The column oven temperature was programmed from 60°C to 220°C with an increase in rate of 3°C/min. The injection volume was 0.02 µL.

Gas Chromatography-Mass Spectrometry

The GC-MS analysis of the volatile oils was performed on Agilent Technology 7890 mass spectrometer (Model 7890) coupled to a gas chromatograph with a 60 m x 0.32 mm x o.2µm, film thickness column (HP-5). Helium was used as the carrier gas (flow rate 1ml/min). The oven temperature was programmed, range being from 60°C to 220°C at 3°C/min. Other conditions were the same as described for gas chromatography. The mass spectrum was recorded using a mass range of 40-600 Daltons. The identification of the constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, C_9-C_{24} under experimental conditions, co-injection with standards (Aldrich and Fluka), MS library search (NIST and WILEY), and by comparing with the MS literature. The relative amounts of the individual components were calculated based on the GC peak area (FID response) without correction factors.

Results and Discussion

Results of the present study are represented in the Table. The study revealed that twelve components were identified in essential oil obtained from leaves of *O. basilicum* harvested in different areas. In case of Jaspur area, the component, Biclogermacrene was found absent. Methyl chevicol was found as major constituents in all the areas, the quantity of which varied between 70.7-76.9%. It was followed by linalool ranging between 18.3- 20.0 %. These constituents including other minor constituents represented total 98.8- 99.5 % of oil. Minor constituents also vary from one location to another location. The percentage composition of Methyl chevicol in *Ocimum Basilicum* was quite high. This finding is consistent to the previous publication[18-20]. *Ocimum Basilicum* species grown in Yemen and Thailand on various chemotypes are being studied. They are found to contain methyl chevicol as the major compound in agreement with the previous study[21]. Seven types of chemotypes presented by Isa Telci *et al.* 2006 also included methyl chevicol (68.3%) in case of *Ocimum Basilicum* varieties grown in Turkey[21]. It is well reported that variations of the essential oils depends on the type of cultivar as well as on the agronomical practices. The environmental conditions also affect the composition of sensory important compounds[21-22]. Regardless of these factors, 1, 8-cineole, methyl cinnamate, methyl chevicol, and linalool[23] are generally the main compounds responsible for the typical basil aroma. On the basis of more than 200 analyses of essential oils isolated from *O. basilicum* L.[24], four major essential oil chemotypes of basil are classified: (1) methyl chevicol-rich, (2) linalool-rich, (3) methyleugenol-rich, (4) methyl cinnamate- rich, alongwith numerous subtypes. The antimicrobial activities of essential oils from *O. basilicum* may

Table- Chemical Composition of *Ocimum basicilium* in Uttarakhand Himalaya

S.N.	Components	RI	Vikas Nagar (Dehradun) %	Rajawala (Dehradun) %	Jaspur (Udham Singh Nagar) %	Khanpur (Haridwar) %
1.	Linalool	1099	20.3	20.0	18.3	20.0
2.	Methyl chevicol	1203	**72.7**	**71.0**	**76.9**	**70.7**
3.	Cis – Citral	1253	0.76	1.3	0.73	1.3
4.	Trans- Citral	1267	1.0	1.8	0.92	1.8
5.	α- Biabolene	1689	1.8	1.9	1.1	1.9
6.	Cis- geraniol	1255	0.06	0.06	0.06	0.11
7.	Trans - Caryophyllene	1431	0.47	0.29	0.27	0.25
8	α- Bergamotene	1439	0.44	0.51	0.24	0.50
9.	α- Humulene	1457	0.08	0.52	0.36	0.52
10.	Germacrene-D	1489	0.11	0.06	0.36	0.56
11.	β- Cubenene	1389	0.55	0.57	0.30	0.59
12.	Biclogermacrene	1530	0.59	0.62	-	0.08
	Total (%)		**98.86**	**98.63**	**99.54**	**98.31**

be in part due to the presence of high content of linalool[25-28].

Conclusion

It is concluded that the chemical composition of major constituents, methyl chevicol and linaloon in the essential oil from the harvested *O. basilicum* was found to be more or less the same in all the different sites under study.

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Cardioactive Potential of *Allium humile* (AH) Methanolic Leaves Extract and Its Fraction in Ischaemia and Reperfusion-Induced Myocardial Injury

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Abstract- In search of isolation of active *cardioactive* compound from *Alium humile,* the present study has been designed to evaluate the efficacy of active fraction from *Allium humile* leaves methanol extract (active) to limit myocardial injury in wister albino rats. Active methanolic extract of Alium humile leaves was purified using chromatographic technique that results four major fractions .These fractions (F1, F2, F3, F4) were screened for myocardial injury. Among these fractions, F4 was found to be hot fraction. In the present study, we are reporting cardioprotective potential of active methanolic extract of *A. humile* leaves and its fraction F4. Fraction F4 of the extract significantly prevented myocardial infarct size, release in LDH and CKMB, level important parameters to establish well said cardioprotective activity as compared with that of standard drug, Ramipril. Fraction F4 significantly attenuated ischaemia and reperfusion induced increase in myocardial infarct size. The present findings suggest that fraction F4 of methanol extract of *A.humile* leaves are significantly effective to ameliorate myocardial ischaemic injury as compared to ischaemia and reperfusion induced control group. Moreover, some extensive work in this direction could also lead to isolation pf active principle from the same and explore the possible mechanism of *A.humile* against ischaemia and reperfusion induced myocardial injury.

Keywords: Ischaemia, Reperfusion, *Allium humile*, Ramipril, Chromatography.

Introduction

The use of herbal preparation is increasing in the treatment of cardiovascular disease because of various possible mechanism involved in the cardio protection. Therefore, herbal extracts that are traditionally used, were evaluated against in limiting the deleterious effects of ischaemia and reperfusion- induced myocardial injury. Furthermore, the results are statistically analysed and validated for prophylactic approaches and as an adjunct to standard treatment of ischaemia and reperfusion-induced myocardial injury[1,2].

Small Alpine Onion *Allium humile (A.nivale; A.gowanianum)* belongs to family Alliaceae and is a species of onion found in the Himalayas at altitudes of 3000-4000 m. Flowers are white, star-shaped, in a rather lax umbel 2.5-4 cm across, borne on a leafy stem. Narrow-elliptic petals, about 1 cm long, spread outwards and are much longer than the stamens. Leaves are many, flat, 2-5 mm broad, blunt, usually shorter at flowering than the stem. The stem itself is 7-25 cm tall. Bulbs are clustered, cylindrical, covered with fibrous leaf-bases. Edible plant part used includes flowers, leaves, root and bulb. Leaves and inflorescences are also used as seasoning agents. There are reports[3,4] about it describing blood purification, anti- inflammatory, anti-asthamatic and anti-diabetic activities. The potential therapeutic value of *A.humile* in ischaemia and reperfusion myocardial injury has yet not been evaluated. Therefore, in search of isolation of active

cardioactive compound from *Alium humile* leaves, the present study has been designed to evaluate the efficacy of active fraction from *Allium humile* leaves methanol extract (active) in terms of reduction in infarct size increase in release of LDH and CKMB biochemical parameters to establish cardioprotective activity.

Material and Methods

Drugs and chemicals

Ramipril is taken as a gift sample from USV Baddi, Himanchal Pradesh, Himanchal, India. All the reagents used in this study were of analytical grade and were always freshly prepared before use.

Plant material

Leaves of *Allium humile* was collected from Chamoli District, Uttarakhand, India. The plant material was identified from Botanical Survey of India, Northern Regional Centre, Dehradun, India with the reference number BSI/NRC 9 (Tech.)/2010-03/839/12796.

Animals

Adult Wister rats of either sex, weighing 250 to 300g maintained on rat feed (Ashirwad Feeds Ltd., Chandigarh, India) and tap water ad libitum were used in the present study. They were exposed to 12h cycle of light and dark. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (Reg. no.273/CPCSEA). Animals were obtained from IVRI Bareilly, India and were maintained under standard laboratory conditions in the departmental animal house of SBSPGI, Dehradun, Uttarakhand, India.

Preparation of extracts

The fresh leaves of *A. humile* were dried in shade and room temperature for 2days followed by drying [40-50^{o}C] for 3-4hrs and then powdered to obtain coarse powder. 980g of powder of *A.humile* leaves were extracted with pet ether, chloroform, acetone and methanol to get four extracts. The solvent was removed by evaporation under reduced pressure to obtain a semi-solid mass. The resultant extracts were kept in a desiccators followed by weighing to give percentage yield of each extract. The methanolic extract was subjected to column chromatography using silica gel mesh size 200-400µ and chloroform: methanol as mobile phase in different ratio led in to the isolation of four fractions, F1, F2, F3 and F4. The cardio protective activity was evaluated for all four fractions in which fraction F4 of methanol extract was found significantly effective.

Acute toxicity study

Albino mice of 10 animals per group and weighing 20-25g were administered graded dose (100-2000 mg/kg body weight, orally) of the methanol extract of *A.humile*. After administration of extract, mice were observed for toxic effects after 48hr of treatment. The toxicological effects were observed in terms of mortality expressed as LD_{50}. The number of animals dying during the period was noted. The LD_{50} of the extract was determined by Litchfield and Wilcoxon, 1949 method. No mortality was observed, therefore the extract was safe to use even at the doses of 2000 mg/kg of body weight orally.

Isolated rat heart preparation[5]

Rats were heparinised (500 IU/L, i.p.) and sacrificed after 20 min by cervical dislocation. The heart was rapidly excised and immediately mounted on Langendorff's apparatus. The temperature was maintained at 37^{o}C by circulating hot water. The preparation was perfused with krebs Henseleit (K-H) buffer (NaCl 118 Mn; KCl 4.7 Mm; $CaCl_2$ 2.5 Mm; $MgSO_4.7H_2O$ 1.2 Mm; KH_2PO_4 1.2Mm; $C_6H_{12}O_6$ 11 Mm), pH 7.4 and bubbled with 95% O_2 and 5% CO_2. The coronary flow rate was maintained 6-9 ml/min and perfusion pressure was kept constant at 70 mm Hg. Global ischemia was produced for 30 min by completely closing the inflow of physiological solution and followed by 120 min of reperfusion. The coronary effluent was collected before

ischaemia, immediately 5 min, 30 min and 120 min after reperfusion for estimation of LDH and CK-MB.

Assessment of myocardial injury

The myocardial infarct size was measured using the triphenyltetrazolium chloride (TTC) staining method. The level of LDH and CK-MB (Siemens Medical Solution Diagnostic Ltd., Baroda, India) in coronary effluents was estimated using commercially available kits. Values of LDH and CK-MB were expressed in international units per litre (IU/L).

Assessment of myocardial infarct size[6]

Heart was removed from the Langendorff's apparatus. Both the auricles and the root of aorta were excised, and ventricles were kept overnight at temperature of -4°C. Frozen ventricles were sliced into uniform sections of 1-2 mm thickness. The slices were incubated in 1% w/v TTC solution in 0.2M Tris-chloride buffer, pH 7.8 for 20 min at 37°C. The normal myocardium was stained brick red while the infarcted portion remained unstained. Infarct size was measured by macroscopic volume method.

Experimental protocol

In all groups, isolated rat heart was perfused with K-H solution and allowed to stabilize for 10min.

Group-1 (Sham control; n=5) After stabilization isolated rat heart was perfused continuously with K-H buffer for 160 min. without subjecting it to global ischaemia.

Group-2 (Vehicle control; n=5) Rats were administered 1% Tween 80 orally for 7 days; thereafter, on the 7th day, isolated rat heart after stabilization, was subjected to 30 min of global ischaemia followed by reperfusion for 120 min.

Group-3 (Standard; n=5) Ramipril (1 mg/kg) was dissolved in 1% Tween 80 and administered orally once daily to rats for 7 days; thereafter, on the 7th day, isolated rat heart after stabilization, was subjected to 30 min of global ischaemia followed by reperfusion for 120 min.

Group-4 (Methanol extract; n=5) Methanol extract (100 mg/kg) was dissolved in 1% Tween 80 and administered orally once daily to rats for 7 days; thereafter, on the 7th day, isolated rat heart after stabilization, was subjected to 30 min of global ischaemia followed by reperfusion for 120 min.

Group-5 (Fraction 4 of Methanol extract; n=5) Fraction 4 of methanol extract (100 mg/kg) was dissolved in 1% tween 80 and administered orally once daily to rats for 7 days; thereafter, on the 7th day, isolated rat heart after stabilization, was subjected to 30min of global ischaemia followed by reperfusion for 120 min.

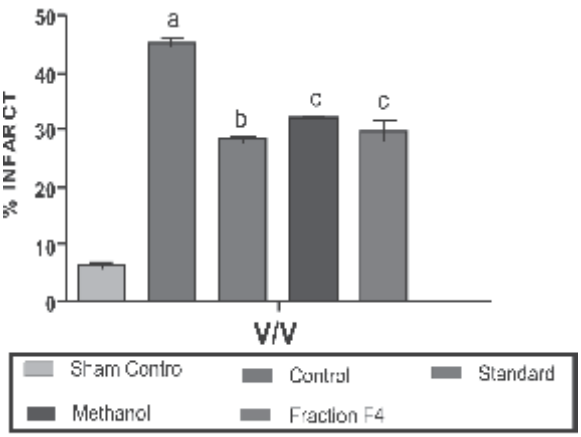

Figure-1 Assessment of Myocardial Infarct Size.

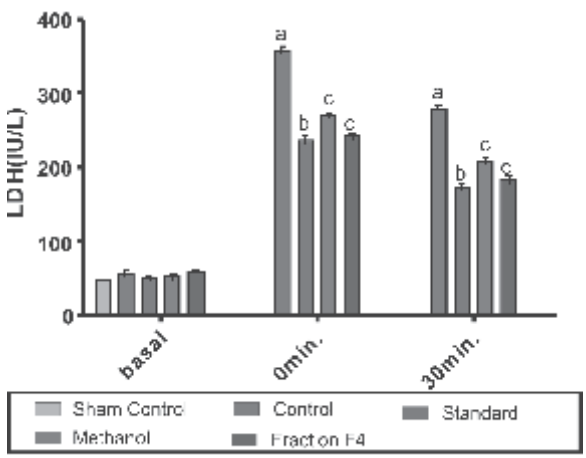

Figure-2 Effect of Ischaemia and Reperfusion on LDH release.

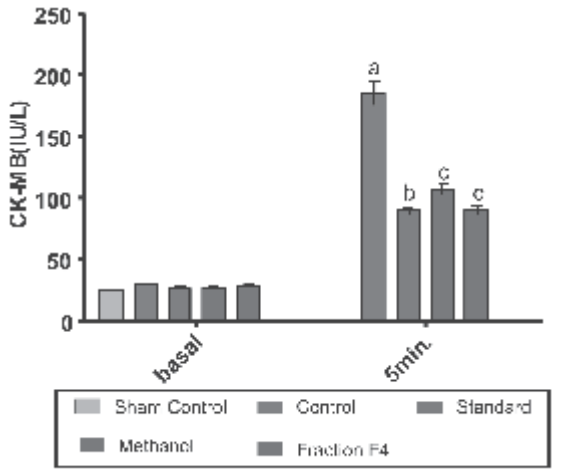

Figure-3 Effect of Ischaemia and Reperfusion on CK-MB release.

Infarct size was measured by volume method. Values are expressed as mean ±SEM. a=P<0.05 vs. Sham control; b= P<0.05 vs. Control; c= P<0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test.

LDH was estimated in coronary effluent after stabilization (Basal), Immediately (Imm'Rep.) and 30 min. after reperfusion (30' Rep.). Values are expressed as mean±SEM. a=P<0.05 vs. Sham control; b=P<0.05 vs. Control; c=P<0.05 vs. Standard. ANOVA followed by Tukey's multiple comparison test.

Statistical Analysis

All values for enzymatic data (LDH and CK-MB) and infarct size were expressed as mean ±SEM. Statistical analysis was performed using Graph Pad Prism Software. The values were statistically analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Value of P<0.05 was considered to be statistically significant.

Results and Discussion

Effect on Myocardial Infarct Size

Active methanol extract of *A. Humile* leaves was purified by using column chromatography which resulted isolation of four fraction viz. F1, F2, F3 and F4 which were again evaluated for above said effect and among all the fractions, fraction F4 significantly attenuated ischaemia and reperfusion induced increase in myocardial infarct size.

Effect on Ischaemia and Reperfusion Induced release of LDH

Active methanolic extract of *A. humile* leaves and its four fractions F1 F2, F3 and F4 were evaluated on ischaemia and reperfusion induced increase in release of LDH in coronary effluent measured immediately and 30 min. after reperfusion. Fraction F4 from extract significantly reduced release of LDH in coronary effluent. Effluent measured immediately and 30 min after reperfusion.

Effect on Ischaemia and Reperfusion Induced release of CK-MB

Active methanolic extract of *A.humile* leaves and its four fractions F1 F2, F3 and F4 were evaluated on ischaemia and reperfusion induced increase in release of CK-MB measured in coronary effluent collected after 5 min of reperfusion. Fraction F4 from methanolic extract significantly reduced release of CK-MB in coronary effluent. CK-MB in coronary effluent collected after 5 min of reperfusion. Moreover, treatment with standard (Ramipril, 1 mg/kg) markedly reduced release of CK-MB in coronary effluent, collected 5 min of reperfusion (Figure-3).

Inspite of the disadvantage of high mortality, high heart rate and high rate of drug metabolism, albino rats are used in the present study because they are small in size having low cost and readily available. Moreover, histological sectioning and quantification is easy in rat hearts due to small size. Isolated perfused rat heart preparation has been employed in the present study because it permits the use of pharmacological interventions without any interference due to change in systemic circulation. Various extracts of *A.humile* leaves viz. petroleum ether, acetone, chloroform

and methanol at a dose level of 100 mg/kg were evaluated for ischaemia and reperfusion induced myocardial injury. Further purification of active extract was carried out using column chromatography which resulted isolation of four fraction viz. F1, F2, F3 and F4. These fractions were again evaluated for above said effect and among all the fractions Fraction F4 significantly attenuated ischaemia and reperfusion induced increase in myocardial infarct size.

Conclusion

The present findings suggest that fraction F4 of methanol extract of *A.humile* leaves are significantly effective to ameliorate myocardial ischaemic injury as compared to ischaemia and reperfusion induced control group. Moreover, some extensive work in this direction could also lead to isolation of active principle from the same and explore the possible mechanism of *A.humile* against ischaemia and reperfusion induced myocardial injury.

Acknowledgement

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Inhibitory Activity of Ophthacare Against Pathogens

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Abstract - Ophtha Care brand eye drops combats infective and allergic ophthalmic disorders and has potent antimicrobial and antihistaminic properties which combat infective and allergic eye disorders. The drug's analgesic property relieves pain, and its anti-inflammatory property is effective in soothing inflammation. In the present study, the Ophthacare was investigated for its *in-vitro* antimicrobial activity. Ophthacare brand eye drops showed significant antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* sp. These findings suggest that Ophthacare brand eye drops can be used in the treatment of various infective ophthalmic disorders.

Keywords: Antimicrobial activity, Eye Drop, Ophthacare

Introduction

Conjunctiva, due to its exposure, is the common site of infection of both acute and chronic types. Inflammation of the conjunctiva manifests in many grades and types but is usually of infective and allergic origin. Conjunctivitis is a contagious ailment, prevalent worldwide and is the most common form of ocular infection occurring in all age groups.

Ophthalmic problems effect a big segment of the population managed with antibiotics and steroids, but prolonged use of these drugs may have potential side effects. Ayurveda has described a number of medicinal plants in the treatment of ophthalmic diseases. Thus, considering the potential side effects of corticosteroidal therapy, it is worthwhile to explore the benefits of herbal drugs. OphthaCare is an herbal formulation with proven herbs.

The plants used in the traditional system of medicine have not been studied extensively using experimental models (Srinivas and Prabhakaran, 1989; Barde, 1994).

In the present study, Ophthacare brand eye drops (The Himalaya Drug Company, Bangalore), a preparation containing *Carum copticum* (seeds) 0.60% w/v , *Terminalia belerica* (fruits) 0.65% w/v, *Emblica officinalis* (fruits) 1.3% w/v, *Curcuma longa* (rhizome) 1.3% w/v, *Ocimum sanctum* (leaves) 1.3% w/v , *Rosa damascena* (petals) 1.1% w/v, *Cinnamomum camphora* 0.05% w/v and honey 3.7% w/v, was studied for its in-vitro antimicrobial activity .

The constituents of Ophthacare brand eye drops are known to possess antimicrobial and anti-inflammatory properties and are used in traditional medicine for the treatment of a variety of ocular disorders. *Carum copticum* has been shown to possess antibacterial activity against *Salmonella typhosa*, *Micrococcus pyogenes* and *Escherichia coli* (Krishnan and Badhwar, 1953). It is also recommended as a potential source of natural antioxidant (Mehta et al., 1994). Fruits of *Terminalia belerica* were found to be useful in the treatment of many ocular diseases. Fully ripe or dried fruit, mixed with honey is used as an external application in ocular diseases (Nadkarni, 1976a). It also possesses significant antimicrobial activity against both Gram-positive and Gram-negative organisms (Valsaraj et al., 1994). Extracts of *Curcuma longa* exhibit anti-inflammatory, antioxidant and antimicrobial properties (Ammon and Wahl, 1991; Bone, 1991). Volatile and fixed oils of *Ocimum sanctum* are known to possess anti-inflammatory activity (Singh and Agrawal, 1991). Essential oil of *Ocimum sanctum* possesses bactericidal activity against Gram-positive and Gram-negative bacteria (Prasad and Rao, 1987). Rose water obtained from petals of *Rosa*

damascene is known for its soothing effect and is also found to be beneficial in ophthalmopathy (Kiritikar and Basu, 1987). The extract of *Cinnamomum camphora* showed antibacterial activity against Gram-positive and Gram-negative organisms (Naqvi et al., 1985). Honey is generally recommended for sore eyes. It is also reported to prevent infection and promote healing, as it has ingredients similar to antibiotics.

Material and Methods

Antimicrobial activity was determined using the Well agar diffusion method. The method involved diffusion of the drug from a vertical hole through the solidified agar layer of a petri plate to such an extent that growth of the added micro-organisms was prevented entirely in a circular area or zone around the hole containing a solution of the drug sample. The agar medium was homogeneously inoculated with a culture of the test organism. Wells (6-8 mm diameter) were dug using a flamed cork borer and aseptically filled with Ophthacare brand eye drops (100 µl/ well). The plates were incubated at 37°C for 18-48 h, after which zone of inhibition was measured. All the tests were performed in triplicate of the micro-organisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella sp*. (Chand et al., 1994)

Results and Discussion

Ophthacare showed strong antibacterial activity against *Salmonella sp*. with a zone of inhibition of 28mm followed by *Staphylococcus aureus* and *Psuedomonas aeruginosa* with 20 mm diameter of zone of inhibition (Table and Plate-1, 2 and 3).

Causative factors of conjunctivitis, particularly *S. aureus* infection, appeared to be very frequent in this study. The disappearance of organism after the treatment reveals that the constituents of the OphthaCare eye drops has antimicrobial activity against *S. aureus.* The symptomatic relief in conjunctivitis, which was found to be significant in terms of inhibition of hyperaemia, reduction in conjunctival discharge, alleviation of conjunctival follicular hyperplasia and chemosis suggests that the additional role of anti-inflammatory properties of various constituents.

The herbs used in OphthaCare eye drops are reported to have various pharmacological activities which in combination has produced a synergistic effect in terms of antimicrobial and anti-inflammatory activities. Hence, OphthaCare eye drops are beneficial in patients with acute and chronic conjunctivitis which appears as epidemic in certain seasons of the year.

Therapy of acute conjunctivitis includes agents effective against Gram-positive and Gram-negative bacteria. While many antibacterial agents have similar spectra of activity, they are not equally potent against several organisms and vary in their pharmacokinetic properties (Neu, 1991).

Plate-1

Table

Test Sample	Diameter of zone of inhibition(mm)		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Salmonella sp.*
Ophthacare eye drops	20	20	28

Plate-2

Plate-3

Conclusion

Ophthacare brand eye drops revealed marked antibacterial and antifungal activity. The effects of Ophthacare brand eye drops against both Gram-positive and Gram-negative bacteria make it a promising drug in the treatment of ocular diseases due to infections.

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In-vitro Phytochemical and Antioxidant Evaluation of *Coriandrum sativum* and *Asparagus racemosus* Extracts

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Abstract-The present study was carried out to evaluate the phytochemicals and antioxidant activities of methanolic extracts of *Coriandrum sativum* and *Asparagus racemosus*. Phytochemical screening of *C. sativum and A. racemosus* showed the confirmation of saponins, alkaloids, tannins, reducing sugars, glycosides, flavonoids, and terpenoids. Antioxidant activity of *C. sativum* and *A. racemosus* extracts was assessed by using DPPH, it increased in concentration dependent manner. *A. racemosus* and *C. sativum* shows maximum percentage inhibition of 44% and 42% respectively. So, both the plant extracts have high medicinal potential. These results suggest that traditional folk medicines could be used as a guide in our continuing search for new natural products with potential medicinal properties.

Keywords: *Coriandrum sativum*, *Asparagus racemosus*, DPPH, Phytochemicals.

Introduction

Medicinal plants are known to have their therapeutic potential for treatment of several human ailments. India has rich tradition of using medicinal herbs and their spices for curing diseases. There are more than 2000 species spread over a vast geographical area with high potential abilities for Ayurvedic, Unani and Siddha traditional medicines. But only very few have been studied chemically and pharmacologically for their potential medicinal value. In Ayurveda and Unani system of medicine, a large number of Indian medicinal plants are used to treat infectious diseases (Padmini *et al*., 2010). Medicinal plants have important phytochemical components such as alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, steroids, tannins etc. These produce different physiological action on human body. One of the main advantages of using products of medicinal plants is that they don't have any side effect and are therefore, safer than antibiotics (Srivasan, 2001). With the emergence of antibiotics resistance microorganism, the infections have increased tremendously and are of great public health concern (Mahesh *et al*., 2008).The forests of Himachal Pradesh said to have been the birthplace of Ayurveda are known to supply a very large proportion of the medicinal plants requirements to the extent as high as 80% of all Ayurvedic drugs, 46% of all Unani drugs and 33% of all Allopathic drugs developed in India (Mazid *et al*., 2012).

Material and Methods

Collection of plant material

The plant materials used were the dried leaves of *Asparagus racemosus*, which were collected from forest region of Bajhol (Solan), Himachal Pradesh, India. The seeds of *Coriandrum sativum* used were purchased from market (Solan) Himachal Pradesh.

Extraction of plant materials

The dried leaves were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were dried in sunlight and then made into fine powder. 10 g powder of both the plants were mixed in 100 ml of methanol and incubated for 48-72 hours at 40°C in shaker. The mixture was filtered after incubation. Methanolic mixture was dried on waterbath for 4 hours to make fine paste for phytochemical and antioxidant investigations.

Phytochemical analysis of C. sativum and A. racemosus plant extarcts

The phytochemical screening of crude extracts from different plant species were carried out to determine the presence of active secondary plant metabolites. The plant extracts were screened for the presence of reducing sugars, alkaloids, saponnin, tannins, flavonoids, anthraquinones, phlobatannin, steroids, terpenoids and cardiac glycosides. Different phytochemicals were estimated qualitatively by the following procedures.

Reducing sugars (Fehling's test)

To 0.5 g of each extract, 5 ml of solvent (methnol) was added. After filtration, 1 ml of Fehling's solution (A and B) was added and test tubes were kept in boiling water bath until brick red color was formed.

Terpenoids (Salkowski test)

To 0.5 g each extract, 5 ml of solvent (methanol) was added. After filtration, 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids.(Siddiqui and Ali, 1997).

Flavonoids

To 0.5 g of each extract, 5 ml of solvent (methanol) was added, after filtration 5 ml of ammonia solution was added. A yellow coloration was observed, which disappeared on standing after adding 1 ml of H_2SO_4 or HCl, indicating the presence of flavonoids.

Saponins

To 0.5 g of each extract, 5 ml of solvent (methanaol) was added, after filtration the solution was shaken vigorously and observed for a stable persistent froth. The froth was mixed with drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion.

Tannins

To 0.5 g of the each extract, 5 ml of solvent (methanol) was added. After filtration a few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Evans and Trease, 2002).

Alkaloids

To 0.5 g of each extract, 5 ml of solvent (methanol) was added, after filtration 2 ml of 10% HCl added and boiled. After boiling for 5 to 10 minutes, Mayer's reagent (1.36 g mercuric chloride and 5 g of potassium iodide) in 100 ml distilled water was added to test tubes. The formation of white creamy precipitate showed the presence of alkaloids.

Cardiac glycosides (Keller-Killiani test)

To 0.5 g of each extract 5 ml of solvent (acetone and water) was added, after filtration 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. Then 1 ml of concentrated sulphuric acid was added to the test tubes. A brown ring at the interface indicated the presence of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Antioxidant activity assay of *C. sativum and A. racemosus* plant extracts

DPPH radical scavenging assay

The antioxidant activity of extract was evaluated by DPPH radical scavenging assay which was originally described by Blois (1958). DDPH is considered as a stable radical because of para magnetism conferred by its odd electron (delocalization of the spare electron over the molecule as a whole). The solution (in absolute ethanol) appears as a deep violet colour and shows a strong absorption band at 517 nm. DPPH radical can accept an electron or hydrogen radical to become a stable diamagnetic molecule and has a pale violet colour. If the substance for testing antioxidant activity is mixed with DPPH solution and gives rise to pale violet colour, it suggests that this substance has antioxidant effect by mechanism of free radical scavenging activity.

The following assay procedure was modified from those described by Blois (1958).

1. DPPH solution (0.004% w/v) was prepared in 95% methanol.
2. A stock solution of methanolic extract and standard ascorbic acid were prepared in the concentration of 10 mg/100 ml (100 µg/ml).
3. Sample diluted for at least four concentration (15, 25, 50, 75 µg/ml).
4. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes.
5. The reaction mixture was incubated in the dark for 30 min and there after, the optical density was recorded at 517 nm against the blank.
6. Calculation of percentage inhibition.
7. % inhibition = OD control – OD sample/ ODcontrol × 100
8. Plotted dose- resonance curve between % inhibition and concentration.
9. Linear regression analysis is carried out for calculating the effective concentration required to scavenge DPPH radical by 50% (ED_{50} value).

Results and Discussion

Phytochemical screening of C. sativum and A. racemosus

Medicinal plants contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of herbal extract has been attributed by phytochemicals which are non nutrient plant compound. Different phytochemicals have been found to possess a wide range of activities which may help in protection against chronic diseases. Qualitative analysis carried out on each plant metabolites with antibacterial potency have been actively investigated as alternative to or in combination with antibiotics in therapy of infection (Sato et al., 2001). The phytochemical screening of the plants studied, showed the presence of flavonoids, reducing sugar, sponinis, alkaloids, terpenoids, tannins and glycosides. *C. sativum* showed the presence of flavonoids, reducing sugars, sponnins, alkaloids, terpenoids, gilcosides and tannins. *A. racemosus* also showed the presence of flavonoids, reducing sugars, sponnins, alkaloids, terpenoids, gilcosides and tannins. Qualitative analysis carried out on each plant extract showed the presence of phytochemical constituents and the results are shown in table-1.

Chaudhary *et al*., (2014) observed the phytochemical screening of methanol and acetone extracts of *Coriandrum sativum* and found the presence of some promising alkaloids, glycosides, flavonoids and amino acids. Pathak *et al*., (2011) also observed the presence of carbohydrate reducing sugar, terpenoids, protein and volatile oil in methanolic extracts of *Coriander sativum*. In the previous study, the phytochemical screening of the *Asparagus racemosus* extracts revealed the

Table-1 Phytochemical analysis of plant Extracts

Sr. No.	Phytochemical	*C. sativum*	*A. racemosus*
1.	Alkaloids	+ve	+ve
2.	Tannins	+ve	+ve
3.	Reducing sugars	+ve	+ve
4.	Terpenoids	+ve	+ve
5.	Flavonoids	+ve	+ve
6.	Sponins	+ve	+ve
7.	Cardiac Glycosides	+ve	+ve

(+ve = Present, -ve = Absent)

Table-2 DPPH radical scavenging activity of *C. sativum* and *A. racemosus*

conc. / Plants	15µg/ml	25µg/ml	50µg/ml	75µg/ml	IC_{50}
Coriandrum sativum	11.86±0.0011	18.64±0.001	32.20±0.0005	44.8±0.0011	88.16
Asparagus racemosus	20.33±0.0015	25.42±0.0015	35.59±0.0015	44.06±0.0015	88.90

presence of flavanoids, tannins, alkaloids, saponins and phenolic compounds (Singh *et al*., 2013).

Antioxidant activity of methanolic extracts of *C. sativum and A. racemosus*

DPPH radical scavenging assay

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *both the plants* is given in table-2. The percentage inhibition was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.

The radical scavenging activities of *plants* extracts / fractions showed that the polar fractions scavenged the DPPH radicals more significantly. The scavenging of radicals increased with increasing concentrations of the extracts/ fractions which showed similar amount of percent radical scavenging afterwards.

Conclusion

In the present study, both the extracts had significant scavenging effect on the DPPH radical, which was generally significantly increasing with the increase in the concentration from 15-75 µg/ml.The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. Wangensteen *et al*., (2004) observed that the antiradical activity of extracts of coriander for the DPPH radical inhibition was only 15% and Karmakar *et al*., (2012) also observed the antioxidant activity of ethanol extract of *Asparagus racemosus* on the basis of the scavenging activity of the stable DPPH free radical and 10% H_2SO_4.These results suggested that both plant extracts have high value of medicinal effect including phytochemical and antioxidant activity.

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Phytochemical Analysis of Different Extracts from the Fruits of *Diospyros peregrina*

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Abstract- *Diospyros* is a large genus of shrubs and trees comprising of 500 species distributed in the warmer regions. It belongs to the family, Ebenaceae. About 41 species occur in India mostly on evergreen forests of Deccan, Assam, and Bengal; only few are found in North India. Leaves are simple, elliptic, ovate, rounded at base, 13.5 cm long and 4.5 cm broad, alternate, exstipulate, coriacious, glabrous, reticulation prominent, petiolate; petiole upto 1 cm long. Phytochemical analysis exhibited the presence of steroids, alkaloids, glycoside, proteins, tannins, phenolic compounds, carbohydrates, gums and mucilage. Maximum number of phytochemicals were found in ethanol extract whereas least phytochemicals were observed in case of chloroform extract. The results so obtained, point out that the phytochemicals from the extracts of fruits of *Diospyros peregrina* may be useful in treatment of various microbial infections. However, the active components in the phytochemicals need to be evaluated.

Keywords: Ebenaceae, *Diospyros peregrina*, Phytochemicals.

Introduction

The fruits of *Diospyros peregrina* fall to ground from June to July onwards and under favourable conditions, the seeds germinate during rainy season. Its fruit is spherical berry with a leathery rind, containing 4-8 seeds embedded in a viscid glutinous pulp. It is yellow when ripe and is covered with a rusty easily detachable scruffiness. A good tree produces about 4000 fruits in a season. Its fully ripe fruits have a mawkish sweet taste and are edible. Unripe fruits are rich in tannin and are employed for tanning hides and dyeing cloth. The fruit and the stem bark possess astringent properties. The unripe fruit is acrid, bitter and oleaginous. An infusion of the fruit is used as gargle in aphthae and sore throat. The juice forms a useful application for wounds and ulcers. It is mainly used as antidiarrheal, aphrodisiac, anti-snake bite and as a tonic in the ancient Indian medicine. Mature fruits play a significant role as tonic and aphrodisiacs in traditional Indian medicine (Kirtikar and Basu, 1975). Ripe fruits are resistant to insect attack (Benthall, 1946). Its fruits are used for making jams, jellies, osmo-dehydrated slices and squash (Reddy, 1959). Products like sweet chutney, dried pieces, milk shake, nectar, blended drinks, pickle, preservative and candy can also be prepared with good sensory quality (Sawant, 1989). Even wine can be prepared from *Diospyros* fruits (Gautam and Chundawat, 1998). Fruits of *Diospyros peregrine* are shown in the Figure-1.

Phytomedicines have been an integral part of traditional health care system in most parts of the world for thousands of years. According to World Health Organisation, greater than 80% of world

Figure-1 Fruit bearing shoots of *Diospyros peregrine*

population depends on traditional medicine for their primary healthcare needs (Duraipandiyan et al., 2006). Studying medicinal plants with ethno-botanical importance and folklore reputation has become the more important need in recent times in order to promote the use of herbal medicines and to determine their potential as source of new drugs (Parekh and Chanda, 2007).

Scientists are shifting their attention to folk medicine in order to find new leads for better drugs against microbial infections. Plant materials are known as source of new antimicrobial agents; as a result search has been to discover new antibacterial drugs of plant origin. A number of compounds like vincristine, quinine, salicylic acid, eligitalis, morphine, and codeine have been derived from plants which are having enormous therapeutic potential (Parekh and Chanda, 2007). Still medicinal properties of many plants are yet to be investigated through phytochemical and pharmacognostic analysis. There is an urgent need to identify lead substances that are active towards resistant pathogens (Recio, 1989).

The ethno medicinal information from herbal practitioners from Pakistan indicated that all parts of this plant specially bark, leaves, flowers and fruits are used in different medicinal preparations. The bark and leaves are anti-inflammatory, febrifuge, depurative, constipating, acrid, astringent, cooling and are used in dyspepsia, leprosy, diarrhoea, dysentery, haemorrhages, skin burning, diabetes, spermatorrhea, vaginal diseases, wounds, flatulence, prolepsis, scabies and as carminative, laxative and tonic. Fruits being bitter in taste are used as carminative, aphrodisiac and indigestive disorders (Warrier *et al.,* 1996).The plant is reported to possess many medicinal properties. The plant has an astringent action and is particularly used for the treatment of diarrhoea and dysentery. Extract of the fruits possesses antibacterial properties, and has also been used for dye making and tanning fishing nets (Anon, 1952; Walt, 1980). The anti-stress activity of an ethylacetate extract of *D. peregrine* was found to be similar to that of panax ginseng. The alcoholic extract of stem barks of this plant has been reported to possess hypoglycemic, diuretic and anti-cancer properties.

The main object of the present study is to evaluate the potential of the plant fruit extracts in different solvents by means of standard phytochemical analysis. The various solvents used in the present study are chloroform, acetone, ethanol, methanol and water.

Material and Methods

Plant material

Diospyros peregrina (Ebenaceae) fruits were collected from Forest Research Institute, Dehradun, India in the month of March to April 2011. The plant was identified by Department of Botany, Forest Research Institute, Dehradun, India and a voucher specimen was deposited in the Department of Botany, Forest Research Institute University, Dehradun, India. The dried fruit was homogenized to powder and subjected to extraction with different solvents.

Crude extraction

Successive chloroform, acetone, ethanol, methanol and aqueous extraction (yield 0.8, 1.09, 5.6, 4.8 and 2.06% respectively) from dried fruits of *Diospyros peregrina* were recovered using Soxhlet apparatus. The extracts were evaporated to complete dryness by vacuum distillation and stored at 4°C in airtight bottles for further use. All the chemicals used were of analytical grade.

Phytochemical analysis

All the five solvent extracts (acetone, chloroform, ethanol, methanol and aqueous) of the fruit were evaluated for the presence of different phytochemicals using procedures of Mukherjee (2002) and Parekh and Chanda (2007).

Results and Discussion

Preliminary phytochemical screening of the fruit extracts studied show that ethanol extract contains most of the phytochemicals like alkaloids, steroids, tannins, saponins, ascorbic acid etc.

Table-1 Phytochemical analysis of different extracts of Diospyros peregrina fruit.

Phytochemical	Chloroform	Acetone	Ethanol	Methanol	Aqueous
Steroid	-	+	+	+	-
Alkaloid	-	+	+	+	-
Cardiac glycoside	-	+	+	-	+
Protein and amino acid	+	+	+	+	+
Tannin and phenolic compounds	-	+	+	+	+
Ascorbic acid	+	+	+	-	+
Fixed oil and fats	+	+	+	-	+
Anthraquinone	-	-	-	-	-
Triterpenoid	-	-	-	-	-
Carbohydrate	+	+	+	+	+
Saponin	+	-	+	+	+
Gums and mucilage	-	+	+	+	-

Phytochemicals, steroid and alkaloid were present in acetone, ethanol and methanol, they were absent in chloroform and aqueous extracts. Aminoacids and carbohydrates were universally present in all the extracts. The active constituents of plants usually interfere with the growth and metabolism of microorganisms in a negative manner (Aboaba et al., 2006). The phytochemicals, ascorbic acid and fixed oil and fats were present in chloroform, acetone, ethanol and aqueous extract, but absent in methanol extract. Anthraquinone and triterpenoid showed negative effect in chloroform, acetone, ethanol, ethanol, methanol and aqueous extract. Tannins and phenolic compounds were absent in chloroform extract and present in acetone, ethanol, methanol and aqueous extracts. Cardiac glycoside was absent in chloroform and methanol extracts but present in acetone, ethanol and aqueous extracts. Saponin was absent in acetone extract and present in chloroform, ethanol, methanol and aqueous extracts. Gums and mucilage were absent in chloroform and aqueous extracts but present in acetone, ethanol and methanol extracts (Table-1).

Phenolic compounds like tannins found in plant cells are potent inhibitors of hydrolytic enzymes used by plant pathogens. Other compounds like saponins also have antifungal properties. Hence, these phytochemicals present in the crude extracts may be responsible for antimicrobial activity of plant extracts which need further investigation.

Conclusion

The present findings on phytochemical analysis of the fruits of *Diospyros peregrina* show fairly good degree of correlation with ethno medicinal uses of the plant. Preliminary results of this investigation appear to indicate that fruits of *Diospyros peregrina* have high potential antimicrobial activity. Novel bioactive compounds from the fruit need to be isolated and screened for their pharmaceutical and biotechnological applications for therapeutic uses.

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Effect of *Cinnamomum Camphora* Leaf Extract on Female Reproductive Organs of Albino Rats

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Abstract– Histopathological studies of the ovary and uterus were undertaken after oral administration of aqueous extract of the leaves of *Cinnamomum camphora* at doses of 100, 250 and 400 mg/kg/day/rat for 30 days. The body weight was not affected significantly but higher doses reduced the ovarian weight significantly. The naked eye observation revealed no change but when examined histologically, there was an evidence of follicular atresia with the absence of corpus luteum at higher dose. Primary and secondary follicles showed degenerative changes. The primordial oocyte population was also reduced. There were no regressive changes in the uterus of the treated rats except at 400 mg/kg/dose.

Keywords: *Cinnamomum camphora*, Herbal drugs, Reproduction, Female contraception, Fertility control, Medicinal plants

Introduction

Cinnamomum camphora Linn., family Lauraceae, (Hindi – Kapoor, Karpur, Kapuram; English – Camphor tree), is a large evergreen tree found mostly at Himalayan region. It is a source of camphor which has been used for medicinal purposes by pharmaceutical industries. The tender leaves contain more camphor than old ones. Kirtikar and Basu (1935) and Chaudhury (1966) included this plant in the list of antifertility plants. Dhawan *et al.* (1977) carried out experiments on the antifertility affect of this plant on female albino rats. Singh in (1989) studied this plant in male albino rats. The tender leaves in powdered form were used in the present study and the effects are reported in this paper.

Material and Methods

Freshly collected air dried and powdered leaves of *C. camphora* were soxhleted with distilled water. The extract was evaporated to dryness under low temperature and reduced pressure on a water-bath and the dried extract as powder was used in this study.

Cyclic adult female albino rats (150-160 g) were selected for the experiment. They were maintained under uniform husbandry conditions with free access of food (Hindustan Lever Ltd.) and tap water. The extract was orally administered (suspended in distilled water) by an intragastric rubber cathetor in three dose levels i.e. 100 mg, 250 mg and 400 mg/kg body weight to (Group II, III and IV respectively) daily for 30 days. Female rats in control group (Group I) received distilled water as vehicle only. Five animals were allotted in each group.

All the animals in each group, 24 hours after the last day's dose, were sacrificed by decapitation. The ovaries and uterii were dissected out, freed from surrounding tissues, blotted on filter paper and weighed quickly on semi-micro balance. For histological studies, ovaries and uterii were fixed in Bouin's fluid. Dehydrated paraffin embedded tissue sections were cut at 6 micron and stained with Ehrlich's Haematoxylin and Eosine. Slides of ovary and uterus were photographed and histopathological changes were described.

The body weight prior to start of experiment and last day of experiment was taken and recorded. The significance of difference of weight between treated and control rats was assessed by the student 't' test taking $p < 0.05$ as significant.

Results

Body weight and organ weight changes presented in the Table.

(i) Ponderal changes

Administration of *C. camphora* at lower doses did not reduce the body weight for 30 days. At 400 mg/kg dose, slight reduction in body weight was observed. The doses 250 and 400 mg/kg for 30 days reduced the ovarian weight significantly. The weight of uterus was not much affected.

(ii) Histological changes

a) Control: The ovarian histology of control rat revealed the normal cellular structures with organised germinal epithelium, all types of follicles, a few atretic follicles, interstitial cells, normal vascularity and loose stroma (Figure-1). The histological structure of the uterus of control group of rats presented the normal features. The endometrium made up of columnar epithelial cells. Lumen was wide, uterine glands were normal and distributed in the stroma. Musculature (myometrium) was well developed and vascularity appeared normal (Figure-2).

b) Treated by *C. camphora* : The administration of 100 mg/kg/day for 30 days caused no histopathological changes in the ovaries. Many developing follicles can be seen with a few atretic follicles. The ovaries at higher doses (250 and 400 mg/day) showed marked follicular atresia of primary and secondary follicles. Graffian follicles were few and appeared not much affected. There were no Corpora lutea. *Primordial oocyte population was significantly* degenerated. Vascularity and stroma were normal (Figure- 3,4).

The administration of *C. camphora* leaf powder did not cause histopathological changes at 100 and 250 mg/kg dose for 30 days. It did not differ much from the picture of uterus of control female rats. At 400 mg/kg dose for 30 days, a regression of uterine elements was noted. The lumen appeared normal and fully distended. The uterine glands were reduced and highly regressed. The musculature and vascularity were not much affected (Figure- 5,6).

Discussion

The results, as noticed in this study, were obtained by Chakraborti *et al.* (1960) when the female rats were fed with the green leaves of *Artobotrys odoratissimus* Linn. Dixit (1977) reported the follicular degeneration and uterine dysfunction by daily administration (25 days) of *Malva viscus conzattii* flower extract. Follicular atresia and other changes were reported by Kholkute and Udupa (1974) and Kholkute *et al.* (1976) following the treatment of extracts of *Hibiscus rosa sinensis* Linn. flower. Bhardwaj and Mathur (1976) and Bhardwaj *et al.* (1980) reported

Table - Effect of *Cinnamomum camphora* leaf extract on body weight (gm) and genital organ weight (mg) of female albino rats administered at different doses for 30 days. Five animals used in each group. Values are mean

Group	Treatment	No. of rats	Body weight (gm)		Organ Weight (mg)	
			Initial	Final	Ovaries	Uterii
I	Control	05	153.80 ± 0.45	159.00 ± 0.71	39.40 ± 0.92	102.00 ± 1.12
II	100 mg	05	157.40 ± 1.91	161.20 ± 2.52	35.50 ± 3.10	100.10 ± 2.70
III	250 mg	05	153.40 ± 1.35	160.00 ± 1.16	24.60 ± 2.90*	98.50 ±1.30
IV	400 mg	05	160.20 ± 0.58	161.60 ± 1.26	20.70 ± 0.60*	95.40 ± 3.70

* p values < 0.05

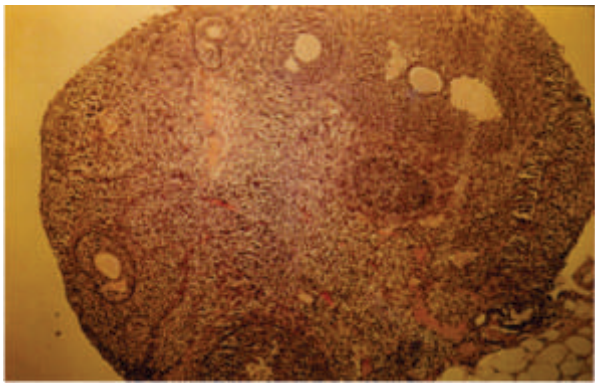

Figure-1 T.S. of ovary of control group of rats. Note the normal histoarchitecture with all kinds of follicles Corporal lutea, stroma and vascularity X 100.

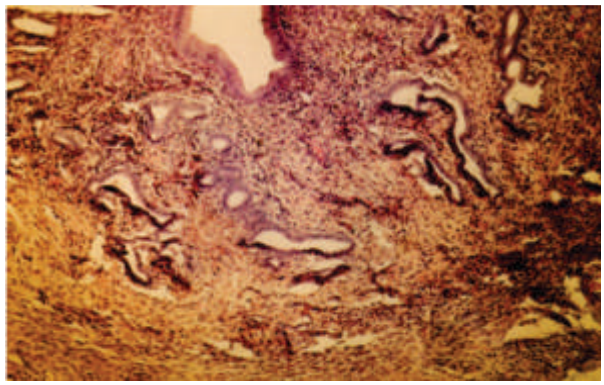

Figure-2 T.S. of uterus of control group of rats. Note the normal histological structure with columnar epithelium, endometrium, myometrium, loose stroma, vascularity, lumen and uterine glands X 150.

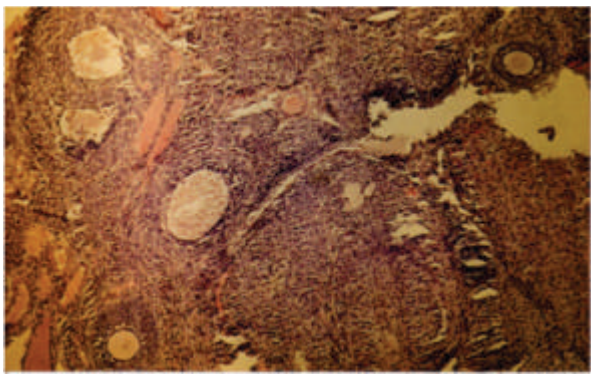

Figure-3 T.S. of ovary of treated group of rats with *C. camphora* at 100 mg/kg/day for 30 days. Note the normal structure and follicles. Corpora lutea are not seen X 150.

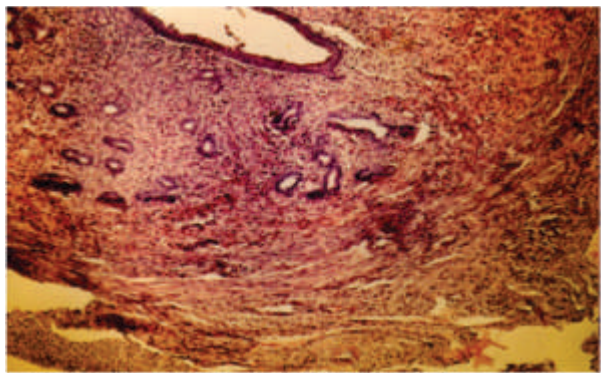

Figure-4 T.S. of uterus of treated group of rats with *C. camphora* at 100 and 250 mg/kg/day for 30 days. Note the normal structure of endometrium, myometrium, lumen and uterine glands X 100.

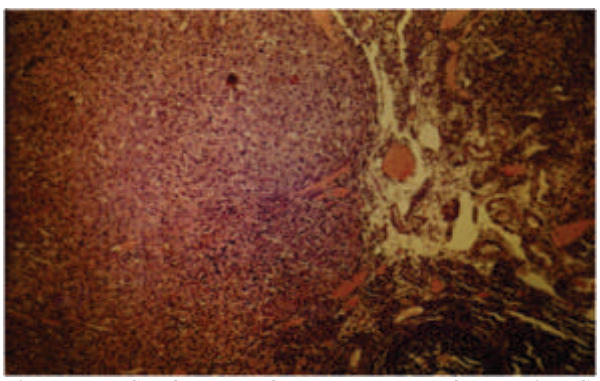

Figure-5 T.S. of ovary of treated group of rats with *C. camphora* at 250 and 400 mg/kg/day for 30 days. Note the histopathological effects such as follicular atresia and arrest of primordial follicles X 150.

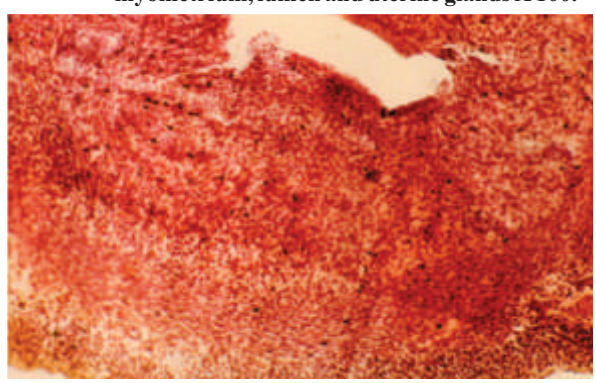

Figure-6 T.S. of uterus of treated group of rats with *C. camphora* at 400 mg/kg/day for 30 days. Note the regressive uterine structures such as endometrium, myometrium, uterine glands and wide lumen X 150.

antifertility effects of *Cassia fistula* fruits in female albino rats.

Both histological and ponderal change in the uterus were reported by Prakash (1979 a,b) on administrating the extracts of *Embelia ribes* (Burn) seeds. It increased the uterine weight and showed uterotrophic activity. Stimulation in the uterine histoarchitecture has also been observed by Munshi and Rao (1972) when an Ayurvedic preparation ROC-101 was administered to adult rats. The present observation are in agreement with Munshi (1974) who had reported an increase in endometrial glands with dialated and irregular lumen after the application of an indigenous preparation, 'Garbh Nivaran Aushadham' composed of *Embelia ribes*, *Piper longum* and Borax. Singh *et al.* (2000) found similar results with feeding of *Randia dumetorum* aqueous extract of seeds. Similar results as found in the present study was also reported by Singh and Singh (2013) with application of *Solanum xanthocarpum* seeds in female albino rats.

Conclusion

It is concluded from the present study that the aqueous leaf extract of *Cinnamomum camphora* inhibit the ovarian function and stimulate the uterine function of albino rats.

Acknowledgement

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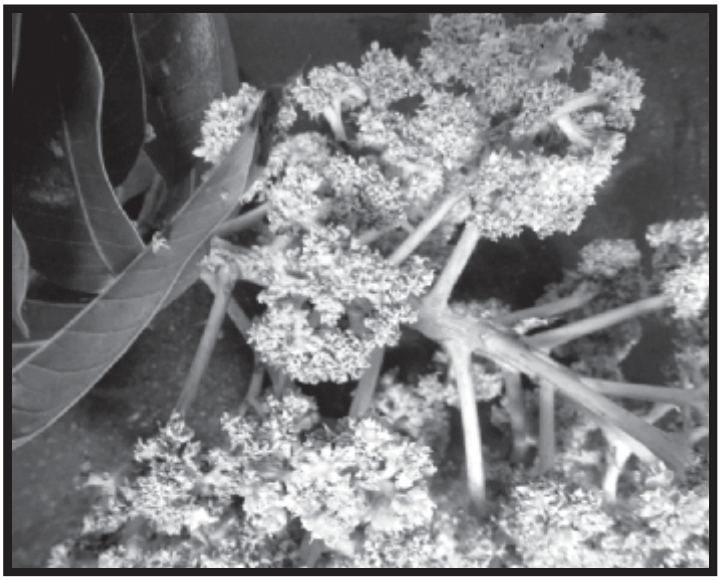
About Flowers (Shown on the cover page)

Antidiabetic herbs



***Mangifera indica* (Mango)**

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Anacardiaceae
Genus:	*Mangifera*
Species:	*M. indica*

Mangifera indica, commonly known as mango, is a species of flowering plant in the sumac and poison ivy family, Anacardiaceae. It is native to the Indian subcontinent where it is indigenous and cultivated varieties have been introduced to other warm regions of the world. It is a large fruit-tree, capable of a growing to a height with crown width of about 100 feet and trunk circumference of more than twelve feet. The mango leaves are very useful for treating diabetes. The tender leaves of the mango tree contain tannins called anthocyanidins which help in treating early diabetes. The leaves are dried and powdered, or used as an infusion to treat the same. It also helps to treat diabetic angiopathy and diabetic retinopathy. Mango flowers look beautiful at the bloom time. The blooming time of the flowers make the mango time beautiful than ever before. The tiny flowers look pretty when seen in close-up. These are used as medicine since many years.

Nasal bleeding can be stopped after inhaling the flowers of mango. It can happen due to various health problems. It mostly happens after eating spicy food during the summers.

Curing Chronic Dysentry: Powder of mango flowers or decoction can be used for curing chronic dysentry and gonorrhea.

1. **Cure Acidity Problems:** Decoction of mango flowers can be consumed for curing acidity related problems like headache, vomiting, allergy due to acidity and fainting.
2. **Curing Urinary Diseases:** Mango flower powder when consumed with one-fourth part of sugar candy helps in curing urinary diseases.
3. **Curing Luecorrhea:** Flowers of grafted mango tree when fried with ghee can be consumed for curing leucorrhea.
4. **Improving Sexual Potency:** Taking 5-10 gm of mango flower with milk helps improving sexual potency.
5. **Curing Diseases:** Diarrhea, eczema and blood related disorders can be cured by consuming the flowers of mango with sugar candy.

***Syzygium cumini* (Jamun)**

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Myrtales
Family:	Myrtaceae
Genus:	*Syzygium*
Species:	*S. cumini*

Syzygium cumini trees start flowering from March to April. The flowers are fragrant and small, about 5 mm in diameter. The fruits develop by May or June and resemble large berries; the fruit of *Syzygium* species is described as "drupaceous". It is oblong, ovoid. Unripe fruit looks green. As it matures, its color changes to pink, then to shining crimson red and finally to black color. A variant of the tree produces white coloured fruit. It has a combination of sweet, mildly sour and astringent flavour and tends to colour the tongue purple.

The seed of the fruit is used in various alternative healing systems like Ayurveda to control diabetes; Unani and Chinese medicine for digestive ailments.

Wine and vinegar are also made from the fruit. It has a high source in vitamin A and vitamin C.

Jamun is traditionally used for the treatment of various diseases especially diabetes and related complications. Flowers of jamun contains oleanolic acid, ellagic acid, isoquercetin, quercetin, kampferol and myricetin.

***Cinnamomum zeylanicum* (Dalchini)**

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Magnoliids
Order:	Laurales
Family:	Lauraceae
Genus:	*Cinnamomum*
Species:	*C. zeylanicum*

It is found in the south western part of the India along the coast line and in Sri Lanka upto the height of 35000 feet. It's found in China, Japan, and also in European countries. According to Ayurveda, three varieties are seen. These are (a) Chinese, (b) Indian and (c) Sinhali or Sri Lankan.

Cinnamon bark is used as a spice. It is employed in cookery as a condiment and flavouring material. It is carminative, astringent, stimulant, antiseptic in action. The essential oil of this herb is a potent antibacterial, anti-fungal, and uterine stimulant. It stops vomiting, relieves flatulence and is useful in diarrhoea and haemorrhage of the womb. Recent studies suggest that consuming as little as one-half teaspoon of Cinnamon each day, may reduce blood sugar and cholesterol level.

Cinnamon is one of the most delicious and healthiest spices on the planet. It can lower **blood sugar levels**, reduce heart disease risk factors, and has a plethora of other impressive health benefits.

Cinnamon has ancient origin, a broad range of folkloric health remedies have been practiced through the years that can be found in various cultures. While not all of its claimed health benefits are supported by science, a number of scientific studies that were done, confirmed that there are indeed some medicinal benefits from Cinnamon.

Oral conditions. Cinnamon has traditionally been used to treat toothache and fight bad breath.

Health tonic. Cinnamon has been used to promote overall health and feeling of well being

Some of the folkloric applications of Cinnamon are as follows.

Memory Booster. Cinnamon can improve cognitive function as well as memory.

Blood Purification. Cinnamon helps in removing impurities from the blood, and is often recommended for pimples.

Promotes Healing. Cinnamon helps to stop bleeding, and facilitates the healing process.

Digestive Tonic. Cinnamon aids in digestion and is effective for indigestion, nausea, vomiting, upset stomach, diarrhea and flatulence. Cinnamon also relieves acidity and morning sickness.

Respiratory problems. Cinnamon helps in cold, flu, influenza, sore throat.

Menstruation. Cinnamon is effective in providing relief from menstrual cramp and discomfort..

In Indian Ayurvedic medicine, Cinnamon is used in the treatment of flatulence, piles, amenorrhea, diarrhea, toothache, amoebiasis, heart diseases, fever, cough, cold, headache and many others.

Anti-Oxidant. Cinnamon is widely believed to be high in anti-oxidants. Regular drinking of Cinnamon tea could be beneficial to oxidative stress related illness in humans,

Diuretic Effects. Cinnamon is diuretic in nature and helps in secretion and discharge of urine.

Aphrodisiac and is believed to arouse sexual desire. It is also believed that cinnamon aids in the secretion of breast milk.

***Cinnamomum tamala* (Tejpatta)**

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Magnoliids
Order:	Laurales
Family:	Lauraceae
Genus:	*Cinnamomum*
Species:	C. tamala

Tejpata spelled as Tej Patta, Tejpatta, and Tejpat, in English it is named as Indian Bay Leaf, and botanically it is called as Cinnamomum Tamala. It is an Indian spice as well as Ayurvedic medicine. It is commonly used in Indian Kitchen for enhancing the taste of the different foods. Additionally, it stimulates the digestive enzymes which helps to improve digestion of food and increases the bioavailability of the nutrients during the digestion process in the intestine.

In Ayurveda, Tejpata is used to reduce AMA toxins in the body and for digestive health problems. It reduces gas formation, flatulence, abdominal pain, indigestion, diarrhea, common cold and asthma. The dried leaves of Cinnamomum Tamala plant/tree are called Tejpatta and used as a spice and in Ayurvedic medicine. The flowers of this plant are also used in folk medicine.

The leaves (Tej Patta) of Cinnamomum Tamala contain volatile oil which contains phytochemical constituents such as monoterpenes and sequiterpenes.

The essential oil and oleoresins in *Cinnamomum Tamala* leaves contain eugenol which is responsible for the anti-bacterial and anti-fungi activities of the Indian Bay Leaf (Tejpatta). So, it can also be used in human yeast infections.

Tejpatta is an antioxidant. When it is added in fat rich food, it increases the shelf-life by preventing oxidative degradation of lipids. So, it can be an alternative to synthetic antioxidants used in food preservation.

Furthermore, it acts as a digestive stimulant, which is likely to induce the appropriate secretion of digestion enzymes. Therefore, it helps to maintain the proper digestion and enhances assimilation (absorbing the nutrients from the food).It is antidiabetic.

Forth Coming Events

1. 17th Annual Medicinal & Pharmaceutical Sciences Congress
   July 05-06, 2018 Bangkok, Thailand
   Theme: New research to uncover opportunities in the medical and pharmaceutical studies
   https://global.acs.org/events/17th-annual-medicinal-pharmaceutical-sciences-congress/

2. ACPB-2018 — Annual Congress on Plant Science and Biosecurity
   July 12 – 14, 2018 Valencia, Spain
   Theme: Plant science conference, plant biology conference, plant bio security conference
   https://acpb2018.com/

3. 4th World Congress on Medicinal Plants and Natural Products Research
   August 08-09, 2018 Osaka, Japan
   Theme: New Frontiers in Transforming Future of Medicinal Plants
   https://medicinalplants.pharmaceuticalconferences.com/

4. 14th World Congress on Pharmacology and Drug Safety
   August 15-17, 2018 Stockholm, Sweden
   Theme: Manifesto for Pharmacology and Drug Safety Surveillance: from Principles to Practice
   https://pharmacology.annualcongress.com/

5. 5th Annual Congress on Clinical Pharmacy & Pharmacology
   September 07-08, 2018 Tokyo, Japan
   Theme: Clinical Pharmacy, Pharmacology and Innovations
   https://index.conferencesites.eu/conference/24443/5th-annual-congress-on-clinical-pharmacy-pharmacology

6. 6th International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products
   September 12-13, 2018 Shanghai, China
   Theme: Pharmacognosy, A Science of Natural Products in Drug Discovery
   https://pharmacognosy-phytochemistry-natural-products.pharmaceuticalconferences.com/

7. 15th International Conference on Pharmaceutical Formulations & Drug Delivery
   (2298th of Conference Series LLC Ltd)
   September 17-18, 2018 | Philadelphia, Pennsylvania, USA
   Theme: *Looming technologies in Pharmaceutical Formulations and Drug Delivery*
   https://formulations.pharmaceuticalconferences.com/

8. Macrocycles 2018: 3rd RSC BMCS Medicinal Chemistry Symposium on Macrocycles
   October 08 – 09, 2018 Stevenage, United Kingdom
   Theme: Macrocycles, medicinal chemistry, biological chemistry, drug discovery, Lipinski's rules, therapeutic approaches http://www.rsc.org/events/detail/30206/macrocycles-2018-3rd-rsc-bmcs-medicinal-chemistry-symposium-on-macrocycles

9. 18th International Conference on Medicinal and Pharmaceutical Chemistry
   October 18-19, 2018 Dubai, UAE
   Theme: Explore the future of Medicinal and Pharmaceutical Science
   https://pharma-medicinalchemistry.conferenceseries.com/

10. 18th Annual Pharmaceutical and Chemical Analysis Congress
    November 05-06, 2018 Madrid, Spain
    Theme: Pioneering Expansion in the World of Pharmaceutical and Chemical Analysis
    https://gmpnews.net/event/18th-annual-pharmaceutical-chemical-analysis-congress/

11. World Congress on Bio-organic and Medicinal Chemistry
    November 12-13, 2018 Dubai, UAE
    Theme: Explore the latest trends in Bio-organic and Medicinal Chemistry
    https://bioorganic-medicinal.chemistryconferences.org/

12. 13th World Congress on Pharmacology and Toxicology
    November 14-15, 2018 Melbourne, Australia
    Theme: Accelerations and Decelerations in Drug Discovery
    https://pharmacology.pharmaceuticalconferences.com/asiapacific/

13. International Conference on Biological & Pharmaceutical Sciences
    November 19-20 , 2018 Sydney, Australia
    Theme: Evolution in Science is Inevitable - Exploring Pharma & Life Sciences
    https://biologicalsciences.pharmaceuticalconferences.com/

14. 23rd International Conference on Pharmaceutical Biotechnology
    December 10-11, 2018 Rome, Italy
    Theme: Shaping the Future with Latest Advancements in Pharmaceutical Biotechnology
    https://biotech.pharmaceuticalconferences.com/

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   iii. Complete formulation details of all crude drug mixtures.
   iv. The herbarium specimen number of plant(s) studied in case of less well known plants be cited using the collector and collection number (eg. Doe 123) as well as indicating the names of the herbarium institution where it has been deposited.
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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 programthe 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 Phytochemistry and Ayurvedic Height for their endeavor in Herbal research.

Dr. Rajendra Dobhal
Director General

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