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Antiobesity Herbs

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Garcinia cambogia

Terminalia chebula

Trigonella foenum graecum

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Editorial

Unemployment has become primary concern today. There are 1,500 Pharma Colleges and 40,000 Pharma Graduates coming up every year. India have great manpower to tackle any situation if we consider this manpower as an asset to the Country, these young Scientists with their innovation and inventions can make wonders. China's crop production is three times more than of India as far as agro-farming is concern .We can enhance herbal cultivation and give better economic condition to all concerned.

The concern for health is increasing due to several channels of communication like Google which has answer to all problems and their treatment including the Ayurvedic medicines. It's a good sign for the health of human beings who may get relief instantly. These days gymnasiums and health clubs are providing steroids to youngsters in the name of food supplements as a source of protein for body building which cause physiological and psychological disorders such as depression, addiction anxiety and even suicidal tendencies. This has to be stopped.Therefore, neutraceuticals are the best solutions.

Journal ACS Nano published a research which describes a new drug compound which kills gram negative *E.coli* including a multi drug resistant pathogen which will give relief to millions in the future.

The father of medicine, Hippocrates had once said “Let food be your medicines and medicine be your food” which is true in Ayurved. Obesity is the major health hazard. Food diet and life style are outcome of increasing life style diseases at alarming rate. Herbal products and food as neutraceutical can treat many general ailments at primitive stage, this is one area which needs exploration and could be of a great help in maintaining good health and longevity.

I hope the deliberations of this seminar will be very useful for all participants and my sincere thanks to all who have contributed their valuable research for publication and make this issue a success. I would like to thank the entire editorial, advisory members and seminar committees for their untiring efforts in bringing out this issue. I offer my best wishes to all those Scientists, Research Scholars, Students and Teachers who contributed for bringing out this issue and also express my sincere gratitude to all Board members who made this Issue a memorable for scientific fraternity of Uttarakhand and the Country as a whole.

Dr. S. Farooq
Chief Editor

CONTENTS

Phytochemical Analysis and Antibacterial Activity of *Murraya koenigii* (L.) Spreng Pulp and Seed Extract Against Multi Drug Resistant Bacteria

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Abstract-The present study is aimed at phytochemical analysis and antibacterial activity of methanolic crude extracts of *Murraya koenigii*(L.) Spreng pulp and seed extract. The shade dried pulp and seed powder were extracted with methanol and subjected to detection of major phytocompounds by colour test, TLC and Fourier Transform Infrared (FTIR) Spectroscopy. The antibacterial activity of pulp and seed extract was determined using standard agar well diffusion method and MIC was determined by broth macro dilution assay against drug resistant bacterial species; *Escherichia coli (ESBL* positive*), Pseudomonas aeruginosa (ESBL* positive*), Pseudomonas aeruginosa* (PAO1), *Klebsiella pneumoniae* (MCC 2451), *Klebsiella pneumoniae* (ATCC-BAA 7005), *Enterococcus faecalis* (MCC 2409) and drug sensitive *Chromobacterium violoceum* (ATCC 12472).). Phytochemical analysis and TLC showed the presence of major phytocompounds such as alkaloids, flavonoids, glycosides and polyphenols in both of the extracts and various functional groups of major phytocompounds by FTIR. The seed and pulp extract showed antimicrobial activity against *Enterococcus faecalis* with inhibition zone around 18mm and 13mm respectively followed by ESBL producing *Pseudomonas aeruginosa*. Minimum Inhibitory concentration(MIC) of *M. koenigii* seed extractwas found 500µg/ml against *E. faecalis* followed by 1000µg/ml against *C.violoceum* comparable to that of MIC of streptomycin. The above investigation on *M. koenigii* seed or pulp extract is reported for the first time and requires further identification of most active compounds to be exploited in the management of antibiotic resistance problem.

Keywords: *Murraya koenigii,* Phytochemistry, TLC,FTIR, Antibacterial activity and MDR bacteria.

Introduction

Currently, infections associated with multiple drug resistant (MDR) bacteria have become a serious global health concern. Such problematic pathogenic bacterium not only reduce the efficacy of conventional antibiotics but also raises the frequency of therapeutic failure and mortality rate (Teerawattanapong et al., 2018). According to an European Union study infections caused by MDR bacteria leads to 25,000 patients death annually (ECDC, 2014). Due to the serious alarm of infections and resistance emergence of MDR bacteria, researchers are in the quest of identifying new antimicrobial substances from natural sources with novel mode of action so that the problem of resistance could be minimized (Gandhi et al., 2010).

Medicinal plants are naturally gifted with invaluable source for new bioactive components in traditional medicinal system for millenema. In an effort of discovering new lead molecules, scientists are continuously evolving plant extracts for the presence of secondary metabolites possessing relevant therapeutic efficacy and also pharmaceutical industries are implicating plant based products as the lead for the development of semi synthetic derivatives (Sut *et al.*, 2018). Indian medicinal plants are known for their antibacterial activities against selected microbial pathogens (Ahmad and Beg, 2001).

Murraya koenigii ("curry tree") locally called as "karivepaku" or curry patta in Indian dialects belongs to family Rutaceae, exhibits tremendous medicinal importance found in India and southeast Asian regions. Curry leaves because of its characteristic aroma used as flavouring agent in various Indian cuisines. In Indian Ayurveda the curry plant is known as "Krishnanimba" and several parts are used as vital ingredients in many Ayurvedic formulations. Traditionally, the leaves has been used to treat various health related ailments like stomachic, analgesic, hepatoprotective, anti-diabetic, anti-inflammatory and anti microbial, anti diarrhoeal and anticancer activities (Chauhan et al., 2017). Recent literature on the phytochemical studies of leaves, bark, stem and root of curry plant suggest that it constitutes rich source of bioactive metabolites predominantly carbazole alkaloids (Patel et al., 2016) along with other constituents like flavonoids (Ghasemzadeh et al., 2014), phenolics (Tan et al., 2017), carbohydrates and essential oils (Brind et al., 2014).

To the best of our knowledge, there are no reports available on pulp and seed for their antimicrobial activities especially against MDR bacteria. Considering the importance of problematic group of MDR bacteria and lack of concerted efforts to evaluate bioactive plant extracts, the present study has been planned to investigate phytochemical analysis and antibacterial activity of curry pulp and its seed extract.

Material and methods

Bacterial Strains used and growth conditions

The bacterial strain used in this study were gram negative MDR strains, two strains *Klebsiella pnuemoniae* (MCC 2451) and Vancomycin resistant *Enterooccus faecalis* (MCC 2409) [purchased from NCMR, Pune, India], two extended spectrum ß-lactamase producing (ESBL) strains of *Escherichia coli* and *Pseudomonas aeruginosa* (provided by Prof. Qazi Mohd. R. Haq, JMI, New Delhi), *Pseudomonas aeruginosa* PAO1 (gifted by Prof. Robert J. C. McLean, USA), carbapenem resistant *Klebsiella pnuemoniae*(ATCC BAA-7005) (JN Medical College, AMU, Aligarh) and *Chromo bacterium violaceum* 12472. The collected bacterial cultures were maintained on Luria bertani (LB) broth at 37ºC and 28 ºC respectively.

Collection of plant material and preparation of Crude extract

Murraya koenigii fruits were collected from the University Campus in the month of August 2018, Medical colony, AMU, Aligarh, India. The collected material was washed thoroughly with running tap water two to three times to remove dust particles. The seeds were separated from the pulp of berries, then both seeds and pulp were air dried at room temperature for a week **(Figure – 1).** The shade dried seeds and pulp were pulverized into fine powder with the

A B

Figure–1. *Murraya koenigii* plant with fruits (A) and shade dried seeds (B).

help of electric grinder. Fifty grams of finely powdered seed and pulp material was macerated in 250 ml of methanol and and kept for 3 days with intermittent shaking. Extracts were filtered using Whatman No.1 filter paper (Whatman Ltd., England). The residual material was again extracted two times to improve the yield and filtrates were pooled. The filtrates obtained were concentrated under reduced pressure using rotator evaporator at 40ºC. The both extracts were determined these extracts were reconstituted in minimum concentration of DMSO (≤0.5%) to perform further experiments.

Preliminary phytochemical analysis of *Murraya koenigii* pulp (MKPE) and seed extract (MKSE)

The freshly prepared methanolic pulp and seed extracts were subjected for qualitative analysis of various bioactive phytochemicals such as alkaloids, glycosides, flavonoids, saponins *etc.* using standard colour test procedures (Ahmad and Beg, 2001)

Detection of phytocompounds by Thin layer Chromatography (TLC)

TLC based detection of common secondary metabolites such as alkaloids, flavonoids and terpenoids of pulp and seed extracts was performed as described previously (Wagner,1996). Chromotagrams were developed upon TLC Silica 60 F254 plates (Merck, Germany) using toluene, ethyl acetate and glacial acetic acid in a ratio of 2:7:1 for seed extract and benzene, ethyl acetate and formic acid in a ratio of 4:6:2 for pulp extract.

2.5.1 Test for Alkaloids

On spraying the TLC chromatogram of both the extract with dragendorff reagent, development of orange brawn colours indicates the presence of alkaloids and heterocyclic nitrogenous compounds.

2.5.2 Test for Flavonoids

Once the chromatograms was developed, TLC plate was dried and sprayed with natural product reagent, then the plates were observed for yellow-green or orange-yellow fluorescence bands on exposure to UV(365nm). The characteristic bands indicates the presence of flavonoids group of compound.

2.5.3 Test for terpenoids

The TLC plate was sprayed with vanilline-sulphuric acid reagent followed by heating in oven at 90ºC for 5-10 minutes. The development of purple to red spots indicates the presence of terpenoids or the components of essential oils.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR was performed to identify the presence of characteristics functional groups or types of chemical bonds present in phytocompounds of crude extracts. A small amount of pulp and seed extract powder was mixed with dry potassium bromide to prepare translucent disc. The prepared sample o each extract was loaded in FTIR spectroscope (Shimadzu, Japan) with scan range from 4000 to 400cm^{-1}. The characteristic peaks obtained were recorded using Perkin Elmer Spectrophotometer (Version 10.4.00). By the interpretation of IR absorption spectra occurrence of various functional groups can be inferred.

Antibacterial Assay

Antibacterial activity of *Murraya koenigii* pulp and seed extract was determined by using agar well diffusion as described earlier (Ahmad and Beg, 2001). An aliquot of 0.1 ml of overnight grown bacterial culture to be tested equivalent to a 0.5 McFarland standard (1.5×10^5 CFU/ml) was spread on the surface of Mueller–Hinton agar plates. Then wells of about 6mm diameter were punched in to the agar medium, sealed with soft agar and wells were filled with each crude extract (1000mg/ml). Streptomycin antibiotic (1mg/ml) was used as positive control while DMSO (0.5%v/v) was served as negative control. The plates are allowed to diffuse the extract at room temperature, then incubated overnight at 37ºC. After incubation the diameter of growth inhibition was measured in mm by zone measuring scale.

Minimum Inhibitory Concentration of plant Extracts

Minimum Inhibitory Concentration (MIC) of Extracts of both of the extracts were determined by broth macrodilution assay (CLSI, 2014). MIC can be defined as the lowest concentration of drug or any agent to be tested at which no visible growth of the testorganisms occur. To determine the MIC of both pulp and seed extracts of *M. koenigii*, serial dilutions in tubes having Mueller Hinton broth (Hi-Media) was made and the concentrations ranged from 0.062-8mg/ml. A 10µl of bacterial culture that accounts for 1.5×10^5 CFU/mL was added in each tube. A positive control of streptomycin was also included. After overnight incubation at 37ºC, the lowest concentration of both of the extracts that inhibited visible growth of tested bacteria was considered as MIC. Data represents at least three independent experiments for each microorganism tested.

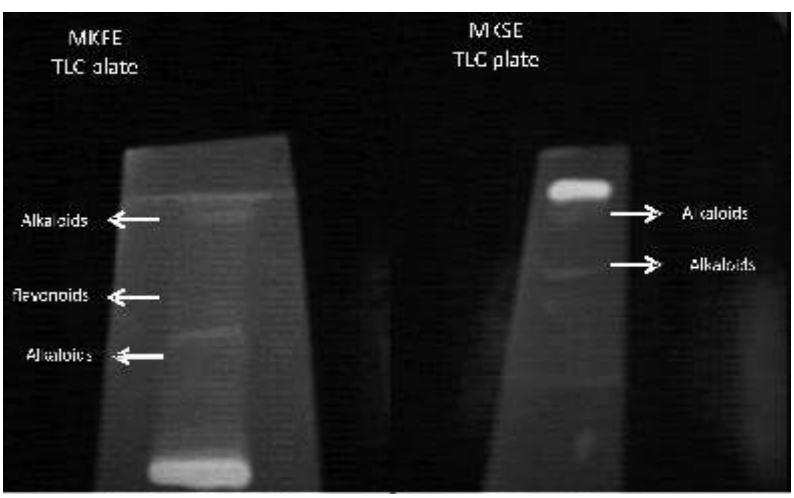
Results and Discussion

Phytochemical analysis of extracts

The results of phytochemical evaluation of both pulp and seed extract with their respective tests are depicted in **table–1**. Both of the extracts revealed the presence ofmajor groups of phytocompound such as alkaloids, flavonoids and phenols. However, saponins, phytosterols and glycosides were only present in seed extract. Further TLC based detection also revealed more phytocompounds in pulp extract **(Figure–2).** Detection of alkaloids and flavonoids in seed and pulp extract was done by observing the TLC plate in UV at 365nm. The pulp extract shows yellow-brawn sharp band of

Table – 1. Phytochemical analysis of *M. koenigii* seed (MKFE) and pulp extract (MKSE) by colour test

Phytochemicals	Plant extract	
	MKSE	MKPE
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	ND
Phytosterol	+	ND
Phenols	+	+
Tannins	ND	+
Saponins	+	ND
+(Present)., ND (not detected)		



Figure–2. TLC based detection of major phtycompounds (TLC plate under UV 365)

flavonoids and orange and blue bands of alkaloids while in seed extract only two bands of blue colour were observed indicating the presence of predominantly alkaloids.

FTIR analysis is to detect the presence of functional groups in *Murraya koenigii* seed and pulp crude extract as depicted in **table – 2**. The absorption spectra of both of the extracts clearly indicate various absorptions peaks **(Figure–3 and 4).** The broad absorption peak at 3433.30 cm^{-1} and 3442.03 cm^{-1} refers to amino acids, alcohols and phenols (O-H stretching) while that of 3905.86 cm^{-1} and 3935.45 cm^{-1} represents N-H stretching of amines or amides. Some shorter peaks 500-1000 cm^{-1} corresponds to C-Br stretching in halogen compounds. The peaks at 1000 cm^{-1} to 1300cm^{-1} determined the presence of aliphatic amines and alkenes. The peak at 2128.56 cm^{-1} in pulp represents the presence of alkynes. The peak observed at 2917.86 $cm^{-1}$and 3004.57 cm^{-1} indicates C-H stretching in aldehydes and alkanes. Both of the spectra showed resemblance with each other. Therefore the FTIR spectra of both the extracts reveal the presence of amines, alcoholic or phenolic group of compounds.

Antibacterial Activity

The results of antibacterial activity of plant extracts assayed by agar well diffusion method against the multidrug resistant bacteria by seed and pulp extract with reference drug

Table – 2. FTIR analysis of *M. koenigii* pulp and seed extract

S.No	Range of frequency	Pulp Extract		Seed Extract	
		Wavenumbers (cm^{-1})	Functional Groups	Wavenumbers (cm^{-1})	Functional Groups
1	500	-	-	-	-
2	500-600	674.43	C-Br Stretch	667.03	C-Br Stretch
3	675-1000	707.17 902.80 953.76	-C-H Bend of Alkenes	925.62	Alkenes
4	1000-1300	1023.09 1316.63	C-N Stretch (aliphatic amines)	1033.82 1055.44 1105.74	C-N Stretch (aliphatic amines)
5	1350-1480	1436.33	C-H bending	1409.60	C-H Bending
6	1560-1640	1647.81	N-H Bend (1º amines)	1635.33	N-H Bend (1º amines)
7	1670-1820	1906.58	C=O stretch Carbonyls		
8	2100-2360	2128.56	C≡C stretching(alkynes)		
9	2850-3000	2917.86	-CH stretching(alkanes and aldehydes)	2941.75	Carboxylic acid -OH group
10	3000-3600	3004.57	Alkenes		
		3433.30	N-H Stretch	3442.03	N-H Stretch
		3935.45	OH group	3905.86	Alcoholic-OH group

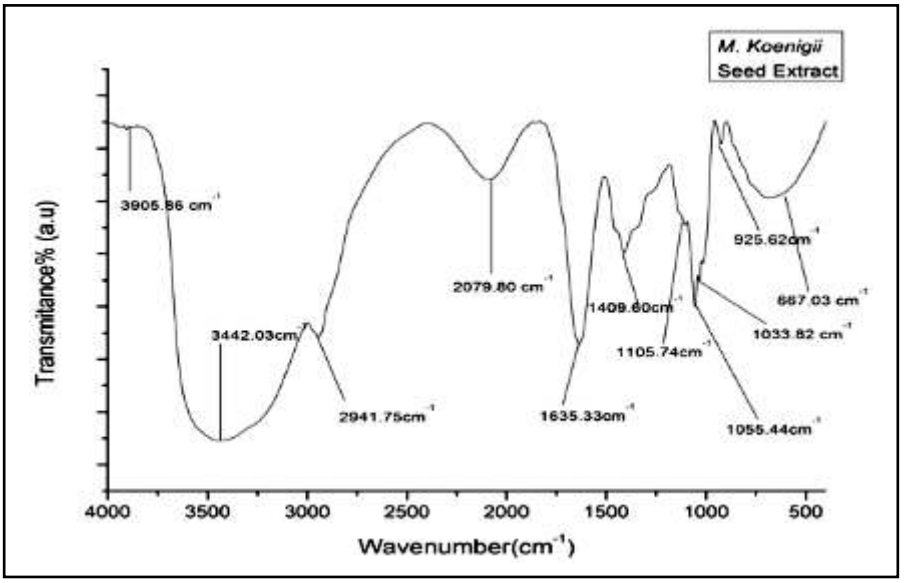


Figure –3. FTIR spectra of *M.koenigii* seed extract.

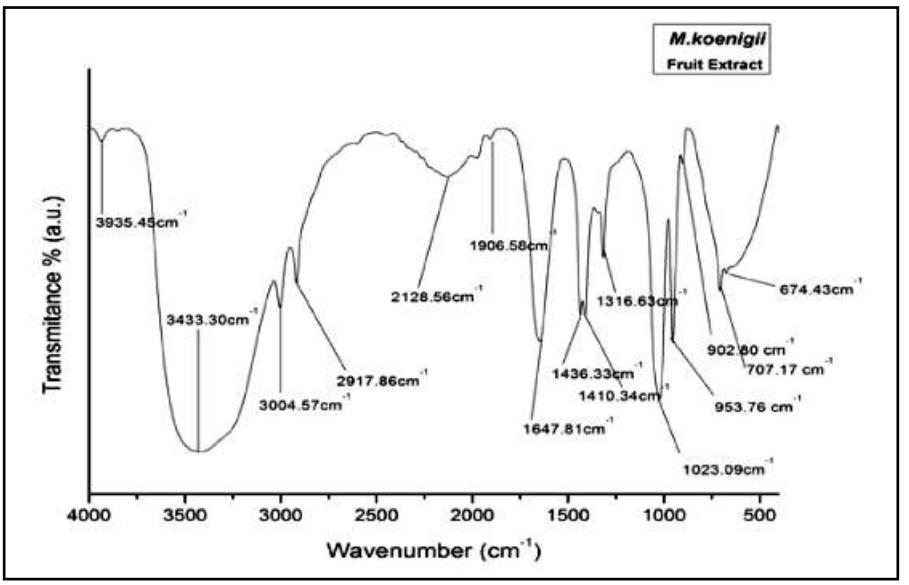


Figure - 4. FTIR spectra of *M. koenigii* fruit extract.

streptomycin are represented in **Figure – 5** and **6**. It is interesting to note that the methanolic seed extract showed highest antimicrobial activity against *Enterococcus faecalis* at 1mg/ml conc. with zone of inhibition upto18mm. No growth inhibitory activity was observed against carbapenem resistant *Klebsiella pneumoniae* by both of the extracts. Minimum inhibitory concentration (MIC) of the seed extract and pulp extract are depicted in **Figure – 7**. The MIC results showed relatively better antimicrobial potency of seed extract as compared to pulp extract. MIC value of *M. koenigii* seed extract ranged from 0.5-8mg/ml.

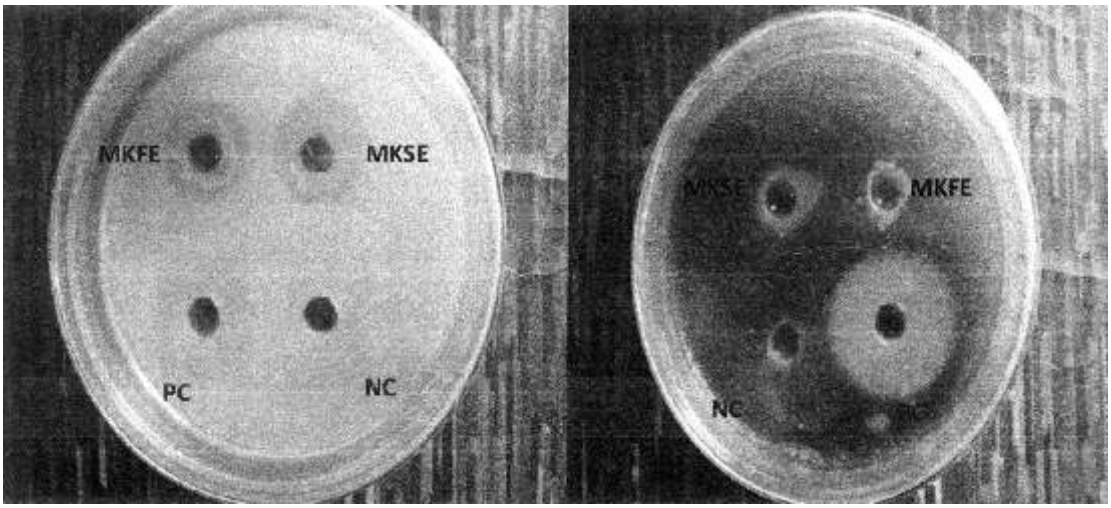


Figure - 5. Showing antibacterial activity of *M. Koenigii seed* extract and *M. Koenigii fruit* extract against *E. faecalis* and *C. violocium* .
NC- Negative Control (DMSO)
PC- Positive Control (Streptomycin)

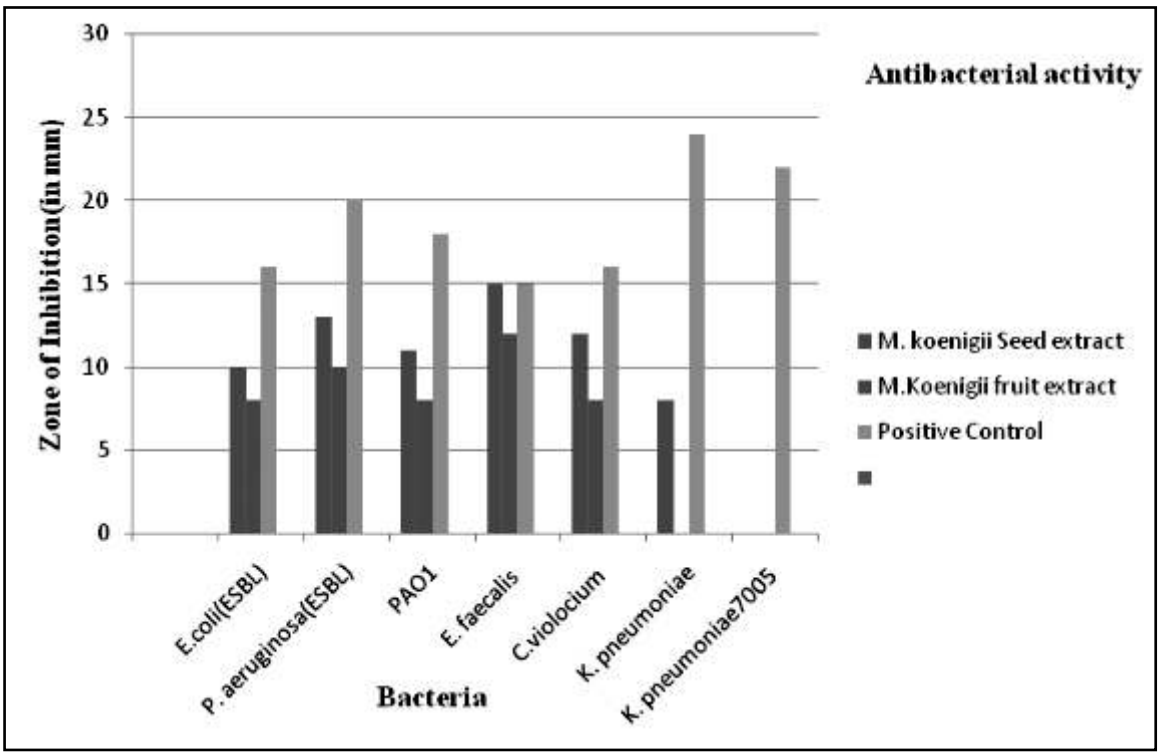


Figure – 6. Antibacterial activity of *M. koenigii* fruit pulp and seed extract against the tested bacterial pathogens.

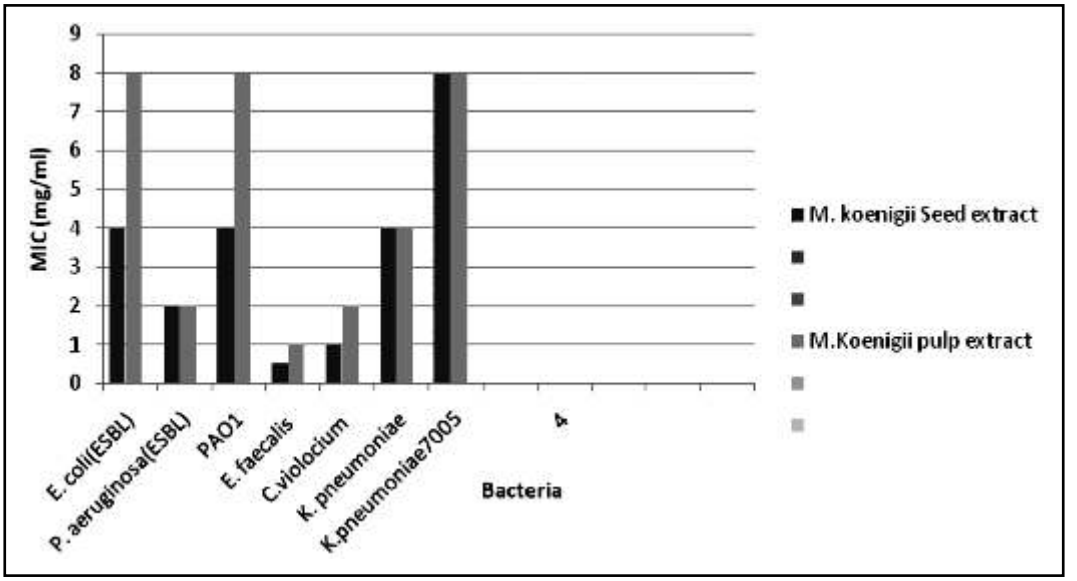


Figure – 7. Minimum Inhibitory Concentration (MIC) of *M. koenigii* fruit pulp and seed extract against the tested bacterial pathogens.

The highest potency was found against *E. faecalis* followed by of *C.violoceum* (1mg/ml), *ESBL* producing *P. aeruginosa* (2mg/ml), *ESBL* producing *Escherichia coli* (4mg/ml),(PAO1) and *K.pneumoniae* and carbapenem resistant *K.pneumoniae* (8mg/ml). On the other hand, similar pattern of MIC range value (1-8mg/ml) was observed with pulp extract.

The antibacterial activity of above extracts is probably reported for the first time. These findings indicate that phytoconstituents present in the seed/pulp extract exhibit specific and selective activity against *Enterococcus faecalis* followed by *ESBL* producing *Pseudomonas aeruginosa.* The presence of tannins in seed extract strongly suggests that the plant is astringent as documented in the literature depicting its antibacterial and antiviral activities (Shekar et al., 2015). The present study has witnessed the prospects of antimicrobial activity against MDR bacteria that are pathogens in humans. However, antimicrobial activity of other parts of the plant such as leaves, shoot and roots have been reported by various workers (Vats et al., 2011; Akula *et al.*, 2016). In the present work, even though the antimicrobial activity of both of the extracts showed pronounced effect on Gram positive bacterial strain, they also show remarkable activity against *Chromo bacterium violoceum*, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. However, many previous reports are available on the antibacterial activity of this medicinal plant, perhaps with best of our knowledge this is the first report of antimicrobial potential of pulp and seed extracts against *ESBL* producing bacterial strains.The MIC results obtained also have clinical importance that ranges from 0.5-8mg/ml by the extracts against the tested pathogen used. It is widely accepted that the plant extracts that show activity at 100mg/ml concentration could be regarded as good potency level (Rios *et al.*, 1988).

There are few reports available on the phytochemistry of pulp extract suggesting the presence of alkaloids and flavonoids (Narsimha *et al*, 1978; Reisch *et al.*, 1992). Similarly the

phytochemistry of seed extracts indicated the presence of major group of carbazole alkaloids possesing therapeutic potential (Patel et al., 2016). The phytocompounds detected in this study are in in agreement with the above reports. Therefore, the antibacterial activity of pulp and seed extracts might be attributed due to the presence of alkaloids, flavonoids, saponins, phenols and glycosides in seeds while alkaloids, flavonoids phenols, glycosides and tannin in pulp. Having traditional and folkloric usage of *M. koenigii* medicinal plant and increased presence of alkaloids in seeds,further studies are essential for the optimum exploitation of their seed as an antimicrobial agent.

Conclusion

Based on the preliminary phytochemical investigation of *M. koenigii* pulp and seed extract, it can be concluded that methanolic extract is rich source of bioactive compounds. The antibacterial activity against MDR bacteria is encouraging and needs further investigation to identify bioactive compounds and explore their therapeutic potential.

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Evaluation of Anti-Anxiety Activity of *Solanum Lycopersicum* Leaves Extract in Rodents

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Abstract-Animal models of anxiety remain a useful tool for evaluating the anxiolytic-like effect of new treatments. Even though many tests are similarly based on exploration tasks, using more than one animal model is all more recommended, since there are qualitative differences between such tests. Furthermore, although many tests are excellent tool for detecting benzodiazepines/GABA compounds, inconsistent results have been reported for 5-HT ligands. Here, this study was performed to investigate the anxiolytic-like effects of Ethanolic extract of *Solanum lycopersicum* leaves(EESL) in mice using the elevated plus-maze model (EPM), light dark model, hole board test, light and dark model. Furthermore, the anxiolytic-like effects of EESL were found to be significant as compared to a known active anxiolytic drug (Diazepam).

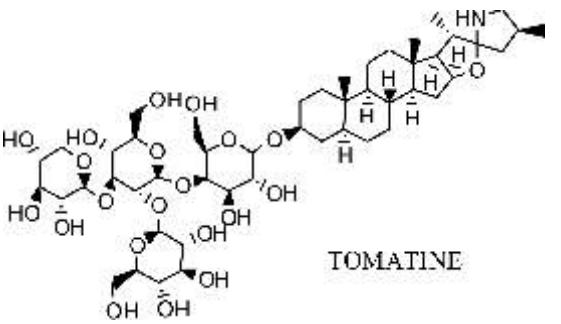
Keywords: Anxiolytic-like effect, *Solanum lycopersicum,* Diazepam, Elevated plus maze, Open field model, Light and dark test, Hole-board test.

Introduction

Mood and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries[1].Anxiety is the most common mental illness affecting one eighth of the total population and has become avery important area of research in psychopharmacology in the current decade. It is a Central Nervous System disorder and a common emotional phenomenon in humans. "Anxiety is an emotional state, unpleasant in nature and is associated with uneasiness,discomfort and concern or fear about some defined or undefined future threat"[2].

A number of biological effects of the extracts oftomato plant (Lycopersicon esculentum *Mill)* on the gastrointestinal tract, liver, urinary tract and its local anaesthetic[3, 4] *and* fungistatic activity[5, 6]have been reported. Since the acetone extract of tomato leaves[7] has already been subjected to comprehensive study, it was considered worthwhile to evaluate, in the present study, the central effects of the alcoholic extract of the leaves. The extract was investigated for behavioural, tranquillising, anticonvulsant, analgesic and anxiolytic activity[8].

The alkaloid tomatine (molecular formula $C_{50}H_{83}NO_{21}$) which is an active principle of tomato leaves[9] devoid of central activity[10] have shown that acetone extract of tomato leaves which has no tomatine in it, is also pharmacologically active. It is also known that tomatine is not soluble in water oralcohol[8]. It seems therefore, aqueous and alcoholic extract possesses central actions due toactive principles other than tomatine[8]. Structure is shown below.



Material and Methods

Animals

Adult male albino mice (20-25 gms) were obtained from the animal house of Rameshwaram Institute of Technology and Management. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed and drinking water was provided *ad libitum*. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The animals were divided into four groups, each consisting of five mice and were used in all sets of experiments. Institutional Animal Ethical Committee-Ref no. [1397/ac/10 CPCSEA] approved the protocol of the study.

Plant Material

The tomato (*Solanum lycopersicum*) plant leaves were collected from the local area of Lucknow in February 2013. The leaves after collection washed to remove the debris and then shade dried and the dried leaves powdered to get a coarse powder. The plant material was identified and authenticated by Dr. Tariq Husian, Senior Principal Scientist, Plant Diversity, Systematic and Herbarium division, NBRI, Lucknow, U.P., India.

Preparation of Extract

The collected plant material was air dried, powdered in mixer grinder. Powder material of S*olanum lycopersicum* was taken in a beaker and sufficient quantity of ethanol was added, then it was kept for maceration (72hrs) and filtered and distilled to obtain a concentrate.

Drugs

Diazepam (Ranbaxy Pharma), (2mg/kg) was used as a standard anxiolytic drugs. It was diluted with saline to the required strength before use and ethanol of analytical grade and distilled water was used as vehicle. Different concentrations of *Solanum lycopersicum* extract were prepared (200mg/kg and 400mg/kg). All the solutions were prepared freshly on test days.

Screening Methods for Anxiolytic Activity

Elevated plus maze

The plus maze apparatus consisted of two open arms, measuring 16 × 5 cm, and two closed arms, measuring 16 × 5 × 12 cm, connected to a central platform (5 × 5 cm). The maze was elevated to a height of 25 cm above the floor. Each mouse was placed individually at the center of elevated plus maze with its head facing toward an open arm and observed for 5 min to record the number of entries into open arm, closed arm and time spent in each arm. In EPM test, the percent time spent on the open arms was determined as follows:

% = 100 × Number of seconds spent on open arms/300 total seconds (5 min observation time)[11].

Open field test

The open field is a 400 × 400 × 300 mm arena with thin black stripes painted across the floor, dividing it into 16 quadratic blocks. Mouse was placed in the centre of arena and an observer quantified the spontaneous ambulatory locomotion of each mouse for 5 min. During this period, the number of squares crossed and number of rearing was measured[11].

Light Dark Box test

The apparatus consisted of two 20cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room.A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse

stepped into the dark box.The apparatus was cleaned thoroughly between trials. All behavioural recordings were carried out with the observer unaware of the treatment the mice had received[12].

Hole Crossed Test

The apparatus was composed of a gray wooden box (50 cm×50 cm× 50 cm) with four equidistant holes 3 cm in diameter in the floor. The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground and divided into squares of 10 cm×10 cm with a water resistant marker. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total locomotor activity (numbers of squares crossed), the number and duration of head-dipping were recorded. A head dip was scored, if both eyes disappeared into the hole[12].

Statistical Analysis

Results were expressed as mean ± standard error of the mean (S.E.M.). All data were subjected to analysis of variance (ANOVA) followed by Dennett's "t" test. P values <0.05(95% confidence limit) were considered statistically significant.

Results

Elevated Plus Maze

Administration of diazepam (2mg/kg) significantly increased the amount of time spent in the open arms and the percentage of open arm entries ($P< 0.001$) compared to saline-treated group. Ethanolic extract of *Solanum lycopersicum* leaves at 200 mg/kg ($P< 0.001$) and 400 mg/kg ($P< 0.001$) significantly increased the time spent in the open arms. Entries in the open arms increased significantly at 400 mg/kg ($P< 0.05$) and. Plant extract at 200 mg/kg (P>0.05) had no significant effects on any of the parameters that were measured on the EPM[Figure – 1].

Open Field Test

Diazepam (2mg/kg) significantly increased the number of square visited in centre ($P< 0.001$) compared to saline-treated group. Significant increase in theno. of square visited in centre ($P< 0.001$ and $P < 0.01$) were seen with administration of 400 and 200 mg/kg of

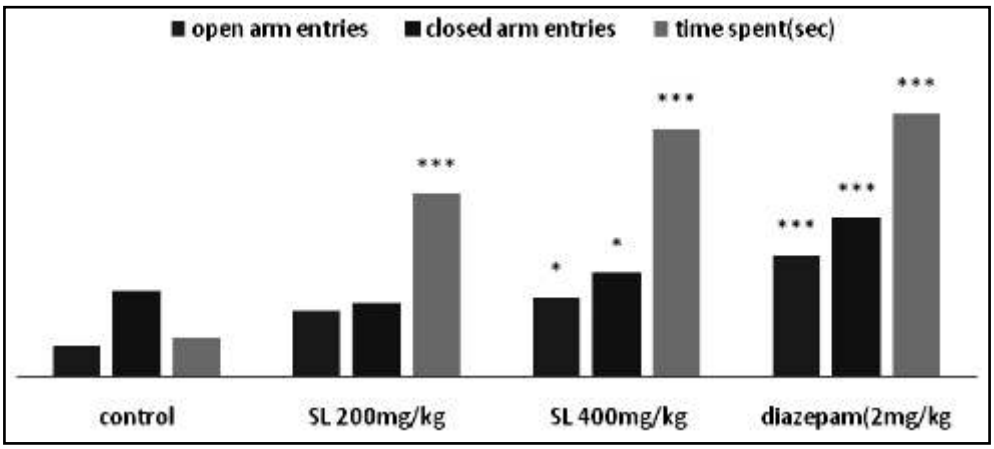


Figure –1. Elevated Plus Maze Model

Values are expressed by mean ± SEM of five animals in each group
Statistical significance: * p<0.05; **p<0.01; ***p<0.001

ethanolic extract of *Solanum lycopersicum* leaves compared to saline-treated group [Figure– 2].

Light and Dark Box test

Diazepam (2mg/kg) significantly increased the time spent in light compartment ($P< 0.001$) compared to saline-treated group. Significant increase in the time spent in the light compartment ($P< 0.001$) was seen with administration of 400 and 200 mg/kg of Ethanolic extract of *Solanum lycopersicum* leaves compared to saline-treated group. Diazepam (2mg/kg) significantly increased the number of Entries in light compartment ($P< 0.001$) compared to saline-treated group. Significant increase in the number of Entries in the light compartment ($P< 0.001$ and $P < 0.05$) were seen with administration of 400 and 200 mg/kg of Ethanolic extract of *Solanum lycopersicum* leaves compared to saline-treated group[Figure – 3].

Hole Crossed Test

The number of head dip count was moderately increased in animals treated with Ethanolic extract of *Solanum lycopersicum* 200mg/kg but statistically nonsignificant and significant ($P <0.001$) increases inanimals treated with Ethanolic extract of *Solanum lycopersicum* 400mg/kg and Diazepam (2mg/kg) significantly increased the number of head dip

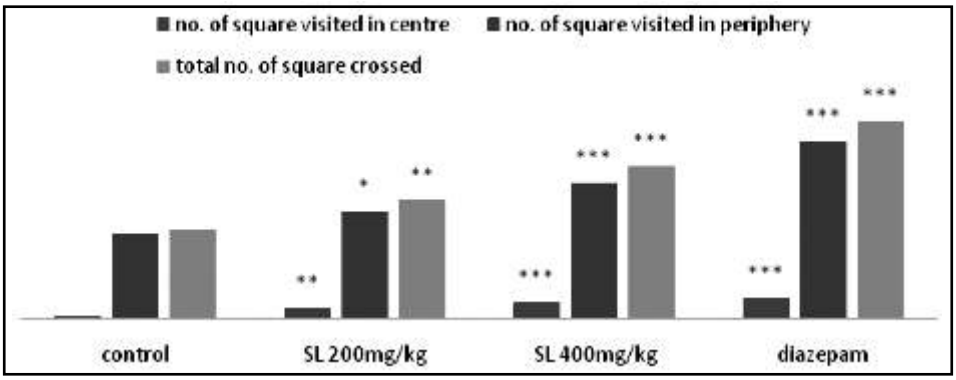


Figure – 2. Open Field Model

Values are expressed by mean ± SEM of five animals in each group
Statistical significance: * p<0.05; **p<0.01; ***p<0.001

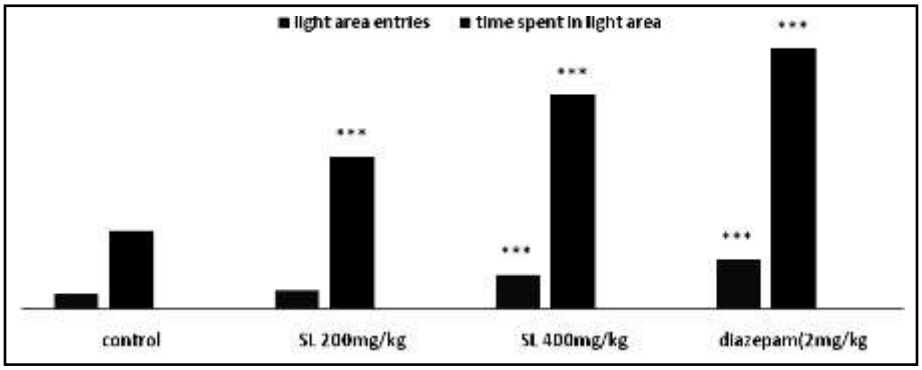


Figure – 3. Light and Dark Box Test

Values are expressed by mean ± SEM of five animals in each group
Statistical significance: * p<0.05; **p<0.01; ***p<0.001

count (P<0.001) when compared to control.The number of head dip duration was moderately increased in animals treated with Ethanolic extract of *Solanum lycopersicum* 200mg/kg but statistically non significant and significant (P <0.01) increase in animals treated with Ethanolic extract of *Solanum lycopersicum* 400mg/kg and Diazepam (2mg/kg) significantly increased the number of head dip count (P<0.001) when compared to control[Figure – 4].

Discussion

The results of the present study demonstrated that *Solanum lycopersicum* has an anxiolytic-like effect in the Elevated plus maze, Open field, Light and dark model and Hole-board test.

The assessment of anxiety related behavior in animal model is based on the assumption that anxiety in animals is comparable to anxiety in humans. One of the most widely used animal models for screening putative anxiolytic is the Elevated plus maze, in which rodents show an avoidance of exposed open areas of the maze, which are presumed to be the most aversive, and a preference for sections enclosed by protective walls[13].

In elevated plus maze open arm and closed arm entries and time ratio provide a measure of fear induced inhibition of exploratory activity. These responses are increased by anxiolytic agents, *Solanum lycopersicum* treated mice exhibited dose dependent significant increases in time spent and no. of entries in open arm and decreased in time spent and no of entries in closed arm in comparison to control mice.

In open field test is also used in rodents as a model for screening anxiolytic drugs. In which mice treated with 200 and 400 mg/kg of *Solanum lycopersicum* showed dose dependent increased in no of square visited in centre with compared to vehicle treated control mice, evincing significant anxiolytic activity of *Solanumly copersicum.* Diazepam also induced significant anxiolytic activity and the effect were found to be more then *Solanum lycopersicum.*

The hole board test has gained popularity as a model of anxiety offering "a simple method for measuring the response of an animal to an unfamiliar environment with advantages that several behaviours can be readily observed and quantified in this test[18.] "In this preliminary study significant increase in head dip count and head dip duration after treatment with 200 and 400 mg/kg of *Solanum lycopersicum.* Thus reinforcing the hypothesis that it has anxiolytic activity.

Light and Dark test is also widely used in rodents as a model for screening anxiolytic or anxiogenic drugs. In the entries in light area and

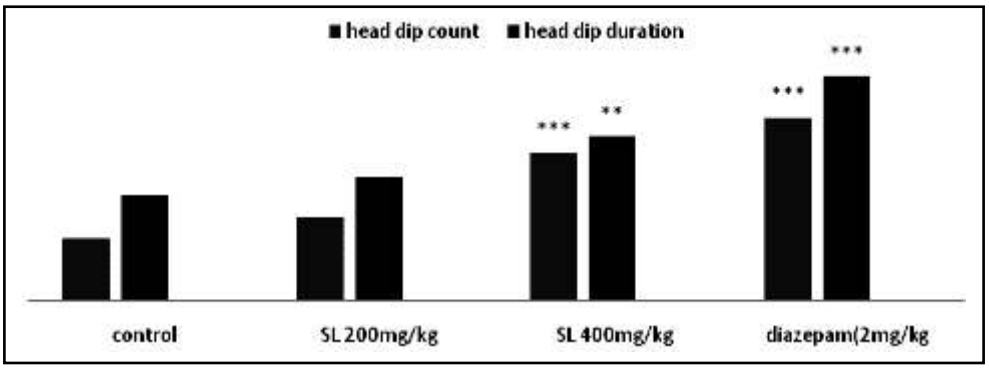


Figure – 4. Hole Crossed Test

Values are expressed by mean ± SEM of five animals in each group
Statistical significance: * p<0.05; **p<0.01; ***p<0.001

dark area and time ratio provide a measure of fear induced inhibition of exploratory activity. These responses are increased by anxiolytic agents, *Solanum lycopersicum* treated mice exhibited dose dependent significant increases in time spent and number of entries in light area and decrease in time spent and no of entries in dark in comparison to control mice.

Conclusion

The present study reveals the central effects of the alcoholic extract of the *Solanum lycopersicum* leaves. The extract was evaluated for anxiolytic activity. To summarize, all the data presented here indicate that *Solanum lycopersicum* could induce anxiolytic-like behaviour in the elevated plus maze, Open field, light and dark model and Hole-board test in mice.

Acknowledgement

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Impact of *Swertia chiraita* Extract on Prevention of Cu^{2+} Mediated LDL Oxidation and Malondialdehyde Formation: *In Vitro*

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Abstract-Diabetes is a multisystem disorder marked by elevated blood sugar level. It has been estimated that the global burden of type 2 diabetes mellitus (T2DM) for 2030 is projected to increase to 438 million ; a 65 % increase. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) as by-products of normal cellular metabolism.The chronic hyperglycaemia, insulin resistance and abnormal lipoprotein profiles found in diabetes may contribute to a decrease of bioavability of vascular nitric oxide (NO), impairing endothelium- dependent vasodilatation documented in and in humans with diabetes. Oxidized LDL may contribute to the progression of atherosclerosis by enhancing endothelial injury. Our results show that extracts of *S. chiraita* reduced LDL oxidation by about 40% which is very close to the efficacy of Atorvastatin (blocked by 51.72%).The management of diabetes nephropathy is extremely expensive and frustrating. Therefore, prevention is better. Sources of antioxidants, especially antioxidant vitamins are available and affordable in most environments. In the present investigation aqueous and ethanolic *Swertia chiraita*, increased total antioxidant power of normal plasma by 1.484 and 1.84 fold,and in diabetics it increased by 1.25 fold and 2.19 fold respectively. Extracts of *S. chiraita* are also very effective in reducing LDL oxidation and MDA formation in plasma also these are free of side effects and very cost effective.

Keywords: *Swertia chiraita*, Atorvastatin, LDL oxidation, MDA, Diabetes, ROS and Hyperglycaemia.

Introduction

Diabetes is a major health problem globally and is one of the top five leading causes of death in most developed countries. According to evidences, it could reach epidemic proportions particularly in developing and newly industrialized countries. The countries covered in American Diabetic Assosiation are as follows: United States (US), China, India, Brazil, Russia, Germany, Pakistan, Mexico, Egypt and Japan. It has been estimated that the global burden of type 2 diabetes mellitus (T2DM) for 2030 is projected to increase to 438 million ; a 65 % increase[1].It is a multisystem disorder marked by hyperglycemia; it also includes cardiovascular disease, renal failure, peripheral neuropathy and retinopathy which may lead to blindness. In case of diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. When the level of reactive oxygen and nitrogen species (ROS/RNS) increases, antioxidant defense is lowered and there is alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus. It can contribute to endothelial, vascular and neurovascular dysfunction[2]. Lipoproteins consist of a hydrophobic core of TG and cholesterol esters (CE) surrounded by a

hydrophilic surface of free cholesterol (FC), phospholipids and apolipoproteins. Plasma lipoproteins are typically classified into five major subclasses on the basis of their densities:chylomicrons (CM), very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)[3]. VLDL particles are synthesized in the liver. VLDL consists mainly of TG, and also contains some cholesterol, one apoB-100 molecule, apoCs and apoE. HDL is synthesized primarily in the liver and small intestine. HDL precursor is (nascent) discoidal particle that comprises phospholipid, cholesterol, apoE and apoA, but is devoid of cholesterol ester. HDL has various potentially antiatherogenic properties including reverse transport of cholesterol from cells of the arterial wall to the liver, inhibition of LDL oxidation by HDL-bound PON1[4]. Low density lipoprotein (LDL) includes a class of proteins which carry cholesterol in the blood and make it available for the use by the cells. LDL is one of the five major groups of lipoproteins (VLDL, IDL, LDL, HDL and chylomicrons) which are classified according to their size and density. LDL is called low-density lipoprotein because LDL particles tend to be less dense than other kinds of cholesterol particles. Each LDL particle contains a single apolipoprotien B-100 molecule, which circulates the fatty acid keeping them soluble in aqueous environment. When cell requires cholesterol, it synthesizes LDL receptors and is inserted into plasma membrane. LDL particles in bloodstream after associating with clathrin- coated pits form vesicles and binds to these extracellular LDL receptors that are endocytosed. Optimal level of LDL in humans: For good health LDL level should be low. The treatment of diabetes with synthetic drugs costs high andhave a lot of side effects so, it is necessary to develop traditional and alternative medicine. *Swertia chiraita* (Family: Gentianaceae) is a valuable herb which is commonly available in India, Nepal and China. The plant is found at an altitude of 1200–3000 meters and available throughout the year. It comprises 170 species which are closely related to each other. Chiraita, also known as Indian gentian is a robust annual herb which grows upto about 1.5 meters in height.It is generally consumed by the older people and/or people with type 2 diabetes mellitus as it is useful for lowering the blood glucose level. Ethanolic extract possesses antidiabetic activity and have significant effect on cholesterol and triglyceride level. In this study, herbal extract of *Swertia chiraita* to assess its antioxidant power and analyzed their impact on prevention of LDL oxidation and malondialdehyde formation. On its contrary, drug Atorvastatin have been also used in comparison to natural extract of *S.chiraita.*

Material and Methods

Chemicals

2, 4, 6-Tripyridyl-s-Triazine, β-Mercaptoethanol, Butylated Hydroxyl Toluene, Ferric Chloride, Heparin, Tris Hydrochloride, Magnesium Chloride, Sodium Citrate, Sodium pyrophosphate, Sulphuric Acid, Malondialdehyde, Tricholoro Acetic Acid, Trisodium Citrate (HiMedia Laboratories Pvt. Ltd., India). Dextran Sulfate, Phenyl Acetate, Thiobarbituric Acid (Sigma-Aldrich Inc., USA). Sodium Dodecyl Sulfate(Bio-Rad Laboratories, USA) Hydrogen Peroxide (RFCL Limited, India) Ethylene diaminetetraacetate (Merck Limited, India) Pyrogallol (Ranbaxy Limited, India). Coommassie Brilliant Blue G 250 (Ployscience Inc., USA). All chemicals and reagents used in this study were of analytical grade. Fresh Stems of S. chiraita were collected from area of Dhanbad (Jharkhand) and specimen was identified and authenticated at the Botanical Survey of India (BSI), Northern Zone, Dehradun (India) with Accession No. 115561 and a sample deposited in the herbarium of BSI.

Collection of blood and plasma

Fresh human blood sample was collected from the pathology laboratory of Sardar Bhagwan Singh Instt., Balawala, Dehradun, UK. India. Plasma was separated by centrifugation at 2500 rpm and the packed erythrocytes obtained were washed thrice with physiological saline and a portion of washed erythrocytes was lysed in hypotonic (10mM) sodium phosphate buffer, pH 7.4. A portion of the washed packed erythrocytes was stored at 40C for future use.

Preparation of the extract

The plant stems (Swertia chiraita) materials were thoroughly washed, shade dried till a constant weight of sample obtained and then powdered mechanically using a blender. 100g of powdered material was soaked in 100ml of aqueous and methanol medium separately for 72 hours. It was filtered by using Whatman no.1 filter paper. The solvent was distilled out completely from the filtrate under the reduction pressure in Rota vapor[25] and the weight of extract was measured.

Estimation

Determination of plasma cholesterol, Measurement of *in vitro* Cu^{++} mediated oxidation of LDL in the absence or presence of *S. chiraita*, Measurement of plasma "total antioxidant power" (FRAP)[7], Determination of Malondialdehyde in erythrocytes of normal and diabetic patients[8].

Protein estimation

The protein was determined by the method of Bradford[8] using bovine serum albumin as standard. Aliquots of LDL and HDL were first precipitated with 10 % TCA. The protein pellets were dissolved in 0.5 N NaOH and suitable aliquots were used for protein determination.

Cholesterol estimatation

Total cholesterol in plasma, LDL subfractions were determined as described by Annino and Giese[9] with a minor modification. For the determination of cholesterol in plasma and lipoproteins, 0.1 volume of plasma was mixed with 1 volume of isopropanol, allowed to stand for 5 min and centrifuged at 3,000 rpm for 10 min. A suitable aliquot of isopropanol extract was used for cholesterol determination in a total volume of 0.75 ml. To each tube 0.25 ml of 7.03 mM ferric chloride dissolved in glacial acetic acid, was added, mixed instantly followed by the addition of 0.8 ml of sulphuric acid with thorough mixing. After 5 min, the absorbance was read at 550 nm in a Beckman DU 640 spectrophotometer. The cholesterol content in the samples was determined by using a cholesterol standard.

Results

Average Value of Age, Weight, Height, Male, Female of Normal and diabetic subjects

The average values of body weight, age, male and female of normal subjects (n=45) were 59.42 ± 8.39kg, 32 ± 1.86 years, 26 and 19 resepectively and of diabetic subjects (n=60) were 64 ± 9.41kg, 58± 3.56, 29 and 31 respectively as shown in **table 1.**

Average value of TC, Total protein, LDL-C, HDL-3, HDL_2-C, HDL_3-C, HDL protein and non – HDL cholesterol in normal and diabetic subject

As shown in table – 2, the average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL2-C,HDL3-C, HDL 3 protien, HDL_2C protein, HDL_3 C protein, Non HDL-C in normal lipidemic subjects were 42.80 ± 2.39 µg/ml, 14.9 ± 2.09 µg/ml, 1.212 ± 0.066 µg/ml, 1.179 ± 0.348µg/ml, 5.36 ± 0.46 µg/ml, 1.186 ± 0.34 µg/ml, 3.172 ± 0.53 µg/ml, 0.301 ± 0.111µg/ml, 0.164 ± 0.00014µg/ml, 0.601 ± 0.018µg/ml and 37.44 ± 1.93µg/ml respectively. The average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL2-C, HDL3-C,HDL 3 protien, HDL_2C protein, HDL_3 C protein, Non HDL-C in hyperipidemic subjects

Table – 1. Average value of Age, Weight, Height, Male Female of Normal and Diabetic subject.

S.NO.	Parameter	Normal Subject (n=49)	Diabetic Subject (n=65)
1.	Age	32±1.53 year	55±3.82 year
2.	Body weight	59.42±8.29	64±9.32
4.	Male	30	43
5.	Female	19	22
6.	Drug taken by patient (pre-treated)	-	Insulin, Metformin, Glycate

Table – 2. Average value of TC, VLDL-C, LDL-C, LDL-Apo-B100, HDL-C, HDL2-C, HDL3-C in normallipidmic and Diabetic subject.

S.NO	Parameters	Normal Value (µg/ml)	Daibetic Value (µg/ml)
1.	**Total cholesterol in plasma**	42.80 ± 2.39	114.66 ± 2.24
2	**Low Density Lipoprotien – Cholesterol (LDL- C)**	14.9 ± 2.09	60.89 ± 0.53
3.	**Total protein in plasma**	1.212 ±0 .066	1.190 ± 0.015
4.	**Total protein in LDL**	1.179 ± 0.348	1.046 ± 0.28
5.	**High Density Lipoprotein cholesterol (HDL)**	5.36 ± 0.46	7.025 ± 0.63
6.	**High Density Lipoprotein2 cholesterol(HDL_2-C)**	1.816 ± 0.34	1.514 ± 0.51
7.	**High Density Lipoprotein3 Cholesterol(HDL_3-C)**	3.172 ± 0.53	4.33 ± 0.247
8.	**High Density Lipoprotein-protein(HDL)**	0.301 ± 0.111	0.332 ± 0.0016
9.	**High density Lipoprotien – protein(HDL_2-C)**	0.164 ± 0.00014	0.183 ± 0.036
10.	**High Density Lipoprotien – protein(HDL_3 –C)**	0.601 ± 0.018	0.304 ± 0.0007
11.	**Non- HDL - cholesterol**	37.44 ± 1.93	107.63 ± 1.61 *

*Indirectly calculated values
All values are mean ± S.D from pooled serum of normal subjects (n=49)
All values are mean ± S.D from pooled serum of diabetic subjects (n=65)

Table – 3. Average Ratio Value of TC/LDL-C, LDL-C/TC, HDL_2-C/HDL_2-C and HDL_3-C/HDL_2-C in Normallipidemic and Hyperllipidemic Subject.

S.NO.	Parameter	Normal Value	Daibetic Value
1.	**TC/LDL -C**	1.15	1.055
2.	**LDL -C/TC**	0.866	0.950
3.	**HDL_2-C/HDL_3-C**	0.233	0.602
4.	**HDL_3-C/HDL_2-C**	3.66	1.66

were 114.66± 2.24µg/ml,10.89±0.53µg/ml, 1.190 ± 0.015µg/ml, 1.046± 0.28µg/ml, 7.025±0.63µg/ml, 1.514± 0.51µg/ml, 4.33 ± 0.247 µg/ml, 0.332 ± 0.0016 µg/ml, 0.183 ± 0.036 µg/ml, 0.304 ± 0.0007 µg/ml, 107.63 ± 1.61 µg/ml respectively.

Average Ratio Value of TC/LDL –C, LDL –C /TC, HDL_2 – C /HDL_3 –C and HDL_3 –C /HDL_2 – C

As shown in table – 3, the average ratio Value of TC/LDL –C ,LDL –C /TC , HDL_2 – C /HDL_3 –C and HDL_3 –C /HDL_2 – C in normal lipidemic subjects were 1.15, 0.866, 0.233, 3.66 and of dyslipidemic subjects were 1.055, 0.950, 0.602, 1.66 respectively.

***In vitro* copper mediated oxidative modification of LDL (at 37°C) isolated from normallipidemic and hyperlipidemic subjects**

Cu^{2+} mediated oxidation of LDL in the presence or absence of glucose Figure – 1

In absence of glucose, 0.24 fold(+24.20%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (376.19 µM/ml)at 120 min. after adding 2.5 mM $CuSO_4$.But in case of LDL+glucose, this increase was of 40%(285.11 µM/ml - 475.59 µM/ml).

Cu^{2+} mediated oxidation of LDL in the presence or absence of Ethanolic chiraita in normal and diabetic subjects Figure – 2

In absence of Ethanolic Chiraita (E.Ch), 0.34

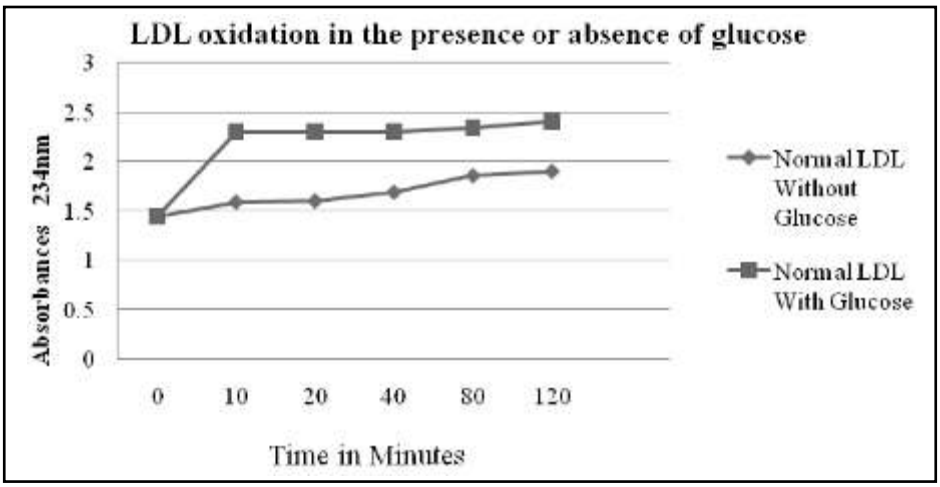

Figure – 1. Cu $^{++}$ mediated LDL oxidation in the presence or absence of glucose.

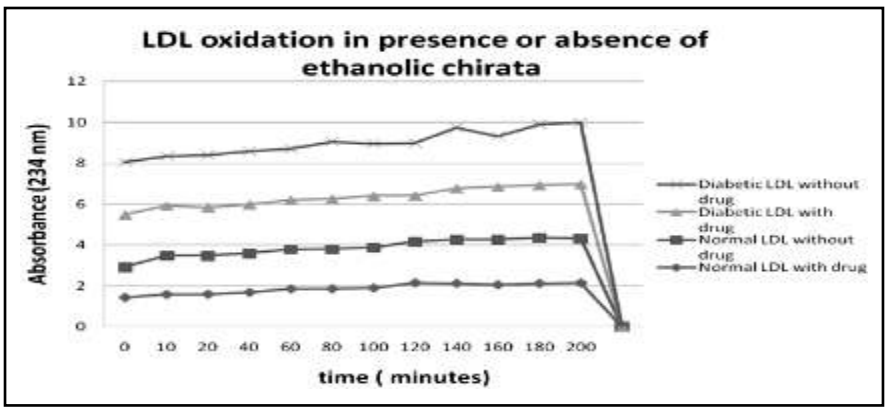

Figure – 2. Cu^{2+} mediated oxidation of LDL in the presence or absence of Ethanolic chiraita in normal and diabetic subjects.

fold(+33.92%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (432.73 µM/ml) at 200 min. after adding 2.5 mM $CuSO_4$. But in presence of E.Ch this increase was only 15%(285.11 µM/ml – 423.01µM/ml), in normal lipidemic subjects. In absence of E.Ch, 0.145 fold(+14.5%) increase in oxidative modification of LDL was observed from basal value (510.12µM/ml) to maximal value (597.22µM/ml)at 200 min. after adding 2.5 mM $CuSO_4$.But in presence of E.Ch this increase was significantly reduced to 3.31%(510.12 µM/ml – 527.57µM/ml), in hyperlipidemic subjects as shown in Figure – 2.

Cu^{2+} mediated oxidation of LDL in the presence or absence of aqueous chiraita in normal and diabetic subjects Figure – 3 and 4

In absence of Aqueous Chiraita(Aq. Ch.)

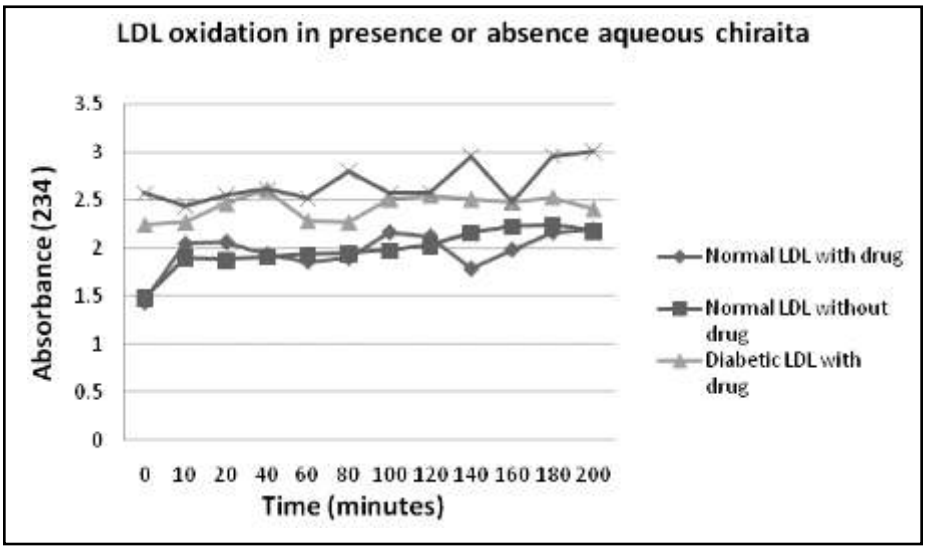

Figure – 3. Cu^{2+} mediated oxidation of LDL in the presence or absence of aqueous chiraita in normal and diabetic subjects.

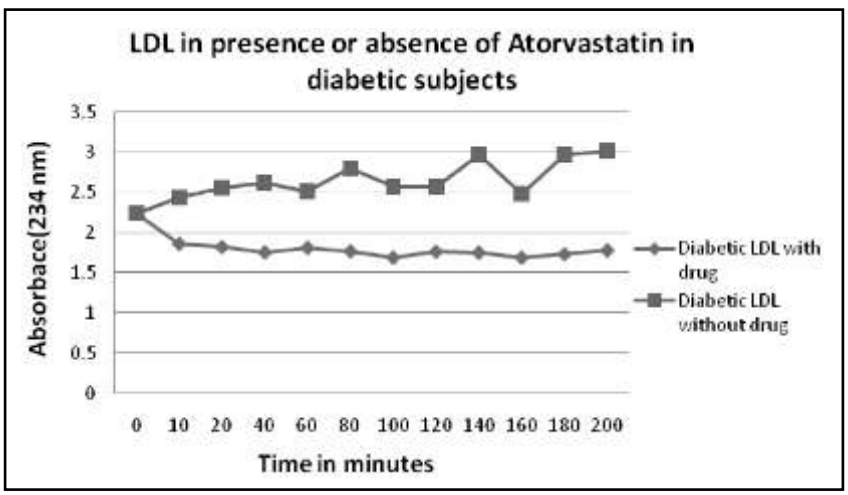

Figure – 4. Cu^{2+} mediated oxidation of LDL in the presence or absence of Atorvastatin in diabetic subjects.

,0.34fold(+34.11%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (432.32 µM/ml)at 200 min. after adding 2.5 mM $CuSO_4$.But in presence of Aq.Ch this increase was only 30.61%(285.11 µM/ml – 434.32µM/ml), in normal lipidemic subjects. in absence of Aq.Ch,0.255 fold(+25.5%) increase in oxidative modification of LDL was observed from basal value (510.12µM/ml) to maximal value (597.22µM/ml)at 200 min. after adding 2.5 mM $CuSO_4$.But in presence of Aq.Ch this increase was significantly reduced to 6.9%(510.12 µM/ml – 477.77µM/ml), on the other hand, same result registered copper mediated LDL oxidation, with Atorvastatin in hyper lipidemic subjects.

Formation of malondialdehyde in plasma of normal and diabetic patients in the presence or absence of *Swertia chiraita* extract and vitamin C figure – 5

Baseline MDA formation at 535 nm, in normal and diabetic subjects without extracts were 265.87 ± 0.552 nM/mg/ml and 341.26 ± 0.611 nM/mg/ml respectively. After addition of extracts, baseline MDA formation in normal subjects were significantly reduced. Reduction in baseline value MDA after addition of E.C leaves, Aq.C leaves, E.Ch, Aq. Ch, E.C bark,Vitamin C, Atorvastatin in normal LDL were 41.9% ,60.5%,53.57% and 67.8% respectively, and reduction in baseline value after addition of E.Chiraita, Aq. Chiraita and Atorvastatin in diabetic subjects were 40.65%,55.43%,49.27% and 69.81% respectively.

Discussion

Diabetes is a multisystem disorder marked by elevated blood sugar level. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms[5]**.***In our baseline study of various physiochemical parameters, the average values of physical parameters like,* of body weight, age, male and female of normal subjects (n=45) were 59.42 ±

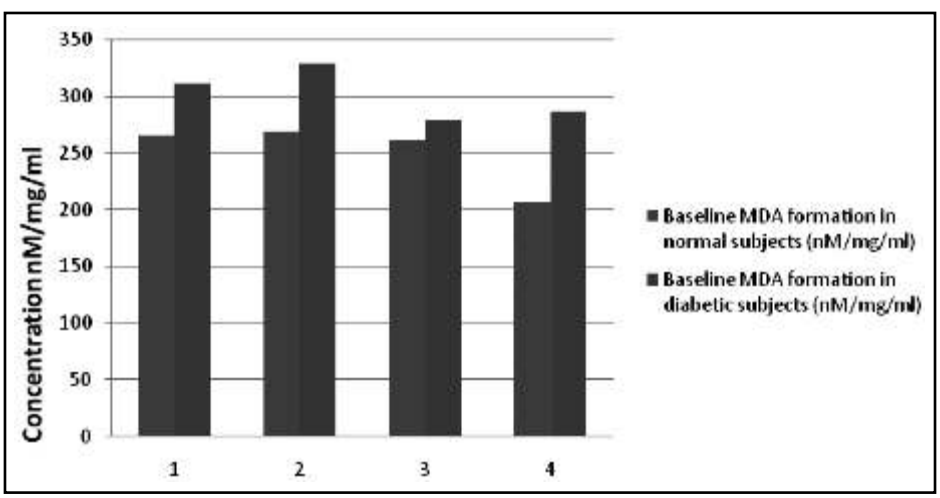

Figure – 5. Formation of malondialdehyde in plasma of normal and diabetic patients in the presence or absence of *Swertia chiraita* extract and Atorvastatin. 1= without drug, 2= ethanolic chiraita, 3= Aq. chiraita, 4= Atorvastatin.

8.39kg, 32 ± 1.86years, 26 and 19 respectively, and of diabetic subjects (n=60) were 64 ± 9.41kg, 58± 3.56, 29 and 31 respectively. Statistical evaluation of the lipid profile among the normal and diabetic patients were compared[10], and the normal range for total cholesterol should be 150-200 mg dl^{-1}. In the present study, the results showed that the lipid and the lipoprotein profiles of the diabetics were higher than that of the controls and they were comparable with the findings of Albrkiet al[12]. The average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL_2-C, HDL_3-C,HDL-C protein, HDL_2C protein, HDL_3 C protein, Non HDL-C in normal lipidemic subjects were 42.80 ± 2.39 µg/ml, 14.9 ± 2.09 µg/ml, 1.212 ± 0.066 µg/ml, 1.179± 0.348µg/ml, 5.36 ± 0.46 µg/ml, 1.186 ± 0.34 µg/ml, 3.172 ± 0.53 µg/ml,0.301± 0.111µg/ml,0.164 ± 0.00014µg/ml,0.601± 0.018µg/ml and 37.44 ± 1.93µg/ml respectively. The average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL_2-C, HDL_3-C, HDL protein, HDL_2C protein, HDL_3C protein, Non HDL-C in hyperipidemic subjects were 114.66 ± 2.24µg/ml,10.89 ± 0.53µg/ml,1.190 ± 0.015µg/ml, 1.046 ± 0.28µg/ml, 7.025 ± 0.63µg/ml, 1.514 ± 0.51µg/ml, 4.33 ± 0.247 µg/ml, 0.332 ± 0.0016 µg/ml, 0.183 ± 0.036 µg/ml , 0.304 ± 0.0007 µg/ml, 107.63 ± 1.61 µg/ml respectively.The average ratio Value of TC/LDL–C , LDL–C /TC , HDL_2 – C /HDL_3 –C and HDL_3–C /HDL_2–C in normal lipidemic subjects were 1.15, 0.866, 0.233, 3.66 and of dyslipidemic subjects were 1.055, 0.950, 0.602, 1.66 respectively. Dyslipidaemia was observed in the diabetic population, but that HDL-C was not significantly decreased. Lipid disorders are very common in both insulin dependent and non-insulin dependent diabetic mellitus. The chronic hyperglycaemia, insulin resistance and abnormal lipoprotein profiles found in diabetes may contribute to a decrease of bioavability of vascular nitric oxide (NO), impairing endothelium-dependent vasodilatation documented in and in humans with diabetes[5]. NO possesses a variety of antiatherogenic properties and loss of these protective mechanisms may lead to an increase in susceptibility to vascular disease. *In vitro* cell mediated oxidative processes usually require the presence of transition metal ions to oxidize the LDL. Oxidized LDL may contribute to the progression of atherosclerosis by enhancing endothelial injury by inducing foam cell generation and smooth muscle proliferation, it also initiate endothelial inflammation leading to atherosclerosis and CVD. Modifications take place either in plasma or in the inner layer of the artery[6].In my present investigation it was found that in absence of glucose, 0.24 fold (+24.20%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (376.19 µM/ml) at 120 min after adding 2.5 mM $CuSO_4$. But in case of LDL+glucose, this increase was of 40% (285.11 µM/ml - 475.59 µM/ml) which suggests that elevated glucose level promote LDL oxidation in normal plasma. *In vitro* treatment was given to both normal lipidemic and dyslipidemic subjects with natural herbal Aq. and ethanolic extracts of *Swertia chiraita* and significant decrease in LDL oxidation was observed. In case of E.Ch. LDL oxidation was reduced by 48.92% in normal lipidemic subjects and dyslipidemic subjects it was reduced by 41.19%. When *in vitro* treatment was given with Aq.Ch.LDL oxidation was decreased by 44% in normal lipidemic subjects and in dyslipidemic subjects LDL oxidation was reduced greatly by 32.4%. Very potent drug, Atorvastatin of statin family was also used for inhibition LDL oxidation. Atorvastatin reduced LDL oxidation drastically by 51.72% in diabetic patients. FRAP is a novel method for assessing “antioxidant power” in which Ferric ion is reduced to ferrous ion at low pH and lead to formation of coloured ferrous tripyridyltriazine complex[7,12,-18].

Total antioxidant power of normal plasma, without any extract increased from 277.88µM/ml to 431.003 µM/ml (0.35 fold increase) with time (from 0' to 5') whereas in diabetic patients it decreased from 185.78 µM/ml to 142.62 µM/ml (0.23 fold decrease) with time (from 0' to 5')[11,18-24]. In case of Aq. and ethanolic *Swertia chiraita*, total antioxidant power of normal plasma increased by 1.484 and 1.84 fold, and in diabetics it increased 1.25 fold and 2.19 fold. Baseline MDA formation at 535 nm, in normal and diabetic subjects without extracts were 265.87 ± 0.552 nM/mg/ml and 341.26 ± 0.611 nM/mg/ml respectively. After addition of extracts, baseline MDA formation in normal subjects was significantly reduced. Reduction in baseline value MDA after addition of E.C leaves, Aq.C leaves, E.Ch, Aq. Ch, E.C bark, Vitamin C, Atorvastatin in normal LDL were 41.9%, 60.5%, 53.57% and 67.8% respectively, and reduction in baseline value after addition of E.Chiraita, Aq. Chiraita and Atorvastatin in diabetic subjects were 40.65%, 55.43%, 49.27% and 69.81% respectively. In conclusion, the present investigation aqueous and ethanolic *Swertia chiraita*, increased total antioxidant power of normal plasma by 1.484 and 1.84 fold, and in diabetics it increased by 1.25 fold and 2.19 fold respectively. Extracts of chiraita are also very effective in reducing MDA formation in plasma, also these are free of side effects and very cost effective.

Acknowledgments

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Variation in Physico-Chemical Properties of *Cymbopogon citratus* (DC. ex Nees) Essential Oil from Different Locations of Uttarakhand, India

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Abstract-*Cymbopogon*, better known as lemongrass, is a genus of Asian, African, Australian and tropical island plants in the grass family. Some species (particularly *Cymbopogon citratus)* are commonly cultivated as culinary and medicinal herbs because of their scent, resembling that of lemons (*Citrus limon*).. Lemongrass oil is used as a pesticide and a preservative. Research shows that lemongrass oil has antifungal properties. Despite its ability to repel some insects such as mosquitoes, its oil is commonly used as a "lure" to attract honey bees. Because of this, lemongrass oil can be used as a lure when trapping swarms or attempting to draw the attention of hived bees. Looking at the importance of essential oil extracted from lemon grass, the present study was undertaken to study variation in physicochemical properties of Cymbopogon citratus (DC. ex Nees) essential oil extracted from leaves in different locations of Uttarakhand. Collection of plant material from different locations was carried out and the essential oil was extracted using hydro-distillation technique and studied it for its physicochemical properties.

Lemon grass leaves from Dehradun had the significant effect on the essential oil content (2.40%) on dry weight basis while lemon grass leaves obtained from Mussoorie and Rishikesh afforded oil at percentage of 2.10% and 2.12% respectively. Eighteen major components were identified in the essential oil which represented 99.29-84.41% of the oil component. The major components of the essential oils (extracted from lemongrass leaves collected from Dehradun, Mussoorie, Rishikesh and Vikasnagar, respectively) were Geranial (14.72, 31.53, 39.86 and 37.24%), Neral (34.52, 30.08, 34.98 and 31.28%) and Myrcene (15.69, 16.61, 14.49 and 15.42%) respectively. The quality of lemongrass is generally determined by its citral content. Comparison of the results showed that different locations had no variations in the major components of the essential oil, but had a significant effect on their percentages.

Keywords: Lemon grass, Physicochemical Property, GCMS, Hydrodistillation

Introduction

Cymbopogon commonly known as lemon grass represents an important genus of about 120 species that grow in tropical and subtropical regions around the world. On account of their diverse uses in pharmaceutical, cosmetics, food and flavor, and agriculture industries, Cymbopogon grasses are cultivated on large scale, especially in tropics and subtropics[1]. Cymbopogon citrates (lemon grass) a well known specie possesses strong lemony odour due to its high content of the aldehyde citral which has two geometric isomers, geranial (citral -a) and neral (citral - b)[2]. As a medicinal plant, lemongrass has been considered a carminative and insect repellent. It is used in herbal teas and other non alcoholic beverages in baked goods and in confections. Essential oil from lemongrass is widely used as a fragrance in perfumes and cosmetics such as soaps and creams. Citral extracted from the oil, is used in

flavoring soft drinks in scenting soaps and detergents, as a fragrance in perfumes and cosmetics and as a mask for disagreeable odors from several industrial products. Citral is also used in the synthesis of ionones used in perfumes and cosmetics. Source of lemongrass oil used to flavor teas, ice cream, candy, pastries, desserts and chewing gum, also used in cosmetics and perfumes. Quick growing clumps can be used instead of the classic lemongrass (Thai) in culinary dishes. Lemongrass oil revitalizes the body and relieves the symptoms of jetlag, clears headaches and helps to combat nervous exhaustion and stress-related condition citral, exhibited high antibacterial activity[3,4] anticonvulsant activity, antiviral activity[5], antifungal activity[6.7], sedative as well as motor relaxant effects[8]. Lemongrass is sour, cooling and astringent. Therefore, it combats heat and tightens tissues of the body[9]. looking at wide variety of activity associated with citral main component of *Cymbopogon citratus* (DC. ex Nees). The present work aims to study the variation in chemical composition and physicochemical characteristics of the volatile oil extracts of *Cymbopogon citratus* (lemon grass) growing in different locations of Uttarakhand.

Material and Methods

The fresh leaves of *Cymbopogon citratus* were collected from different locations of Uttarakhand namely, Dehradun, Mussoorie, Rishikesh, Vikasnagar in first half of March 2018 (growing season).

Extraction procedure

Fresh and dried leaves of *Cymbopogon citratus* (DC. ex Nees) (100 gm) were subjected to hydro-distillation for three hours using Clevenger apparatus. The extracted essential oils were dried using anhydrous sodium sulphate and stored in sealed vials at low temperature (2 ^{0}C) before analysis.

Physico-Chemical Properties of Essential Oil

Physical parameters of the essential oils extracted from leaves *Cymbopogon citratus* (DC. ex Nees) were determined using the methods described by [10,11]. These parameters are the density, refractive index, rotatory power and acid Index.

Density at 20°C

The density measure was carried out using a mettler toledo precision balance.

Refractive index at 20°C

The refractive index was determined by means of the refractometer, Carl Zeiss Jena 234678.

Rotatory power at 20°C

The measurement was made by Carl Zeisspolarimeter, 128291.

Acid index Ia

The material used to determine the acid index was constituted by phenolphthalein, neutralized ethanol, potassium hydroxide (0.05N) and a graduated burette. The index acid calculation was done using the following formula

$Ia = 5.61xV / m.$

V = Volume in mL of the ethanolic solution of

potassium hydroxide m = Mass measured in gram of essential oil charged.

Gas chromatography

GC analyses were performed using a HP 6890 GC gas chromatograph equipped with a fused capillary column (30 m ·320 lm i.d., film thickness 0.25 lm) coated with 5% Phenyl Methyl Siloxane (HP-5). Oven temperature was held at 50 ^{0}C for 2 min and then programmed to 240^{0}C at a rate of 8 C/min. Detector (FID) temperature was 280^{0}C and injector temperature was 240^{0}C; nitrogen was used as carrier gas with a linear velocity of 30 ml/min. The percentages of compounds were calculated by the Area normalization method.

Gas chromatography–mass spectroscopy

GC–MS analyses were carried out using a Varian 240 GC–MS system equipped with a VF-5 fused capillary column (30 m · 0.25 mm i.d., film thickness 0.25 lm); oven temperature was 50–180 ^{0}C at a rate of 5^{0} _C/min, transfer line temperature 250 ^{0}C, carrier gas was helium with a flow rate of 1 ml/min, spilt ratio 1:20, ionization energy 70 eV, and mass range 35–390 a.m.u. The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances

Results and Discussion

The table–1. shows the values of the four physico-chemical factors measured. In the table–2. are presented the results of chromatographic analysis of essential oils extracted from leaves of *Cymbopogon citratus* (DC. ex Nees) grown in different locations of Uttarakhand, India.

Table – 1. Physico-Chemical Properties of Essential Oil of Cymbopogon *citratus* (DC. ex Nees).

S.No	Sample	Yield (%)	Density	Refractive Index (at 20°C)	Rotary power(at20°C)	Ia (mg de KOH/g)
1	Dehradun	2.40	0.943	1.4839	- 44.8	2.596
2	Rishikesh	2.12	0.940	1.4835	- 44.87	2.562
3	Mussoorie	2.10	0.941	1.4842	- 44.84	2.597
4	Vikas Nagar	2.34	0.899	1.4845	- 44.88	3.000

Table – 2. Chemical Composition of Essential oil of Cymbopogon *citratus* DC. ex Nees from Different Locations of Uttarakhand

No.	Compounds	RT	Dehradun	Mussorrie	Rishikesh	Vikasnagar
1	Myrcene	9.15	15.69	16.16	14.49	15.42
2	Limonene	9.78	0.41	0.42	0.43	0.40
3	E,E-cosmene	10.37	0.20	0.23	0.21	1.26
4	Z-b-Ocimene	10.60	0.97	traces	0.17	0.22
5	E-b-Ocimene	10.94	0.41	0.28	0.26	0.27
6	a-Terpinolene	12.87	1.02	1.09	1.09	1.06
7	Citronellal	13.16	0.60	2.06	2.03	3.01
8	Cis-Verbenol	14.10	0.15	0.15	0.15	0.18
9	Linalool	14.66	1.03	2.06	2.03	2.44
10	Cis- Carverol	15.25	1.18	1.49	1.35	1.47
11	Atrimesol	15.41	0.26	0.15	T	0.19
12	Nerol	16.15	0.17	0.27	0.22	0.29
13	Neral	17.15	34.52	30.08	34.98	31.28
14	Geraniol	17.44	0.53	0.86	0.95	1.31
15	Geranial	18.01	14.72	31.53	39.86	37.24
16	Carveol	18.48	0.18	0.65	0.38	0.73
17	Geranyl acetate	21.38	0.51	0.7	0.49	0.67
18	Caryophellene	22.66	0.28	0.23	0.20	0.21

RT: Retention time

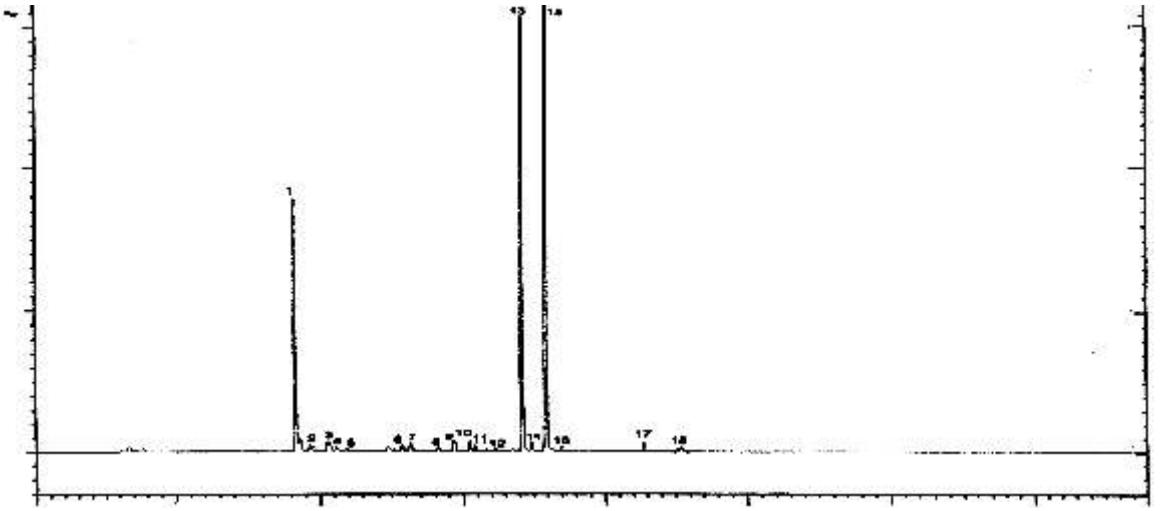

Figure – 1 Gas Chromatogram of Cymbopogon *citratus (*DC. ex Nees).

Cymbopogon citratus (DC. ex Nees) leaves were collected from different locations. The varied quantities (yield) of essential oil are shown in Table – I. The results indicated that lemongrass leaves from Dehradun had the highest essential oil content (2.40%) on dry weight basis while lemongrass leaves from Mussoorie and Rishikesh afforded oil at percentages of 2.10% and 2.12% respectively with no significant difference between their physico chemical properties i.e refractive index, rotary power, density and acid value, well in agreement with lieterature. The quality of lemongrass is generally determined by its Citral (Geranial and Neral) content. Eighteen components were identified in the essential oil of leaves of Cymbopogon citratus (DC. ex Nees) in different locations which represented 99.29–88.41% of the oil components. The chemical constituents of oils are presented in (Figure – 1) and (Table – 2). The components are listed in order of their retention time on the VF-5 column. The major components of the essential oils were Geranial (14.72, 31.53, 39.86 and 37.24%), Neral (34.52, 30.08, 34.98 and 31.28%) and Myrcene (15.69,16.61, 14.49 and 15.42%) in oils extracted from lemongrass leaves collected from Dehradun, Mussoorie, Rishikesh and Vikasnagar, respectively. The proportions of Geranial and Neral in the essential oil of Rishikesh lemon grass leaves were more pronounced than those in the other three areas while Myrcene content was higher in Mussorrie area. This variation in composition could be due to climatic and soil conditions of the different locations.

Conclusion

Variation in chemical composition of essential oil from different locations was observed according to the place of collection of plant species in terms of their percentage content. In general, essential oil collected from Rishikesh predominated in Citral content of the oil, one of very important factors for assessing biological activity associated with the essential oil. Thus, essential oil sample collected from Rishikesh locality can be regarded as better quality of essential oil in comparison to other oil samples.

Acknowledgement

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Cell Cycle Arrest and Gene Expression in MDAMB231 Cells Treated with *Curcuma Longa* Silver Nanoparticles: Nanotechnology Approach

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Abstract-Recently nanoparticles (NPs) have been widely utilized for biomedical applications, such as diagnostics, drug delivery and tissue engineering due to their unique pharmacological properties. The several in-vitro studies supported silver nanoparticles (AgNPs) applications to cancer therapy. Therefore, the present study aimed to investigate the anticancer and anti-metastatic potential of *Curcuma longa* Silver nanoparticles (CUR/AgNPs) on breast cancer cells (MDAMB-231) with the help of expression level of MMP-2, MMP-9, TIMPs-2 and TIMPs-3. This study also found that Silver nanoparticles of *Curcuma longa* (CUR/AgNPs) significantly induces apoptosis and cancer cell cycle arrest in G1 phase and increase the expression level of TIMPS by several folds and at the same time lowers the expression of MMPS by several folds. The high dose of CUR/AgNPs (B) i.e. 14µg/ml showed more activity in lowering the expression level of MMPS and increasing the expression level of TIMPS as compared to dose 10 µg/mlof CUR/AgNPs (A) in breast cancer cell line MDAMB-231.

Keywords: Apoptosis, *Curcuma longa*, Nanoparticles, Cell cycle

Introduction

Breast cancer, a malignant tumor associated with metastasis, high mortality rate, comprises of ~7-10% of all systemic malignant tumors[1,2]. The metastasis involves the disruption of several collagen-endowed tissue barriers. However, physical obstruction to tumor metastas is the basement membrane (BM) that lines vascular endothelial cells. Matrix metalloproteinases (MMPs) are a family of at least 20 zinc-dependent endo-peptidases have the ability to hydrolyze extracellular matrix (ECM) components[7]. The MMP-2 and MMP-9 major members of (MMPs) family, mainly secreted by tumor cells and stromal cells in the form of zymogens plays important roles in tumor invasion and metastasis by degrading extracellular matrices[9,10]. These MMPs after activation v degrade basement membrane (BM) type IV collagen while disturbing the ability of BMs to hinder tumor cell movement[8]. The natural inhibitors of MMPs are Tissue inhibitor of metalloproteinase (TIMPs), and an imbalance in local MMP and TIMP concentrations may induce an increase in MMP expression and a decrease in TIMP production at the same time[9,10]. Although, some modern treatment modality such as surgical resection along with radiotherapy and chemotherapy are effective therapy but the recurrence and metastasis rates associated with poor prognosis remain high in breast cancer patients[3,6.] In recent years, with the developments in evidence-based medicine effective treatment methods have been developed to combat breast cancer. In the same context currently noble metal nanoparticles (NPs) have been studied for its biomedical applications, such as diagnostics, drug delivery and tissue engineering due to their novel therapeutical properties[1,4]. Among various metal nanoparticles, biologically synthesised silver nanoparticles (AgNPs) have received great attention in a variety of applications, including

their applications to cancer therapy supported by several studies in a variety of cancer cells including breast cancer cells[7,10]. Therefore, the present study aimed to investigate the anticancer and anti-metastatic potential of *Curcuma longa* Silver nanoparticles CUR/AgNPs on breast cancer cells (MDAMB-231) with detection of apoptosis and quntifying the expression level of MMP-2, MMP-9, TIMPs-2 and TIMPs-3.

Material and Methods

The Modified Eagle's media (MEM), fetal bovine serum (FBS), HiMedia antibiotics for animal cell culture. Silver nitrate Sigma Aldrich (USA), ethanol, and other chemicals were obtained from HiMedia, Primers from IDT primer, cDNA and Mastermix from Thermofisher. The breast cancer cells MDAMB231 were obtained from National Centre for Cell Sciences (NCCS) Pune, India. *Curcuma longa* filtrate used for the formation of two sample of C*urcuma longa* silver nanoparticles and these formulations named as CUR/AgNPs (A) and (B). Bio-based synthesised and characterized monodispersed *Curcuma longa* silver nanoparticles induces targeted anticancer activity in breast cancer cells. (Phcog Mag., 2018,14:S340-5)

Cancer cell culture

Human breast cancer cells (MDAMB231) were obtained from NCCS, India. These cells were grown in MEM, 10% FBS and 1% antibiotic and incubated at 37°C in 5% CO_2 with 95% humidity. The cells were maintained in vented tissue culture flask.

Flow cytometry assay

Flow cytometry assaywas performed to check the cell cycle arrest in MDAMB231 cells. Seeded cells in 6-well plates were left overnight for attachment followed by replacement of serum-free media and 48 hrs treated cells with or without IC_{50}dose of CUR/AgNPs(A) and 14µg/ml dose of CUR/AgNPs(B). After 48 hrs of treatment, decanted the media and washed adherent cells with Phosphate Buffered Saline (PBS) and added 200µl trypsin for 5 min at room temperature. Then centrifuged the cells at 3,000 rpm for 5 min and drained the supernatant. Cell pellets were washed and fixed in 1 ml ice-cold 70% ethanol, stored at -20℃ for 24 hrs. For staining, cells were centrifuged at 4000 rpm for 5 min and decanted the supernatant and re-suspended in 40 µg/ml PI and 0.1 mg/ml RNase in a dark room for 30 min at 37℃. Results were analyzed by Mod Fit LT 3.0 software[11].

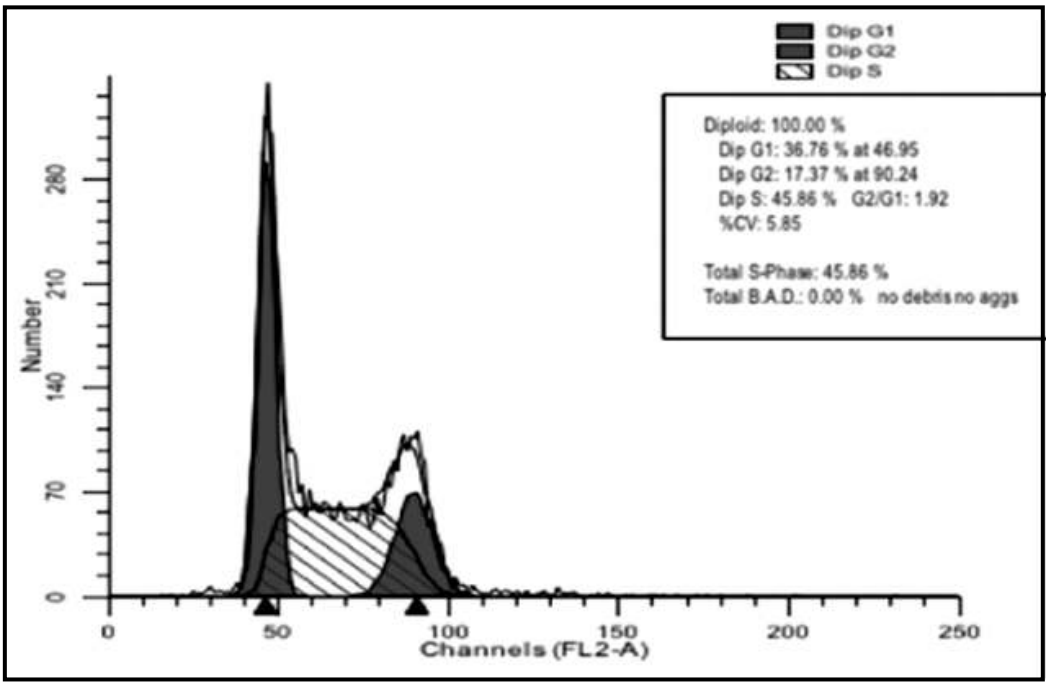
RT PCR assay RNA isolation and RT-PCR

Total RNA was extracted from the cancer cells by TRIzol reagent and RNA (1 µg) was used for RT-PCR (Invitrogen). Primers MMP-2, MMP-9, TIMP-2, TIMP-3, and GAPDH, a housekeeping gene were designed. The polymerase chain reaction (PCR) reaction mixture contained 2 µL of each cDNA sample, 10 pM each of sense and antisense primers, and other PCR reagents in a final volume of 20 µL. Biorad T100 Thermal Cycle used for the reactions as follows. PCR cycles run at 94°C for 5 minutes, then 35 cycles of denaturation at 94°C for 30 Sec, annealing 1 minute at 54°C and polymerization at 72°C for 1 minutes, followed by 72°C for 7 minutes. The RT-PCR products were visualized on 1% agarose gels electrophoresed in 0.5× TBE buffer containing 0.5 µg/mL ethidium bromide[12].

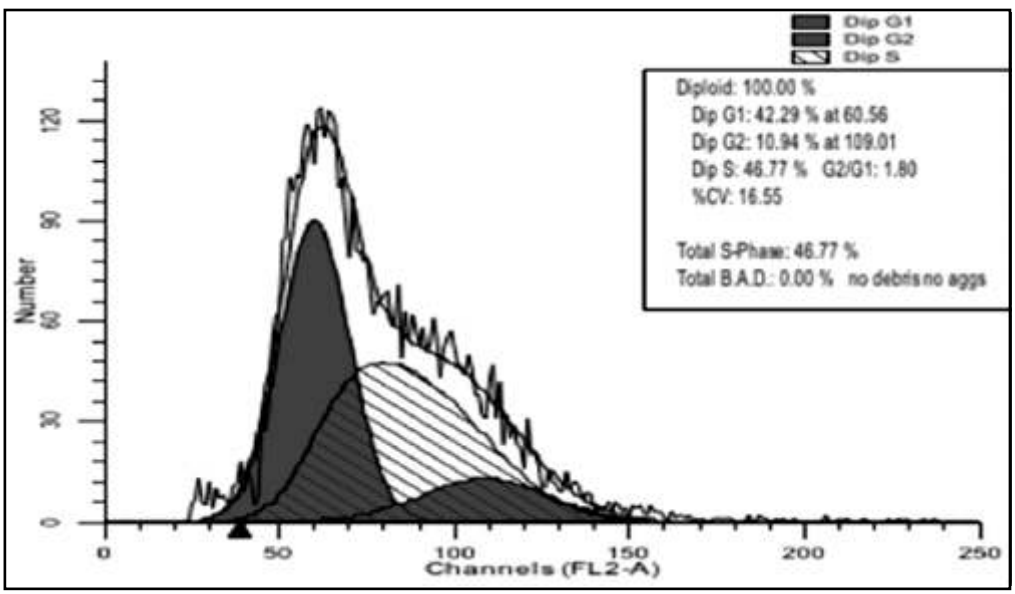
Results

Flow Cytometry

Flow cytometer used to detect the effect of *Curcuma longa* silver nanoparticles sample (A) and sample (B) on cell cycle distribution. Flow histograms[(1,2)] represent cell cycle distribution in breast cancer cells after 48 hours exposure. *Curcuma longa* silver nanoparticles sample (A) and sample (B) exposed to MDAMB231 cells resulted in 42.29% and 45.37 % of cells arrest at G0/G1, as compared to control.

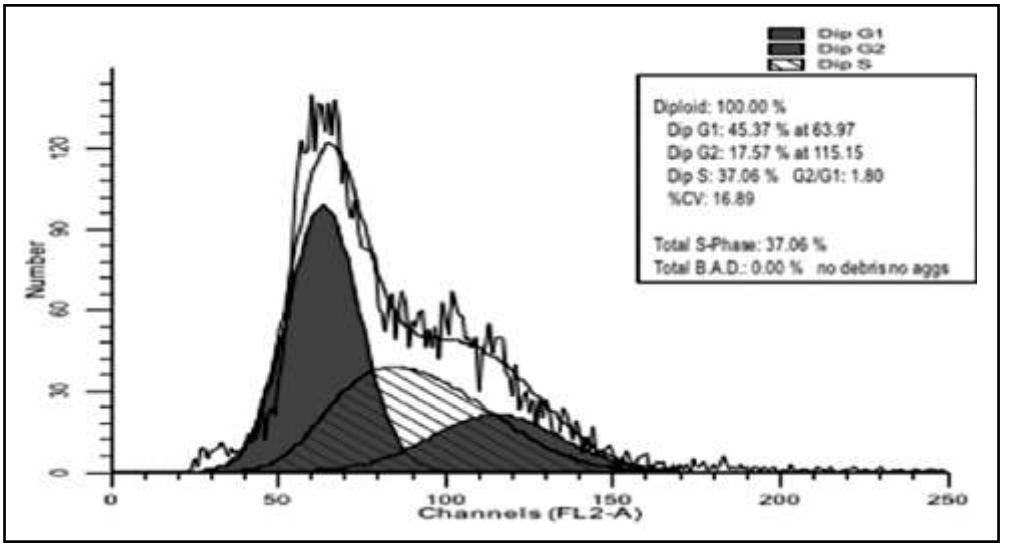


Control: MDAMB231 breast cancer cells



Flow Histogram – 1.

Treatment of Curcuma *longa* silver nanoparticles sample (A) i.eCUR/AgNPs (A) at a **10µg/ml** dose showed G0/G1 phase arrest in cell cycle of MDAMB 231 breast cancer cells



Flow Histogram – 2.

Flow cytometry showed a significant arrest of cells in G1 phase of treated MDAMB231 cells as compared to untreated control. When treated with *Curcuma longa* silver nanoparticles sample (B) at a **14µg/ml** dose arrest more cells in comparison of sample (A).

Effect of CUR/AgNPs(A) and (B)on MMPs and TIMPs Gene Expression of MDAMB231 Cells by Reverse Transcription PCR.

mRNA expression levels by RT-PCR

RT-PCR results showed that IC_{50} dose treatment of CUR/AgNPs A and B significantly lowers the expression levels of MMP-2 and MMP-9 as compared to control in MDAMB231 breast cancer cells. The IC_{50} dose treatment of CUR/AgNPs sample A (10 µg/ml), and sample B(14µg/ml) revealed higher expression levels of TIMP-2 and TIMP-3. The positive control used for the study is Doxorubicin (0.21 µg/ml) which also lowers the expression of MMP-2, MMP-9 and increases the expression of TIMP-2 TIMP-3. The study also showed that the 14µg/ml dose of CUR/AgNPs sample (B) is more effective on expression levels of MMPs and TIMPs as compared to its 10µg/ml dose of CUR/AgNPs sample (A) in MDAMB231 breast cancer cell lines.

In Figure – 1 and 2C is (Control), PC is (Positive Control), A is (10µg/ml dose of CUR/AgNPs Sample A), B is (14µg/ml dose of CUR/AgNPs Sample B). The expression levels of MMP-2 and MMP-9 were significantly lower than in the control group after the treatment of CUR/AgNPs A and B. The expression levels of TIMP-2 and TIMP-3 in MDAMB231 were significantly higher than in the control group after the treatment of CUR/AgNPs. A and B ($p < 0.05$).

Discussion

The MMP-2 and MMP-9 degrade type IV collagens promotes invasion and metastasis of tumor cells[16]. After activation MMP-2 degrades type IV collagen in BMs and also degrades type V, VI, X collagens as well as gelatins[13,14]. Whereas, MMP-9 destroy BMs and extracellular matrixes, hence affecting the adhesion ability of tumor cells[15]. On the basis of advanced breast cancer study on 168 cases of postmenopausal that was associated to clinical staging, pathology type and hormone receptor status showed high expression of MMP-9[19]. Li et al[20] detected MMP-2 and MMP-9 expression

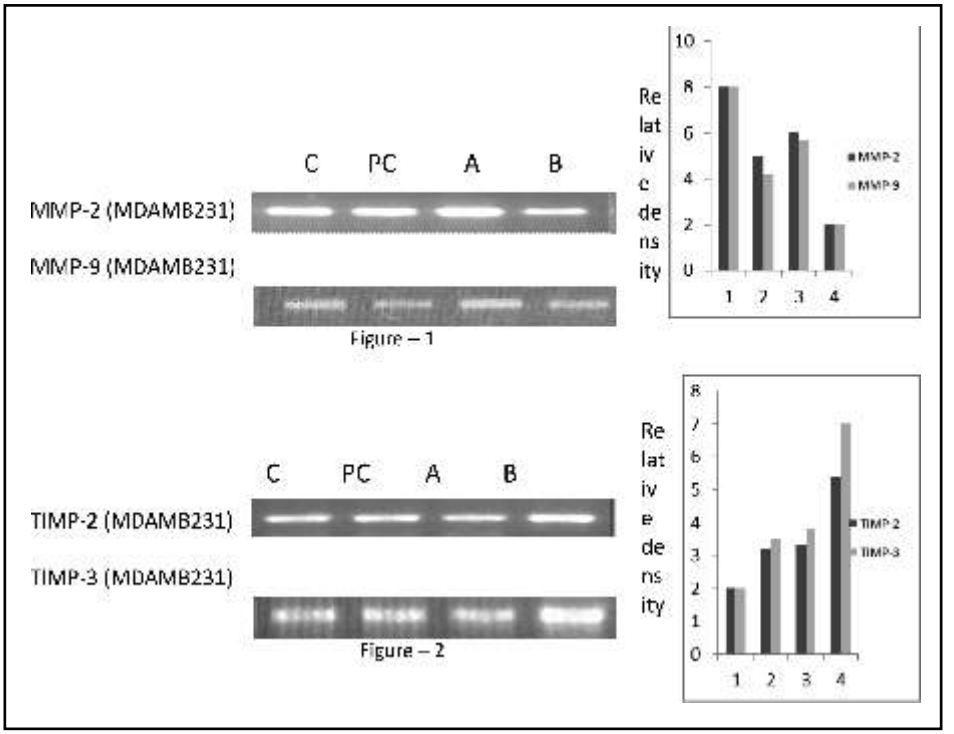


Figure – 1 and Figure – 2. The results of reverse transcriptase polymerase chain reaction for matrix metalloproteinase (MMP-2, MMP-9) and tissue inhibitors of metalloproteinase (TIMP-2, TIMP-3) in breast cancer cell lines.

levels in 270 cases of axillary lymph node-negative breast cancer. Number of studies supports MMP-2 and MMP-9 expression levels were significantly higher in fibrous adenomas in breast cancer tissues and significantly highly expressed in patients with infiltrative breast cancer and lymph node metastasis as compared to patients with non-infiltrative cancer and non-lymph node metastasis[17,18]. However, MMPs are specifically inhibited by a family of small extracellular proteins known as the tissue inhibitors of metalloproteinases (TIMPs). The TIMP family has four members i.e. TIMP-1, -2, -3 and -4 inhibits various MMPs. TIMPs play a key role in regulation of MMPs including effects on cell growth, differentiation, apoptosis, cell migration, angiogenesis and growth factors on cell phenotype in various physiological and pathological conditions[13,19, 20,21, & 22]. Therefore, MMP-2 and MMP-9 can be used as reference indicators for breast cancer treatment and estimating prognosis. Several research studies that have shown curcumin, an anticancer compound of *Curcuma longa* significantly inhibits metastasis in various types of cancers by as potential anti-metastatic includes inhibition of transcription factors and their signalling pathways. While its bioavailability always arises question in order to show anticancer effects. So, in order to use its therapeutical property, we have prepared the Silver nanoparticles of Curcuma longa (CUR/AgNPs) that significantly decreases the cancer cell proliferation, induces apoptosis and halts the cancer cell cycle in G1 phase. The present study also found that CUR/AgNPs increases the expression level of TIMPS by several folds and at the same time lowers the expression of MMPS by several folds. The higher dose of CUR/AgNPs (B) i.e. 14µg/ml

showed more efficacy in lowering the expression level of MMPS and increasing the expression level of TIMPS as compared to dose of CUR/AgNPs (A) i.e. 10 µg/ml in breast cancer cell line.

Conclusion

The present findings conclude that the dose dependant treatment of CUR/AgNPs directly effects the expression of MMP-2, MMP-9, TIMP-2 and TIMP-3 which are associated with the progonosis, treatment, tumor staging and metastasis in breast cancer cell.

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Chemical Composition and Antifungal Activity of Essential Oil from Leaves of *Eupatorium adenophorum* (L.) Spreng.

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Abstract-The chemical composition of essential oil from leaves of *Eupatorium adenophorum* was determined by GC-MS. Altogether, 54 components including monoterpenoids, sesquiterpeniods, monoterpene esters representing 98.22% of the oil were indentified. The major constituents identified include germacrene D (14.32%), -phellandrene (12.25%),bornyl acetate (8.04%), (-)-spathulenol (7.23%), δ-cadinene (6.55%), p-cymene (5.05%), and camphene (3.86%). The essential oil showed remarkable antifungal activity against pathogenic fungi viz., *Aspergillus flavus*, *A. niger*, *Alternaria solani*, *Drechslera halodes* and *Fusarium solani* in a dose dependent manner. The essential oil exhibited highest growth inhibition in all the tested fungi at test concentration of 2000 µg/ml which was almost at par with positive control. Among the tested fungi, maximum growth inhibition was recorded in *Aspergillus flavus* followed by *Fusarium solani* with all the tested concentrations of the essential oil.

Keywords: *Eupatorium adenophorum*, Leaves, Essential oil, GC-MS, Antifungal activity

Introduction

Inappropriate and irrational use of synthetic antimicrobials has resulted in emergence of resistant microbial populations. Owing to adverse side effects of synthetic antimicrobials coupled with peoples' inclination towards safe and efficacious alternatives, interest in herbal antimicrobial agents is witnessed across the world. Plant products including crude extracts or essential oil could serve as an alternate source of resistance modifying agents owing to the wide variety of secondary metabolites (Lopez-Reyes *et al.*, 2013; Nazzaro *et al.*, 2013; Elshafie *et al.*, 2015; Mishra *et al.*, 2016; Tripathi *et al.*, 2018). They exert their activity not only by killing the microorganism but also by affecting key events in the pathogenic process thereby reducing the ability of microorganisms i.e. bacteria, fungi and viruses to develop resistance against such botanicals. The use of essential oils to preserve food from microbial spoilage and to combat infection is common, even since the earliest civilizations. In nature, essential oils play an important role in the protection of plants pathogenic microorganisms. Essential oils are usually complex mixtures of several natural compounds well-known for their antiseptic, antimicrobial and several other therapeutic properties. Both humans and plants are susceptible to fungal infections by pathogenic fungi. Use of available synthetic fungicides for their control is limited by the emergence of resistant fungus strains and toxic impacts of some fungicides on environment and human health. On account of rising public concern over the health and environmental hazard associated with production and use of such synthetic chemicals, research is now directed towards development of new antifungal agents and recently, there has been a great interest in using essential oils as possible natural substitutes for conventional synthetic fungicides.

Eupatorium adenophorum Spreng.(syn. *Ageratina adenophora* (Spreng) King & H.Rob.) is a perennial semi-shrubby herbaceous plant belonging to the family Asteraceae.

Commonly known by several names like Crofton weed, Cat weed, Sticky snakeroot or Mexican devil the plant is native of Mexico and Costa Rica in Central America but now widely distributed worldwide from tropical to temperate regions including Australia, South Africa, Europe, China, and India as noxious invasive weed (Lu and Ma, 2006; Sang *et al.*, 2010).Various medicinal properties and therapeutic uses have been attributed to the plant in Traditional Medicine Systems of India, Nigeria and other parts of the world. It is pharmacologically regarded as antimicrobial, antiseptic, blood coagulant, analgesic, antipyretic and phenobarbitone induced sleep enhancer (Chopra *et al.*, 2006). *E. adenophorum* have been phytochemically investigated for a number of bioactive chemical constituents (Zhang *et al.*, 2008; Liu *et al.*, 2015) having varied pharmacological properties (Wei*et al.*, 2011; Shi et al., 2012; Kundu *et al.*, 2013; Liu *et al.*, 2016; Wang *et al.*, 2016; Tripathi and Saini, 2019). A number of sesquiterpenes have been isolated and characterized from leaves of the plant; of which the major sesquiterpenes namely chlorogenic acid, neochlorogenic acid and cryptochlorogenic acid have been investigated for anti-inflammatory (Chagas-Paula *et al.*, 2011), anti-bacterial (Wang *et al.*, 2009), and anti-obesity (Cho*et al.*, 2010) properties. The volatile oil from aerial parts of the plant reported to have insecticidal and antibacterial properties (Kurade *et al.*, 2010; Ahluwalia *et al.*, 2013). The present study was aimed at determining the bioactive chemical composition and evaluating the antifungal property of the essential oil from leaves of *E.adenophorum* to provide a rational basis for traditional therapeutic uses.

Material and Methods

Plant material

Fresh leaves of *E. adenophorum* were collected from the Botanical Garden of Forest Research Institute (FRI), Dehradun, Uttarakhand, India (GPS data: elevation 437 m; longitude $77^0$99'79" E and latitude $30^0$34'41" N) and were authenticated by Systematic Botany Section of Forest Botany Division, FRI, Dehradun, India under accession No. 144853. A voucher specimen of the collected material is preserved in the Chemistry and Bioprospecting Division for future reference. Collected leaves were properly cleaned under running tap and then dried in shade at ambient temperature with regular turning.

Extraction of essential oil

Leaves of *E. adenophorum* were subjected to hydrodistillation for 6 hours using an all glass Clevenger-type apparatus to isolate essential oils,an official method used as a reference for the quantification of essential oils (Harborne, 1998). The essential oil extracted was separated from the aqueous layer and dried over anhydrous sodium sulphate. Extraction experiment was replicated thrice and average yield of essential oil was calculated on dry weight (dw) basis. The oil was stored in opaque sealed vials at low temperature (4^0C) till further analysis.

Analysis of essential oil

Gas chromatography-mass spectrometry (GC-MS) analysis of the *E. adenophorum* leaf essential oil (EALEO) were done with 7890B gas chromatograph coupled with 5977A mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector (FID) and DB5 msuicapillary column (coated with 5% phenyl polysiloxane, 30 m length 0.25 mm internal diameter; 0.25 m film coating) fused silica capillary column. Nitrogen was used as a carrier gas at 101.2 kPa flow pressure and 1.21 ml/min. Temperature programming was done from 60 to 260°C at 3°C/min with initial hold time 18 min, 4°C/min Ramp. Injector and detector temperatures were 250°C and 280°C, respectively, split ratio was 1:40. The percentage of the individual constituents was

calculated by electronic integration of the FID peak areas without response factor correction. Mode of ionization was electron impact ionization (EI), with ionization energy 70 eV. Identification of the constituents was carried out on the basis of retention time under identical experimental conditions. Individual components were identified by mass spectra library search and comparing their MS with those of standard matching database of NIST (National Institute of Standards and Technology, U.S. Department of Commerce) and Flavour & Fragrance Natural & Synthetic Compounds (FFNSC) GC/MS library (Mondell, 2015).

Determination of Antifungal activity

Test Fungi

Antifungal activity of EALEO was evaluated gaainst some important and frequently occurring pathogenic fungi viz., *Aspergillus flavus, A. niger, Alternaria solani, Drechslera halodes* and *Fusarium solani*. Targeted fungi were isolated from infected maize seeds following 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 target fungal species were made separately and maintained on Potato Dextrose Agar (PDA, HiMedia) slant medium at 4^0C for 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 1-5x105 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

The antifungal activity was determined using the disc diffusion method (Murray *et al.*, 1995). The medium was prepared by dissolving potato dextrose agar (Hi Media) in distilled water and autoclaving at 121^0C for 15 minutes. 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm dia.) and allowed to solidify which were used for antifungal assy. Spore suspension was prepared in 0.9% saline water and adjusted to give a final concentration of 1-$5x10^5$ cfu/ml. The essential oil was diluted with Tween 40 to obtain the final concentrations of 2000, 1000, 750, 500, 250, 100, 50, 25 µg/ml, respectively. A plug of 1-week-old fungal culture (5 mm diameter) was placed on the centre of the sterilised plates containing PDA. About 10 µl of each concentration was injected to the sterile disc papers (6 mm diameter). Then the prepared discs were placed on the culture medium. Carbendazim (2 mg/ml) and Tween 40 were served as positive and negative control respectively. The plates were then incubated at 30°C for 4-5 days, and colony diameter was measured and recorded after 5 days. The growth inhibition of each fungal strain was calculated as the percentage inhibition of a radial growth relative to the control as:

$$\text{Inhibition (\%)} = [(1 - A / B] \times 100$$

Where A = mean diameter of fungal colony in treatment (mm); B= mean diameter of fungal colony in control (mm). All experiments were performed in triplicate.

Determination of MIC

The minimum inhibitory concentration (MIC) was determined through the broth dilution method (Gatsing *et al.*, 2010). 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 EALEO 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.

Results and Discussion

Extraction and compositional analysis of essential oil

The oil isolated by hydrodistillation of the *E. adenophorum* leaves were found to be light yellow with yields 0.46% (w/w) on dry weights basis. Its composition was identified by GC/MS analysis in combination with retention time.The oil constituents identified are summarized in Table – 1. Constituents are listed in order of their elution from a DB-5 capillary column along with their retention time, and percentage composition.

GC–MS analysis of *E. adenophorum* leaf essential oil led to identification of altogether 54 Chemical constituents including monoterpenoid, sesquiterpeniod, monoterpenes esters representing 98.22% of the oil (Table – 1). Based on the percentage composition, the essential oil of *E. adenophorum* leaves was characterized by the presence of major constituents including germacrene D (14.32%),

Table – 1. Chemical composition of *E. adenophorum*leaf essential oil

Peak No.	Constituents Identified	RT	% Composition
1.	?-Fenchene	16.76	0.42
2.	?-Phellandrene	17.42	0.35
3.	?-Terpineol	21.00	0.43
4.	Bornyl acetate	24.17	0.98
5.	Di-epi-α-cedrene	26.39	0.29
6.	Lavandulol	27.63	0.24
7.	Methyl thymol	28.66	2.16
8.	Linalool	29.09	1.05
9.	Borneol	29.33	0.96
10.	Isoledene	29.52	0.23
11.	δ-Cadinene	29.81	6.55
12.	p-Cymene	30.54	5.05
13.	(-)-Spathulenol	30.66	7.23
14.	?-Limonene	31.05	2.19
15.	8,9-dehydrothymol	31.22	0.23
16.	Bornyl acetate	31.52	8.04
17.	?-Caryophyllene	31.75	0.42
18.	Camphene	31.96	3.79
19.	γ-Amorphene	32.08	1.03
20.	Thymol	32.43	1.58
21.	Nerol	32.56	0.82
22.	Cubebol	32.64	0.74
23.	?-Guaiene	33.03	0.83
24.	?-Terpinolene	33.61	0.77
25.	?-Muurolene	33.73	0.37
26.	?-Ocimene	33.98	0.28

Contd.....

Peak No.	Constituents Identified	RT	% Composition
27.	?-pinene	34.35	0.61
28.	?-Gurjunene	34.66	1.07
29.	β-Bisabolene	34.86	3.86
30.	?-Cubebene	35.04	0.28
31.	Germacrene D	35.33	14.32
32.	?-Guaiene	35.6	0.36
33.	Zingiberene	35.75	1.55
34.	Borneol	35.94	0.63
35.	Carveol	36.2	0.92
36.	Eremoligenol	36.55	1.03
37.	?-Phellandrene	36.79	12.25
38.	Allo-spathulenol	37.17	0.26
39.	α-Bisabolol	37.63	2.29
40.	?-2-Carene	37.73	1.74
41.	Thymol	38.12	0.89
42.	Nerylisobutyrate	38.42	0.26
43.	α-Cadinol	39.07	0.63
44.	?-Chamigrene	39.21	0.29
45.	?-Cadinene	39.46	1.52
46.	?-Cadinene	39.63	0.43
47.	?-Eudesmol	39.88	1.55
48.	?-Eudesmol	41.14	0.43
49.	?-Gurjunene	41.32	1.45
50.	Caryophyllene oxide	41.41	0.27
51.	Thymyl methyl oxide	42.95	0.81
52.	?-Eudesmol	44.35	0.59
53.	Muurol-4-en-3,8-dione	47.17	0.44
54.	Eudesma-11-en-4?,6?-diol	50.64	0.46
	SUM		98.22

RT : Retention Time

-phellandrene (12.25%), bornyl acetate (8.04%), (-)-spathulenol (7.23%), δ-cadinene (6.55%), p-cymene (5.05%), and camphene (3.86%). Other representative components of the oil were identified as α-bisabolol (2.29%), limonene (2.19%), methyl thymol (2.16%), -2-carene (1.74%), thymol (1.58%), zingiberene (1.55%), -gurjunene (1.45%), -gurjunene (1.07%), linalool (1.05%) and γ-amorphene (1.03%).

Previous works by Kurade*et al.* (2010) have reported 1-napthalenol (17.50%), α-bisabolol (9.53%), bornyl acetate (8.98%), *β*-bisabolene (6.16%), germacrene-D (5.74%), *α*-phellandrene (3.85%) and a di-*epi*-*α*-cedrene (2.98%) as major constituents of essential oil of *E. adenophorum* leaves from India. Weyerstahl *et al.* (1997) studied the composition of the essential oil from flowers of the plant and recorded *α*-phellandrene (15.3%), camphene (12.2%), bornyl acetate (10.6%), *p*-cymene (8.5%), γ-curcumene (4.5%) and 2-carene as the major constituents.Pala-Paul *et al.* (2002) reported *p*-cymene (11.6%), α-phellandrene (5.7%), γ-curcumene (5.0%), δ-2-carene (5.0%), camphene (4.8%), and endo-bornyl acetate (4.4%) as main components of essential from aerial parts. Ahluwalia *et al.* (2013) have carried out GC-MS compositional analysis of essential oils from inflorescences and roots of *E. adenophorum* and reported that the oil from

inflorescences is dominated by sesquiterpenes including -cadinene (18.4%), -muurolene (11.7%), 3-acetoxyamorpha-4,7(11)-diene-8-one (7.4%) and bornyl acetate (6.3%) as the major constituents whereas the oil from the roots contained both sesquiterpenes (34.3%) and monoterpenes (32.5%) in almost equal proportions with E,E-cosmene (19.9%), -muurolene (10.1%), isothymol(7.5%), -cadinene (7.0%) and -phellandren-8-ol (5.9%) as the major constituents. The composition of the essential oil of *E. adenophorum* leaves as recorded in the present study is supported by finding of earlier work on this species of different origin. However, presence and/or absence of certain constituents as well as difference in the nature and relative content of various constituents as compared with previous findings might be representing different origin, maturity, and chemotype.

Antifungal Activity

The antifungal activity of *E. adenophorum* leaf essential oil determined against pathogenic fungi, *Aspergillus flavus*, *A. niger*, *Alternaria solani*, *Drechslera halodes* and *Fusarium solani* by disc diffusion method. The growth inhibitory activities of the essential oil against the tested fungi at different concentrations are summarized in Table – 2.

The results of antifungal activity assay clearly indicate that the essential oil has varying degree of antifungal activity against the all the tested pathogenic fungiin a dose-dependent manner (Table –2).The MIC values ranged from 25.5 to 75µg/mL. From the results, it is also evident that the essential oil exhibit highest growth inhibition in all the tested fungi at test concentration of 2000 µg/ml and it is almost at par with positive control. The growth inhibition in all the tested fungi was found to be moderate with 1000 µg/mL and 750 µg/mL treatment concentration of EALEO. Among the tested fungi, maximum growth inhibition was recorded in *Aspergillus flavus* followed by *Fusarium solani* with all the tested concentrations of the essential oil. The percentage growth inhibition in the tested fungi *Aspergillus niger*, *A. flavus*, *Alternaria solani*, *Drechslera halodes* and *Fusarium solani* with different treatment doses (25-2000µg/ml) of EALEO ranged between 1.27-95.15, 2.65-97.53, 2.23-94.69, 1.33-95.67, and 2.35-96.63 % respectively.

A number of studies have established the antimicrobial efficacy of plant products including essential oil and its terpenoid constituents (Srivastava and Tripathi, 2003; De Martino *et al.*, 2009; Termentzi *et al.*, 2012).

Table – 2. Antifungal activity of EALEO

Conc. (µg /ml) of EALEO	Antifungal activity (% inhibition) Mean ± SD				
	Aspergillus niger	*Aspergillus flavus*	*Alternaria solani*	*Drechslera halodes*	*Fusarium solani*
25	1.27 ±0.23	2.65±1.21	2.23±1.09	1.33±1.41	2.35±0.53
50	2.61 ±1.15	3.33 ±0.71	2.53±0.21	2.50± 0.25	2.91 ±0.87
100	4.25± 0.16	5.27± 0.83	4.61±0.21	7.50± 0.25	4.52± 0.23
250	15.37± 0.55	16.25± 1.25	11.50± 0.63	16.97± 0.35	13.85± 1.17
500	27.93± 0.21	29.45± 0.43	26.47± 0.21	24.85 ±0.46	24.29 ±0.33
750	46.55± 0.33	48.16± 0.25	45.27± 1.25	45.8 7±1.35	47.63± 0.27
1000	65.53± 0.21	67.85± 0.15	65.21± 0.23	65.85± 1.15	67.5 3±0.25
2000	95.15± 0.33	97.53 ±1.23	94.69± 1.27	95.6 7±0.35	96.6 ±1.21
(+) Control	100 ±.0.0	100 ±.0.0	100 ±.0.0	100 ±.0.0	100 ±.0.0
(?) Control	0.0	0.0	0.0	0.0	0.0

Antifungal activity of extracts of different parts of *E. adenophorum* and essential oil were previously investigated. Leaves and stems extracts of the plant have been reported to inhibit fungal strains including *Fusarium moniliformae, F. eroliferum, F. proliferatum* and *F. oxysporum* (Bhattarai N. and Shrestha, 2009). Volatile oil of *E. adenophorum* has been reported to inhibit four types of fungal pathogens (Tian*et al*. 2007). Petroleum ether extract of leaves showed antifungal activity against *Aspergilus niger, A.candidus* and *Candida albicans*. Compound 5-O-trans-o-coumaroylquinic acid methyl ester further found to display *in vitro* anti-fungal activity against spore germination of *Magnaporthe grisea* (Zhang *et al.*, 2013). Some of the sesquiterpenes constituents of found in the EALEO reported to exhibit antifungal activity (Kundu, et al., 2013). Antifungal action of the essential oil of *E. adenophorum* against the pathogenic fungi, *Sclerotiumrolfsii*, *Macrophomina phaseolina, Rhizoctoniasolani, Pythium debaryanum* and *Fusarium oxysporum* has also been reported (Ahluwalia *et al.*, 2013). In conclusion, EALEO found to be remarkably effective antifungal in accordance to the inhibition action against all tested pathogenic fungi. The fungi toxicity of EALEO might be due to the presence of mono and sesquiterpenoids constituents.

Conclusion

The yield of essential oil from *E. adenophorum* leaves was 0.46% higher than those reported earlier. The GC-MS analysis of the essential oil obtained from *E. adenophorum* leaves allowed the identification of altogether 54 compounds representing 98.22% of the total composition of the oil among which,germacrene D, -phellandrene, bornyl acetate, (-)-spathulenol, δ-cadinene, p-cymene, and camphene have been identified as the major constituents. Other components of the oil were identified as α-bisabolol, -limonene, methyl thymol, -2-carene, thymol, zingiberene, -gurjunene, -gurjunene, linalool and γ-amorphene. The essential oil *E. adenophorum* leaves was found effective against all the tested pathogenic fungi in a dose dependent manner. The antifungal activity of leaf essential oil of the plant might be attributable to the presence of terpenoid constituents including cadinenes and muurolene in the oil. Essential oils primarily made up of monoterpenes or sesquiterpenes are antibacterial in nature. The mode of antimicrobial action of terpenoids is not clearly defined, but it is ascribed to disruption of the membrane in microbes (Termentzi *et al.*, 2012). It is, therefore, important to stress the importance of further studies, mainly for determining the mechanism of action of *E. adenophorum* leaf essential oil as well as the action of its individual constituents.

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Conflict of Interest

Declared none.

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Physical Factors and Biochemical Composition of the Fruits of *Diospyros peregrina* at Different Ripening Stages

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Abstract-*Diospyros peregrina* is a large genus of shrubs and trees comprising of 500 species distributed in the warmer regions. It belongs to the family, Ebenaceae and possesses medicinal value. About 41 species occur in India mostly in evergreen forests of Deccan, Assam, and Bengal; only few are found in North India. Ripening stage of this fruit affects its physical factors which ultimately change the quality of processed products prepared from them. Hence,an experiment was undertaken to study the effect of different stages of ripening on physical factors and biochemical composition of *Diospyros peregrina* (Ebenaceae) fruits. In this study, it was observed that the ripening stages of the fruits cause variations in volume and weight of fruit, specific gravity of its pulp as well as composition of its reducing sugar, tannin, and ascorbic acid. These physical factors and biochemical composition variations in fruit on ripening may be taken as maturity indices to judge its maturing stages and therapeutic value of this fruit.

Keywords: Ebenaceae, *Diospyros peregrina*, Physical factors, Biochemical composition.

Introduction

The fruits of *Diospyros peregrina* fall to ground from June to July onwards and under favourable conditions its 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 scurfiness. 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.

Its fruits are used for making jams, jellies, osmodehydrated slices and squash (Reddy, 1959). Products like sweet chutney, dried pieces, milk shake, nectar, blended drinks, pickle, preserve and candy can also be prepared with good sensory quality (Sawant,1989). Even wine can be prepared from *Diospyros* fruit (Gautam and Chundawat, 1998).

The Department of Chemistry, Forest Research Institute, Dehradun carried out research work(1988) on karonda fruit and on sapota Honde (1995) for juice recovery. Experiments at Dehradun have shown that the viability of seeds is high when fresh but suffers during storage.

Paralkar (1985) worked on sapota and pawar (1988) on karonda. It was noted by them that there was decreasing trend in pulp to seed ratio and also an increasing trend of weight of pulp, weight of skin and specific gravity at different shapes of ripening. Observation analogous to this findings was reported by Raut (1999) in Sapota fruits.

The findings of increasing trends in weight, volume, length and diameter of sapota fruit are supported by Honde (1995). Similar observation have been reported by Joshi et al. (1996) for karonda fruits.

The colour of fruit in *Diospyros peregrina* changed from green in immature stage to dark orange in fully ripe stage with the ripening, has also been reported by the researchers (Suryanarayana and Gond, 1984; Raut, 1999)

In the present investigation, the collection of fruits of *Diospyros peregrina* at different stages during their ripening was done. They were stored and kept into deep freeze in the laboratory at –20°C temperature. These fruits of *Diospyros peregrina* at different stages of ripening were used for study of their physical factors and biochemical composition.

Material and Methods

The fruits of *Diospyros peregrina* used in the study were collected at four different stages viz., immature green, mature green, colour initiation and fully ripe from the Forest Research Institute garden, Dehradun, India. The experiment was conducted by following the complete randomized design with 4 levels of ripening stages in 5 replications. To analyse the physical factors and chemical composition, 10 *Diospyros peregrina* fruits from each ripening stage were randomly selected and examined individually for various physical factors and chemical composition. The average of 10 fruits has been reported for each physical parameter. Fruits of *Diospyros peregrina* which were collected at different stages of ripening – immature green, mature green, colour imitation and fully ripe were stored in deep freeze at -20^{0}C.

The weights of fruits of all stages were measured individually by electronic balance. Volume of fruits were measured by measuring cylinder by liquid displacement method: water was poured into measuring cylinder upto a fixed point and then the fruit was poured and volume difference mark was measured between initial water level and after pouring fruit into measuring cylinder, the increase in volume of water for each stage is the volume of the fruit. The weight of skin of fruit was measured by electronic balance. Skin of every fruit of four stages were removed with knife and their weights was measured by electronic balance individually. Length and diameter by vernier callipers, pH by pH meter at different stages of fruit ripening. The weights of fruit pulpand seeds were also measured by electronic balance at different stages of fruit ripening. The pulp to seed refers was also determined.

Ripening affects fruit physical parameters which ultimately affect the quality of processed products prepared from them. Hence, studies were undertaken to study the effect of different stages of ripening on physical parameters of *Diospyros peregrina* fruits. The fruits were collected at different stages of fruit ripening.The changes in different physical parameter with ripening were observed viz., Weight of fruit, Weight of pulp,Weight of skin, Weight of seed, Volume of fruit, Specific gravity, Length of fruit, Diameter of fruit, Colour, pH, Pulp to Seed ratio were determined.

Results and Discussion

It is seen from Table-1 that all the ripening stages of *Diospyr*os fruit had significant influence on physical parameters of *Diospyros* fruit. Weight of fruit and seed, volume of fruit, length and diameter of fruit showed increasing trend from immature stage to fully ripe stage during ripening of *Diospyros* fruit and significantly decreasing trend of pulp: seed ratio. Increasing trend of weight of pulp, weight of skin and specific gravity at different stages of fruit ripening in *Diospyros* fruit has been shown to be significant.

This increasing trend obtained during ripening of *Diospyros* fruit may be attributed to gain of moisture due to respiration and transpiration during ripening process. The present findings of increasing trends in weight, volume, length and diameter of fruit are supported by the research students of Pawar (1988) on karonda fruit and Honde (1995) on sapota for juice recovery.

Minimum weight of skin observed at mature stage(1.15g) was the impact of loss of moisture in skin. However, increase in specific gravity of fruits from 1.16 (immature stage) to 1.34 (fully ripe stage) during ripening indicated that the increase in weight of fruit was more than the corresponding increase in its volume. But, specific gravity of *Diospyros* fruit at different stages of ripening did not show significant difference. Similar observations have been reported by Joshi *et al.* (1986) for karonda fruits. Weight of pulp and pulp: seed ratio of *Diospyros* fruit at different stages of ripening did not show significant difference.

On the average the decrease in pulp: seed ratio observed during ripening of *Diospyros* fruit may be due to increase in weight of pulp as compared to seed. The colour of *Diospyros* fruit changed from dark green (immature stage) to yellow (fully ripe stage) during ripening. Identical observations during ripening were also reported by Suryanarayana and Goud (1984) and Raut (1999) on sapota.

The pH in immature stage (6.25), mature stage (5.82), colour initiation (6.49) and fully ripe (6.10) found during ripening of *Diospyros* fruit may be attributed to the decrease in acidity during ripening. Results of the present study are supported by Paralkar (1985) in sapota and Pawar (1988) in karonda. The pH values were significant at different stages during fruit ripening in *Diospyros* fruit.

It is observed from Table-2 that all the chemical parameters studied showed significant difference with respect to ripening stages of fruit except moisture content of fruit. Total and reducing sugar content of *Diospyros* fruit at different stages of ripening increased significantly from immature green(13.10% and 07.70%) to colour initiation stage(18.12%and 10.08%) with a slight decline at fully ripe stage (09.87%) in case of reducing sugar and increase (19.30) in case of total sugar. An increase in sugars during ripening process in *Diospyros* fruit may probably be due to accumulation of more sugars in the fruit due to hydrolysis of starch and slight decline at over ripestage was due to utilization of sugars during respiration process. The results of this investigation are in agreement with the results obtained by Raut(1999) in sapota fruit.

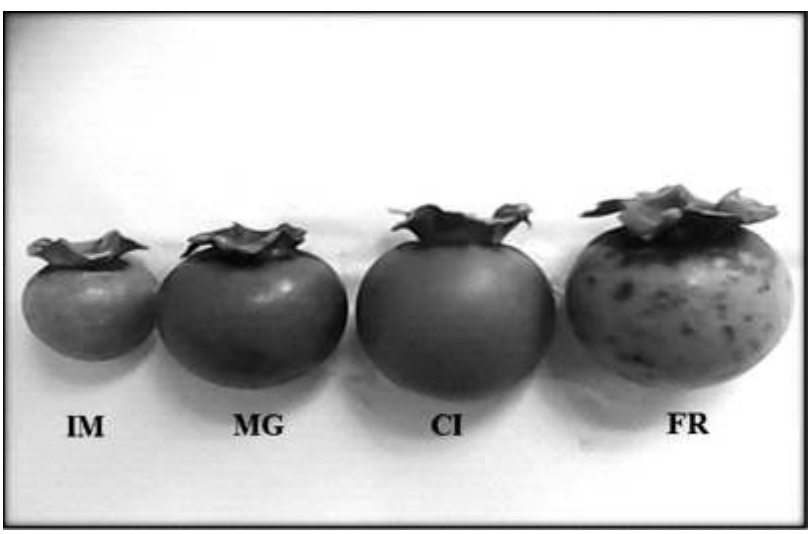

Figure – 1. Immature Green (IG), Mature Green (MG), Colour Initiation (CI) and Fully Ripe (FR) fruits of *Diospyrosperegrina.*

Table – 1. Effect of different stages of ripening on physical factors of *Diospyros peregrina* fruits

Ripening stage	Weight of fruit (g)	Weight of pulp (g)	Weight of skin (g)	Weight of seed (g)	Volume of fruit (ml)	Specific gravity	Length of fruit (cm)	Diameter of fruit (cm)	Colour	pH	Pulp: Seed ratio
1st	15.08	4.90	1.21	1.19	13	1.16	4.3	4.9	Dark green	6.25	4.11
2nd	27.84	15.42	1.15	2.74	23	1.21	4.8	5.3	Light green	5.82	5.62
3rd	31.23	13.53	1.42	4.85	28	1.11	5.0	5.6	Yellowish green	6.49	2.78
4th	51.03	27.49	3.82	7.50	38	1.34	6.2	7.5	Yellow	6.10	3.63

1st-Immature green stage, 2nd-Mature green stage, 3rd-Colour initiation stage, 4th-Fully ripe stage

Table – 2. Effect of different stages of ripening on Biochemical composition of *Diospyros peregrina* fruits

Ripening stage	Total sugars (%)	Reducing sugars (%)	Titratable acidity (%)	Ascorbic acid (mg/100g)	Tannins (%)	Moisture (%)
1st	13.10	7.70	0.23	20.40	0.42	75.60
2nd	15.56	8.80	0.20	14.22	0.29	74.10
3rd	18.12	10.08	0.13	11.97	0.18	72.20
4th	19.30	9.87	0.10	07.27	0.13	69.60

1st-Immature green stage, 2nd-Mature green stage, 3rd-Colour initiation stage, 4th-Fully ripe stage

Ascorbic acid content of *Diospyros* fruit declined throughout the ripening process from 20.40 mg/100g (immature green stage) to 07.27 mg/100g (fully ripestage) due to oxidative destruction of ascorbic acid by enzymes, mainly ascorbic acid oxidase, during ripening (Hulme, 1970). Identical observations during ripening were also reported by Suryanarayana and Goud (1984) and Raut (1999) in sapota. Sharp decrease in tannins was observed during ripening of *Diospyros* fruit. This may be due to the fact that tannins are hydrolyzed into components like sugars, acids and other compounds during ripening and also due to its results were nonsignificant. Decline in moisture of *Diospyros* fruit during ripening could be attributed to the loss of moisture through respiration and transpiration. Similar findings were also reported by Raut (1999) in sapota fruits cv. Kalipatti.

Conclusion

From the present study, it is concluded that physical parameters *viz.* weight of fruit, pulp, skin and seed, volume of fruit, specific gravity, length and diameter of fruit, fruit colour, and pulp: seed ratio and the chemical parameters *viz.* total and reducing sugars, titratable acidity, ascorbic acid, tannins and moisture of *Diospyros* fruits may be considered as maturity indices to judge the ripening stages of *Diospyros* fruit for various value added products preparation and for power to cure diseases viz., sore throat, wounds and ulcers.

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Comparative Phytochemical and Chromatographic Studies on Herbs Traditionally used as Antiobesity Medicines

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Abstract-Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide, resulting into a significant increase in mortality and morbidity related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke and cancers. Synthetic drugs used in the treatment of obesity have various side effects which enables the researchers to find a treatment with lesser side effects and those that are economical and beneficial to mankind.

Various plant products have been found to be effective in controlling obesity. A good portion of fruits, vegetables, spices and herbs need to be included in the regular diet. Plant derived molecules or phytochemicals are blessed with strong anti-obesogenic, anti-carcinogenic and anti-inflammatory properties. There are a plethora of phytochemicals which could tackle obesity. Traditional medicinal plants and their active phytoconstituents have been used for the treatment of obesity and their associated secondary complications.

Various plants showing potent anti-obesity action are described in Ayurvedic and Unani system of medicine. The medicinal plants used in the present study are *Commiphora mukul; Garcinia cambogia; Terminalia chebula* and *Trigonella foenum-graecum* exhibits promising weight loss through different mechanisms.

The present study was designed to comparatively evaluate the phytochemical and chromatographic analysis to explore the bioactive markers. Chromatographic analyses were carried out on the HPLC system. Further studies on them help to define the pharmacology and the active constituents responsible for weight loss.

Key words: Obesity, Antiobesity Herbs, Phytochemicals, Phytomedicine

Introduction

Obesity is a complex health issue to address becoming one of the most prevalent health concerns among all populations and age groups worldwide, it is a serious and chronic disease that can have a negative effect on many systems in the body resulting into a significant increase in mortality and morbidity related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke and cancers.

Obesity is increasing at an alarming rate throughout the world. In the present scenario, obesity is the major public health problem with about 1.9 billion adults (18 years and older) worldwide are overweight and about 600 million of them are clinically obese.

The potential of natural products for the treatment of obesity is still largely unexplored and can be an excellent alternative for the safe and effective development of antiobesity drugs (Birari and Bhutani, 2007).

Commiphora mukul, a highly valuable medicinal plant yields an oleo-gum resin important in Ayurvedic medicines (Chakravarty 1975). Medicinal importance of Commiphora mukul is known since ancient times as it is mentioned by Sushruta 3000 years ago as a valuable drug in Ayurveda have been found useful in curing many diseases like rheumatism, arthritis, hyperlipidemia, obesity, inflammation, anti-bacterial, antimicrobial, anti-oxidant, anti-arthritic, anti-malarial, muscle relaxing,

larvicidal and are responsible for lipid lowering properties in human blood and help in the treatment of diseases such as hypercholesterolemia, hypertension, obesity and diabetes (Satyavati 1969; Abbas ,2007; Madhvi Bhardwaj et al, 2013;).

Garcinia gummi-gutta is a tropical species of *Garcinia* native to Indonesia. Common names include *Garcinia cambogia* (a former scientific name), as well as brindleberry, Malabar tamarind and *kudampuli* (pot tamarind). The fruit looks like a small pumpkin and is green to pale yellow in color. It has received considerable media attention purporting its effects on weight loss. (*Tinworth, et al. 2010).* Amyriad of health effects have been attributed to *Garcinia* (including *G. cambogia,* such as antiobesity effects, antiulcerogenic, antioxidative, antidiabetes, antimicrobial, antifungal, anti-inflammatory, and anticancer effects. In particular, the antiobesity. (Mahendran, et al. 2002).

Terminalia chebula has been extensively used in ayurveda, unani and homoeopathic system. The Sanskrit name for Terminalia Chebula is 'Haritaki' which means yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and it is known to cure (harayet) all the diseases (Das,1991).

In Sanskrit 'Haritaki' is also known as 'Abhaya' which refers to the 'fearlessness', as it provides in the face of the disease. In Indian mythology, this plant has been known to be originated from the drops of ambrosia (Amrita) which fell on the earth when Indra was drinking it. (Srikanthmurthy, 2000).

Terminalia chebula (Combretaceae) is medium to large-sized tree distributed throughout tropical and sub tropical Asia, including China and Tibet. This tree is wild in the forests of Northern India, Uttar Pradesh, Bengal, Southern Maharashtra, Tamil Nadu and Karnataka. The fruit is used medicinally.(Varrier *et al*,1996).

It is considered to be a rasayana (with literal meaning: Path (ayana) of the Juice (rasa), or Elixir vitae) for Vata, balances tridoshas (loosely translated to three energetic forces in the body), enhances digestion (dipanapachana), sharpens the senses (medhyam), displays alterative (medicinal substance that acts gradually to nourish and improve the system), astringent, expectorant, anti-inflammatory, anodyne, cardiotonic, laxative, antiseptic and antiemetic properties (Jagtap and Karkera.1999; Ahmad et al 1998 ; Sato et al. 1997).

Trigonella foenum-graceum Linn.(methi) is an ancient plant has been used throughout the world as medicine, food and spice.

The herb fenugreek (*Trigonella foenum-graecum* L., Fabaceae family) is used both in cooking and for the treatment of diabetes in many parts of the world especially in China, Egypt, India and middle eastern countries. (Saxena A, 2004, Wang E, 2008) In India, it is widely used in Bangladesh. Active compounds of fenugreek included soluble fiber (Neeraja A, 1996, Raghuram TC, 1994,) Hypoglycemic activities have mainly been attributed to dietary fiber (Neeraja A, 1996, Raghuram TC, 1994) and Saponin . Fenugreek is a widely used herbal medicine for diabetes, but its efficacy for glycemic control remains unclear. Fenugreek (*Trigonella foenum-graecum*) being rich in phytochemicals has traditionally been used as a food, forage and medicinal plant (Puri D, 1998). The component called fenugreekine a steroidal sapogenin peptide ester has hypoglycemic properties . Thus it is best use is to control blood sugar in both insulin dependent (type–1) and noninsulin dependent (type–2) diabetes (Anuradha CV, 2001, Sharma RD, 1996)

Keeping in view of the importance and extensive use of these herbs in weight loss and control of obesity a comparative study of phytochemicals of these herbs is required. Therefore, the present study is undertaken to explore bioactive molecules using different

methods for phytochemical analysis applying the High Performance Liquid Chromatographic (HPLC) evaluation.

Material and Methods

Procurement of plants material

The plant materials used for this study were procured from Himalaya Drug Company, Dehradun, and identified by the department of Pharmacognosy, Himalaya Drug Company.

Preparation of plant extracts

Commiphora mukul

Plant samples gum were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 0C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4^0C for future use. 50 g of powdered samples of gum were extracted with methanol by soxhlation method at 60^0 to 80^0C. The three filtrates were separately concentrated in water bath at 40^0C and evaporated under reduced pressure.

Garcinia cambogia

The fruit rinds of ***Garcinia cambogia*** was washed with water, shade dried at room temperature and powdered coarsely. Exactly 10g of the course powder of fruitrinds were taken in 100ml various solvents such as ethanol, hydroalcohol, aqueous and ethyl acetate. The extracts were refrigerated for 72 hours and filtered through Whatmann filter paper No.1. Qualitative tests were conducted on these extracts. Powder analysis were conducted inethyl acetate extract of these plants. Fluorescent characteristics features of fruitrinds and leavesin ethyl acetate extracts were conducted under UV light at 360nm.

Terminalia chebula

Fruit extracts were prepared according to the method described by (Ahmad *et al.*1998) with minor modification. The fruit extract thoroughly washed with distilled water and then dried under shade condition. The dried fruit were powdered and stored in air sealed plastic containerat room temperature until the time ofextraction. The fruit powders were subjected to extraction using organic solvents. 5g of powered plant material was soaked in 10ml of solvent for 72hours, with stirring every 24 hours. At the end of extraction period, it was centrifuged and supernatant was filtered through Whatman No.1 paper. This extraction was repeated three times. Filtrates were pooled and evaporated to air dry and stored at 20^0C for further use.

Trigonella foenum-graceum

The seeds were thoroughly washed with distilled water and then dried under shade condition.

The dried seeds were powdered and stored in air sealed plastic container at room temperature until the time of extraction. The seeds powder was subjected to extraction using organic solvents. 5g of powered seeds was soaked in 10ml of solvent for 72 hours, was filtered through Whatman No.1 paper. This extraction was repeated three times. Filtrates were pooled and evaporated to air dry and stored at 20^0C for further use

Phytochemical screening

Phytochemical screening procedure Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids, Proteins and Anthraquinone were performed according to the procedure described by Harborne et al. (1973). Mayers test, Wagner s test for Alkaloids, Shinodas test for flavonoids, Benedicts test, Molisch s test for carbohydrates, Keller-Killani test for cardiac glycosides, Froth test for saponins, Lead acetate test for tannins, Salkowski test for terpenoids, Ninhydrin test and Biuret test for protein and Ammonia test for anthraquinone were performed.

Alkaloids

To 1ml of extract, added 2ml of Con. HCL then few drops of Mayers reagent was added. Positive test shows green colour or white precipitate.

Tannins

To 1ml of extracts, added 2ml of 0.1% Ferric chloride. Positive test shows brownish green or blue black colouration.

Saponins

To 1ml of extract, added 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shake for 10 minutes, it indicates the presence of saponins.

Anthraquinones

To 1ml of the extract, added 10ml of benzene, filter and add 5ml of 10% (v/v) ammonia to the extracts and shook well. Development of pinkish coloured solution indicates the presence of anthraquinones.

Phenolic flavonoids

To 1ml of extracts added 5ml of Folins ciocalteau reagent 4ml of sodium carbonate Appearance of blue colour shows the presence of phenol. Flavonoids: To 1ml of extract added 2ml of 1% aluminium solution. Appearance of yellow colour indicates the presence of flavonoids.

Carbohydrates

a) To 1ml of extract, added 5ml of Benedict's reagent and boil for 5 minutes. Bluish green colour indicates the presence of carbohydrates.

b) To 1ml of extract added few drops of Molischs reagent and few drops of concentrated sulphuric acid which gives purple colour. Amino acids: To 1ml of filterate, few drops of 0.2% ninhydrin was added and heated for 5 minutes. Formation of blue colour indicates the presence of aminoacid.

Steroids

To 1ml of the filterate added 10ml of Chloroform and 10ml of sulphuric acid slowly by the sides of the test tube. Positive indication if upper layer turns red and sulphuric acid layer showed the yellow colour with green fluorescent.

Terpenoids

Take 1ml of filterate added 2ml of chloroform and carefully added few drops of concentrated sulphuric acid. An interface with a reddish brown colouration is formed showing presence of terpenoids.

Cardiac glycosides

To 1ml of extract, added 1ml of Ferric chloride reagent and few drops of concentrated sulphuric acid. Greenish blue colour appears within few minutes indicating presence of cardiac glycosides.

Phlobatannins

To 1ml of extract, added few drops of 1% aqueous hydrochloric acid. A red precipitate is formed indicating the presence of phlobatannins.

Chromatographic analyses were carried out on the HPLC system (Shimadzu).

Estimation of Gallic acid by HPLC

HPLC Conditions required for analysis:

1.0 **Column** : C18

2.0 **Mobile Phase** : 10mM hexane- 1- sulphonic acid sodium salt containing 1% acetic acid and 0.13% triethylamine

3.0 **Flow Rate**: 1.0ml/min

4.0 **Detection**: 254nm

5.0 **Volume of injectio**n: 20µl of standard and sample solution

Standard Gallic Acid Solution

Weighed accurately 50mg of standard Gallic acid in 100ml of demineralized water and

dissolve. (Filter the solution through 0.20 µm syringe filter).

Sample solution

Weighed accurately 500mg of the harad powder in 100ml of demineralized water. Sonicated the solution to ensure complete solubility of Gallic acid. But do not heat the solution.(Filtered the solution through 0.20 µm syringe filter).

Method of Analysis

Stabilized the instrument with the above mentioned mobile phase and injected 20µl of working standard solutions. Record the chromatogram. Injected 20µl of the sample solution and recorded the chromatogram. Calculated the AUC of the standard Gallic acid peak and corresponding peak in the sample.

Calculation: % content of Gallic acid=

$$\frac{\text{AUC of sample peak}}{\text{AUC of standard peak}} \times \frac{\text{Standard concentration}}{\text{Sample concentration}} \times \text{\% Purity of standard}$$

Estimation of Hydroxy citric acid by HPLC

HPLC Conditions required for analysis:

1.0 **Column** : C18

2.0 **Mobile Phase** : 10mM hexane- 1- sulphonic acid sodium salt containing 1% acetic acid and 0.13% triethylamine

3.0 **Flow Rate**: 1.0ml/min

4.0 **Detection**: 254nm

5.0 **Volume of injectio**n: 20µl of standard and sample solution

Standard Solution

Weighed accurately 10mg of standard Hydroxy citric acid in 10ml of volumetric flask. Added about 5.0 ml of 1% hydrochloric acid in water and dissolve by sonication for about 10 minues. Made the volume up to the mark with 1% hydrochloric acid in water.

Sample solution

Weighed accurately 50mg of standard Hydroxy citric acid in 50ml of volumetric flask. Added about 30-40ml of 1% hydrochloric acid in water and dissolved by sonication for about 10 minutes. Made the volume up to the mark with 1% hydrochloric acid in water.

Method of Analysis

Stabilized the instrument with the above mentioned mobile phase and inject 20µl of working standard solutions. Recorded the chromatogram. Injected 20µl of the sample solution and recorded the chromatogram. Calculated the AUC of the standard peak and the corresponding peak in the sample.

Calculation: % content of Hydroxy citric acid=

$$\frac{\text{AUC of sample peak}}{\text{AUC of standard peak}} \times \frac{\text{Standard concentration(mg/ml)}}{\text{Sample concentration(mg/ml)}} \times \text{\% Purity of standard}$$

Estimation of Guggulsterone in *Commiphora mukul*

HPLC Conditions required for analysis:

1.**0 Column** : C18

2.0 **Mobile Phase** : Acetonitrile: Water (60:40)

3.0 **Flow Rate**: 1.0ml/min

4.0 **Detection**: 241nm

5.0 **Volume of injection**: 20µl of standard and sample solution

Standard Solution

Weighed about 50mg of standard Guggulsterone in 50ml of volumetric flask. Added about 40 ml of acetonitrile and dissolved by sonication. Made the volume up to the mark with acetonitrile. Filtered the solution through 0.20 µm syringe filter.

Sample solution

Made fine powder of guggul with the help of mortar and pestle and weighed about 250mg of sample in 1a 100ml beaker. Added 20ml of acetonitrile and dissolve by sonication. Decant the dissolved extract into 50 ml of volumetric flask. Repeated the same process of extraction with each 15 ml acetonitrile and decanted into same 50ml volumetric flask. Make the volume up to the mark with acetonitrile. Filtered the solution through 0.20 µm syringe filter.

Method of Analysis

Stabilized the instrument with the above mentioned mobile phase and injected 20µl of standard solution and recorded the chromatogram. Two major peaks obtained in the chromatogram correspond to guggulsterone E and Z isomers. In the similar manner Injected 20µl of the sample solution and record the chromatogram. Added the AUC of both the major peaks (Isomer E and Z) for the purpose of calculation.

Calculation: % content of Guggulsterone=

$$\frac{\text{AUC of sample peak}}{\text{AUC of standard peak}} \times \frac{\text{Standard concentration(mg/ml)}}{\text{Sample concentration(mg/ml)}} \times \text{\% Purity of standard}$$

Results and Discussion

The oleo-gum resin of guggul was extracted with alcohol and fractionated with ethyl acetate and petroleum ether. All the fractions were subjected for preliminary phytochemical screening, using various qualitative tests.

Preliminary phytochemical screening was done for petroleum ether, ethyl acetate, and alcoholic extracts of CM using various qualitative tests. The results of phytochemical screening are presented in **Table – 1.**

In the present study, the preliminary phytochemical analysis revealed that the presence of carbohydrates, proteins, tannins, and flavonoids in hydroalcoholic fraction. Ethyl acetate fraction showed positive results toward flavonoids, alkaloids, proteins, and steroids.

Table–4. shows the qualitative analysis of alcoholic extracts of *Garcinia cambogia*. It is observed from table–4. that the ethanolic extracts contain high amounts of flavonoids, phenols, terpenoids and saponins.The phytochemical evaluation can be used for further assessment of secondary metabolites. This investigation will help in further extraction analysis of compounds from various parts of *Garcinia cambogia*.

The present study reveals that all the extracts

Table – 1. Preliminary phytochemical analysis of fractions of guggul

Test for	CMP	CME	CMA
Alkaloids	-ve	+ve	-ve
Flavonoids	-ve	+ve	-ve
Carbohydrates	-ve	-ve	+ve
Proteins	-ve	+ve	-ve
Tannins	-ve	-ve	+ve
Steroids	-ve	+ve	+ve
Fats and oils	+ve	-ve	-ve
CMA: *Commiphora mukul* with alcoholic, CME: *Commiphora mukul* with ethyl acetate, CMP: *Commiphora mukul* with petroleum ether			

Table-2. Phytochemicals Analysis of *Terminalia chebula* fruits

S.No.	Phytochemicals	Observation
1.0.	Phenolic compounds &Tannins	+
2.0.	Saponins	-
3.0.	Alkaloids	-
4.0.	Flavonoids	-
5.0.	Glycosides	+
6.0.	Phytosterols	+
7.0.	Triterpenoids	+
8.0.	Carbohydrates	+

Table – 3. Phytochemical analysis of *Trigonella Foenum-graecum*

S.No.	Phytochemicals	Total Ethanolic extract
1.0.	Alkaloids	+
2.0.	Carbohydrates	+
3.0.	Steroids	-
4.0.	Tannins	+
5.0.	Terpenoids	+
6.0.	Flavonoids	+
7.0.	Saponins	-

Table – 4. Phytochemical analysis of *Garcinia cambogia*

S.No.	Phytochemicals	Ethanolic extract
1.0.	Flavonoids	+
2.0.	Terpenoids	+
3.0.	Tannins	+
4.0.	Saponins	+
5.0.	Sterols	+
6.0.	Carbohydrates	+
7.0.	Glycosides	+

have significant amount of phytochemical properties. The major phytochemical constituents in *Garcinia cambogia*is Hydroxy citric acid (Table – 5) This principle acid has been found to suppress the fatty acid synthesis, lipogenesis, food intake and promote glycogenesis, gluconeogenesis and induced weight loss.

Herbs may be very effective in the fight against obesity. They help in inhibition of lipase, down-regulation of adipogenesis, thermogenesis, metabolism of lipids and modulation of various signalling pathways leading to weight gain

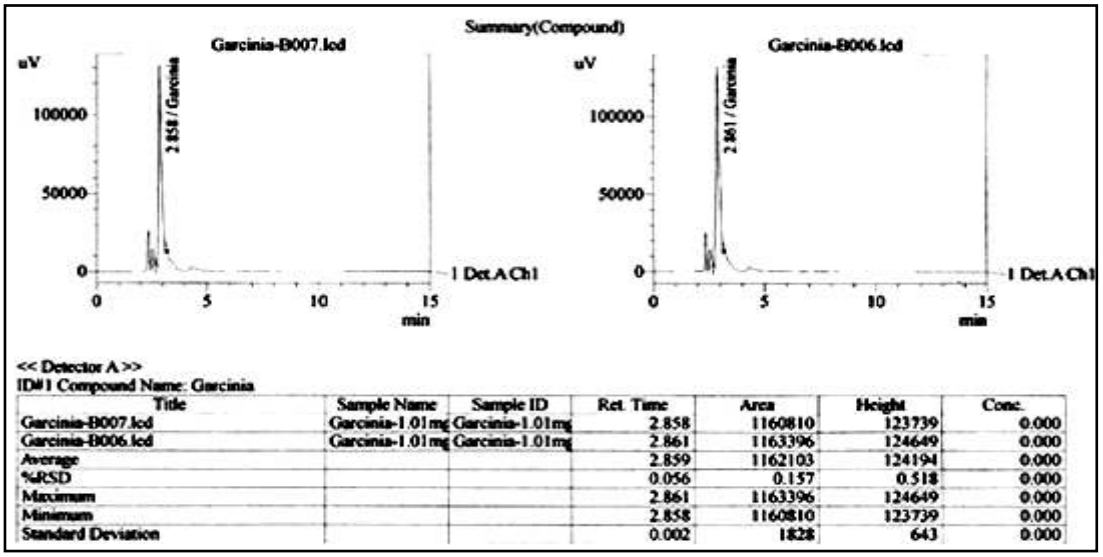
At present, the potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective anti-obesity drugs In course of

search for a plant used as enzymes inhibitor, the investigation was directed towards the anti-obesity activity

The present study was carried out with the extraction of dried leaves of methiusing 70% ethanol by maceration process. The phytochemical screening was performed to identify the phytoconstituents and revealed the presence of alkaloids, tannins, flavonoids and terpenoids. (Table – 3).

Table – 5. Estimation of Bioactive markers through HPLC

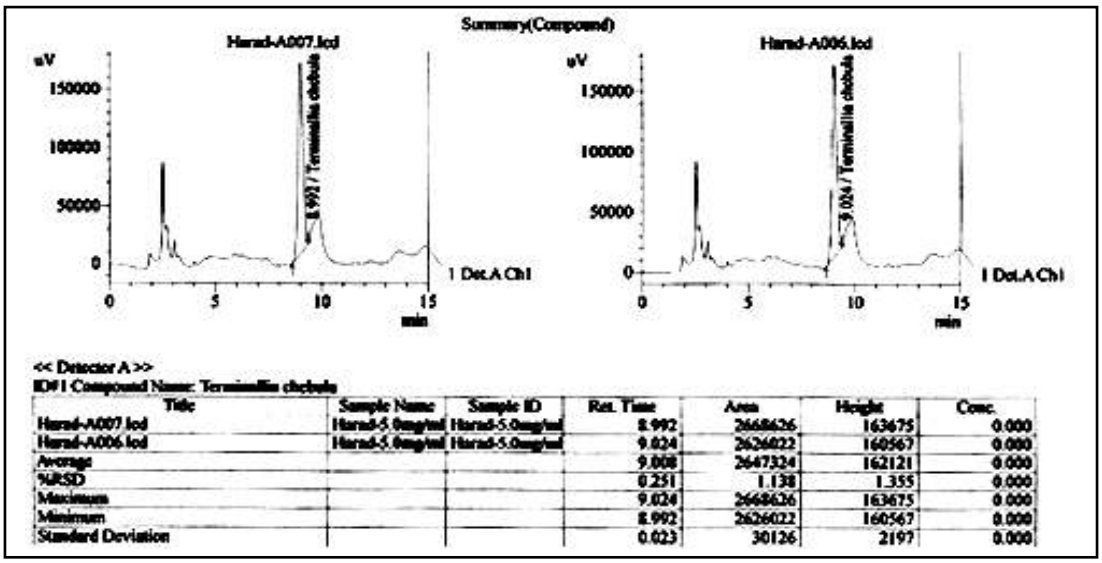
S.No.	Plant	Bioactive markers	Value
1.0.	*Commiphora mukul*	Guggulsterone	1.49%
2.0.	*Terminalia chebula*	Gallic acid	2.66%
3.0.	*Garcinia cambogia*	HCA	65.38%



<< Detector A >>
ID# 1 Compound Name: Garcinia

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
Garcinia-B007.lcd	Garcinia-1.01mg	Garcinia-1.01mg	2.858	1160810	123739	0.000
Garcinia-B006.lcd	Garcinia-1.01mg	Garcinia-1.01mg	2.861	1163396	124649	0.000
Average			2.859	1162103	124194	0.000
%RSD			0.056	0.157	0.518	0.000
Maximum			2.861	1163396	124649	0.000
Minimum			2.858	1160810	123739	0.000
Standard Deviation			0.002	1828	643	0.000

Figure – 1. Estimation of HCA through HPLC



<< Detector A >>
ID# 1 Compound Name: Terminalia chebula

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
Harad-A007.lcd	Harad-5.0mg/ml	Harad-5.0mg/ml	8.992	2668626	163675	0.000
Harad-A006.lcd	Harad-5.0mg/ml	Harad-5.0mg/ml	9.024	2626022	160567	0.000
Average			9.008	2647324	162121	0.000
%RSD			0.251	1.138	1.355	0.000
Maximum			9.024	2668626	163675	0.000
Minimum			8.992	2626022	160567	0.000
Standard Deviation			0.023	30126	2197	0.000

Figure – 2. Estimation of Gallic acid through HPLC

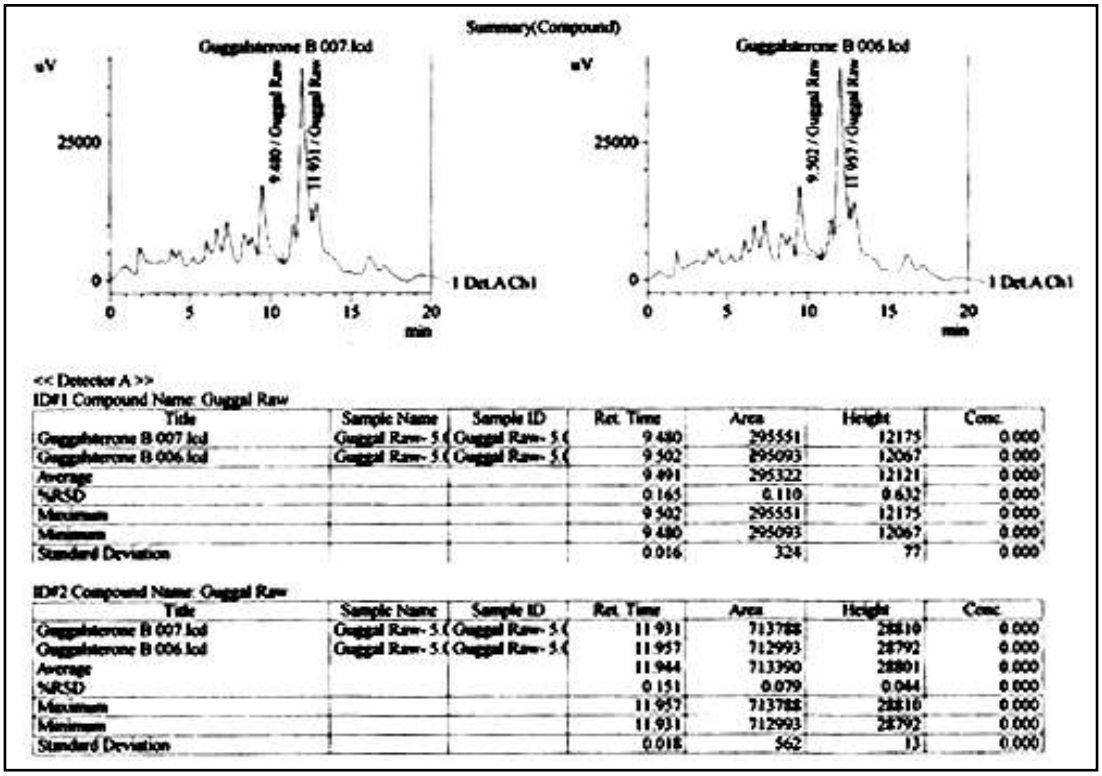


Figure – 3. Estimation of Guggulsterone through HPLC

Chromatographic analyses were carried out on the HPLC system and the results are depicted in (Table – 5).

The major phytochemical constituent in *Garcinia* is HCA (Hydroxycitric acid) 65.38% (Figure–1); the bioactive marker in *Terminalia chebula* is Gallic acid which is 2.66% (Figure–2) and the bioactive marker in *Commiphora mukul* is Guggulsterone 1.49% (Figure – 3).

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Phytochemical Screening and Chromatographic Studies on *Perilla Fructescens* an Omega Fatty Acid Rich and Therapeutically Active Mountainous Herb

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Abstract-*Perilla frutescens* L. belongs to the family Lamiaceae and commonly known as banjira in Uttarakhand. The whole plant is very nutritious like the seeds of the plant supplies nutritious cooking oil. It also contains omega – 6 and omega – 9 fatty acids. *Perilla frutescens* also shows the medicinal properties like antiseptic, antimicrobial, antioxidant, detoxicant, carminative etc. Banjira leaves are edible and also utilized as folk medicine to treat intestinal disorder and allergies, particularly in traditional Chinese medical practice. Qualitative phytochemical screening of methanol, hexane and aqueous extract of leaves and seeds was performed to explore scientific basis of medicinal potential. It confirms the presence of many phytochemicals like tannins, flavanoids, alkaloids, terpenoids, carbohydrates, steroids, Saponins, glycosides, starch and protein in different extracts. The most of the phytochemical were found in methanol extract. Thin layer chromatography was perfomed for the methanolic extracts of leaves and seeds. The finding provided the evidence that *Perilla frutescens* is potent source for some medicinally important phytochemicals and justifies its use as a medicinal plant.

Keywords: *Perilla frutescens*, Phytochemical screening, HPTLC

Introduction

Perilla frutescens L. belongs to the family Lamiaceae. P. *frutescens* is an annual herb found in China, Korea, Japan and the Himalayan region of India and Nepal. In India, it is mainly found in Uttarakhand commonly known as Banjira, Himachal Pradesh and Kashmir states**.** It is an aromatic plant with a strong minty smell. Growing up to four feet tall when in bloom, the stems are square, reddish-purple and branching. The leaves are large, up to 15 cm in diameter, dark green tinted red to purple, and hairy (Foster & Duke, 1990; Manandhar, 2002; Diggs et al., 1999). Various perilla varieties are traditionally used by local people. Leaves of *P. frutescens* are used as a vegetable. *P. frutescens* var. *crispa*is more often used than var. *frutescens* in China for its medicinal properties which may be differentiated by their different leaf and stem colors, which vary from green and red and to purple. The seeds from the plant supplies nutritious cooking oil. The essential oil of the plant is used as a food flavoring. The entire plant is very nutritious with vitamins and minerals (Asif & Kumar, 2010). The medicinal uses of perilla as an antiasthmatic, antidote, antimicrobial, antipyretic, antiseptic, antispasmodic, antitussive, aromatic, carminative, diaphoretic, emollient, expectorant, stomachic, and tonic substance has been shown (Asif, 2011). The essential fatty acids have been associated with benefits in a wide range of inflammatory conditions, heart diseases, colitis/Crohn's disease, asthma, allergies, antimicrobial, anticancer etc. Perilla is rich source of omega-3 polyunsaturated fatty acids (PUFAs), specifically alpha-linolenic acid (ALA). It also contains omega 6 and omega 9 fatty acids. Omega fatty acids are the essential for our health.

Perilla leaves have shown to be detoxicant, antitussive and antipyretic (Liu et al., Nakamura et al., 1998) and are also utilized as folk medicine to treat intestinal disorder and allergies, particularly in traditional Chinese medical practice (Nakazawa a 2000). Perilla

leaves are also edible and commonly used in the preparation of raw fish dishes (e.g. sushi and sashimi) in Japan (Omer et al., 1998) and are also used as spice cooked or fried and combined with rice and soups in many Korean and Chinese dishes (Park et al., 2013). The entire plant is very nutritious with vitamins and minerals (Asif, & Kumar, 2010) and have medicinal properties Present study has aimed to screening of the phyto-chemicals present in the Perilla seeds and leaves with their HPTLC study

Material and Methods

Collection of material

The seeds as well as fresh leaves of *Perilla frutescens* L for study were collected from the local farmers of village Sahiya near Chakarata, Dehradun Utatrakhand, India. The foreign materials and dust and dirt were removed and cleaned seeds are crusted in mortar and pestle and leaves were washed thoroughly with de-ionized distilled water, dried in a shade, and compressed into a powder with the help of a grinder (Zurera et al., 1987).

Sample Preparation for Phytochemical analysis

The crusted seeds and powered leaves were extracted in aqueous, methanol and hexane solvents and filtered using whatmann filter paper. Preliminary phytochemical tests in aqueous, methanol and hexane were carried out for Tannins, Favonoids, Allkaloids, Terpenoids, carbohydrate saponins, steroids, glycosides, starch and Protein.

Phytochemical screening

Phytochemical screening procedure and Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids, Proteins and Anthraquinone were performed according to the procedure described by Harborne et al. (1973). Mayer's test, Wagner's test for Alkaloids, Shinodas test for flavonoids, Benedict's test, Molisch's test for carbohydrates, Keller-Killani test for glycosides, Froth test for saponins, Lead acetate test for tannins, Salkowski test for terpenoids, Ninhydrin test and Biuret test for protein were performed.

Alkaloids

To 1ml of extract, added 2ml of Con. HCL then few drops of Mayers reagent was added. Positive test shows green colour or white precipitate.

Tannins

To 1ml of extracts, added 2ml of 0.1% Ferric chloride. Positive test shows brownish green or blue black colouration.

Saponins

To 1ml of extract, added 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shook for 10 minutes, it indicates the presence of saponins.

Starch

Dissolved 0.015 of iodine and 0.075 g of potassium iodide in 5ml of distilled water, added 2-3 ml of an aqueous extract of the drug. A blue colour is produced.

Flavonoids

To 1ml of extracts added 5ml of Folins ciocalteau reagent 4ml of sodium carbonate Appearance of blue colour shows the presence of phenol. Flavonoids: To 1ml of extract added 2ml of 1% aluminium solution. Appearance of yellow colour indicates the presence of flavonoids.

Carbohydrates

a) To 1ml of extract, added 5ml of Benedicts reagent and boiled for 5 minutes. Bluish green colour indicates the presence of carbohydrates). To 1ml of extract added few drops of Molischs reagent and few drops of concentrated sulphuric acid which gives purple colour. Amino acids: To 1ml of filtrate, few drops of 0.2% ninhydrin was added and heated for 5 minutes. Formation of blue colour indicates the presence of amino acid.

Steroids

To 1ml of the filterate added 10ml of Chloroform and 10ml of sulphuric acid slowly by the sides of the test tube. Positive indication if upper layer turns red and sulphuric acid layer showed the yellow colour with green fluorescent.

Terpenoids

Take 1ml of filterate added 2ml of chloroform and carefully added few drops of concentrated sulphuric acid. An interface with a reddish brown colouration is formed showing presence of terpenoids.

Glycosides

To 1ml of extract, added 1ml of Ferric chloride reagent and few drops of concentrated sulphuric acid. Greenish blue colour appears within few minutes indicating presence of cardiac glycosides.

Protein

To 5ml of extract, 1ml of concentrated nitric acid was added and boiled, a yellow precipitate was formed, after cooling it, 40% sodium hydroxide solution was added, orange colour was performed

Sample Preparation for HPTLC profile

Weighed accurately 1.0gm of each part of bangira in a 250ml flat bottom flask separately. Add 10 ml of methanol and refluxed it on a water bath at $80^0C\pm 2^0C$ for 30 minutes. Filtered the extract through whatmann filter paper no. 41.

Chromatographic system

TLC plate type:Pre coated thin layer silica plate $60F_{254}$, 10×10cm, E-Merck

Mobile Phase: Chloroform: Methanol= 90:10

Spotting volume:10µl

Application

Applyed the sample and standard solution as 10-12mm band,in a distance of 12mm from the bottom of a pre coated thin layer silica plate of uniform thickness,made a marked up to a distance of 8.5 cm from the application point as a development mark using pencil.

Preparation of development tank

Camag made twin trough development tank (10×10cm) was used. Covered one side of the inside chamber with required size of whatman no.41 filter paper. Measured 20ml of mobile phase and transfered into the chamber from the side of the filter paper.

Visualization and Documentation

Visualized the dried plate under UV 254 nm and 366nm using cabinet. Exposed the plate to saturated iodine vapours for 10 minutes .The image of the plate capture under UV 254 nm and 366nm before exposing and at white light after exposing vapours.

Evaluation

With Camag TLC scanner and winCATS evaluation software: scanning done by absorbance with Tungsten/Deuterium lamp at 200nm, slit dimension 6.0 x 4.5mm, scanning sppped 20 mm/s

Results and Discussion

Aqueous (water), methanol and hexane extract of *Perilla frutescens L* seeds and leaves were subjected to phytochemical screening for Tannins, Flavonoids, Alkaloids, Terpenoids, Carbohydrate, Saponins, Steroids, Glycosides, Starch and Protein according to standard procedure. The results of phytochemical analysis of *Perilla frutescens L* seeds and leaves are presented in Table-1 and Table-2 respectively.

The methanol, aqueous and hexane extract of the leaves and seeds of banjira were subjected to the phytochemical screening. The methanolic extract of seeds of bangjira shows the presence of variety of phytochemicals viz tannins, flavanoids, alkaloids, Saponins, glycosides, starch and protein. Aqueous extract shows the presence of tannins, alkaloids, and starch but the

hexane extract does not show any phytochemicals in the seeds (Table-1).

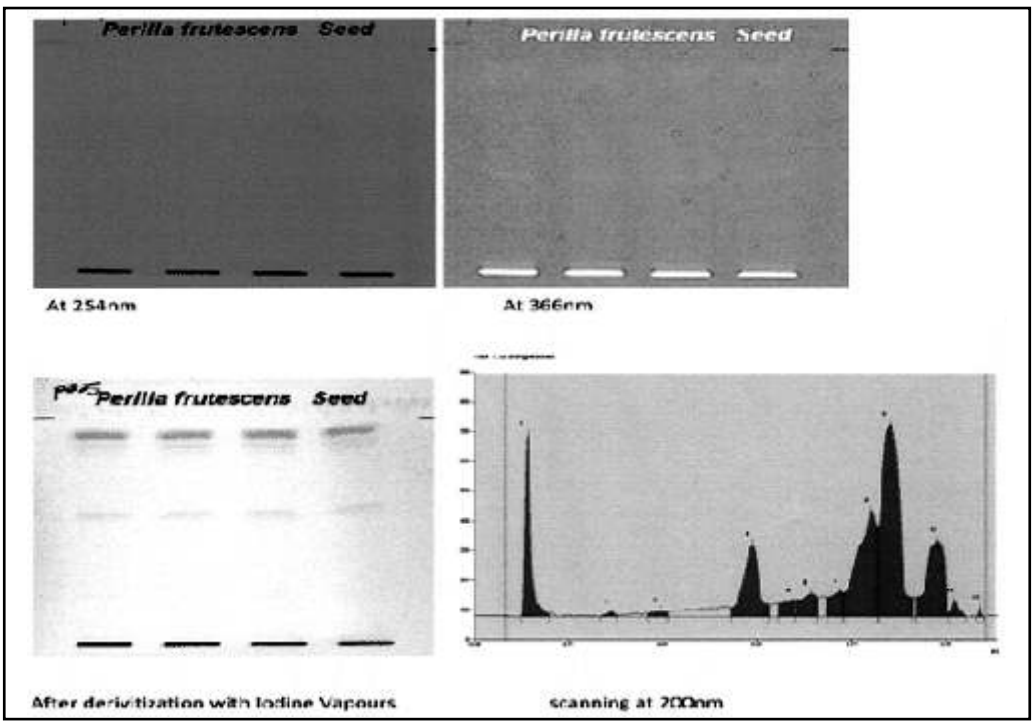
The methanolic extract of leaves of bangjira shows the presence of flavanoids, alkaloids, glycosides, starch and protein. Aqueous extract also shows the variety of chemical constituents like flavanoids, alkaloids,glycosides, carbohydrates starch and proteins but the hexane extract does not show any phytochemicals (Table -2).

Table – 1. Phytochemical screening of Banjia Seeds

Sr. No.	Tannins	Flavonoids	Alkaloids	Terpenoids	Carbohydrate	Saponins	Steroids	Glycosides	Starch	Protein
Aqueous Extract	+	-	+	-	-	-	-	-	+	-
Methanol Extract	+	+	+	-	-	+	-	+	+	+
Hexane Extract	-	-	-	-	-	-	-	-	-	-

Table – 2. Phytochemical screening of Banjira Leaves

Sr. No.	Tannins	Flavonoids	Alkaloids	Terpenoids	Carbohydrate	Saponins	Steroids	Glycosides	Starch	Protein
Aqueous Extract	-	+	+		+	-	-	+	+	+
Methanol Extract	-	+	+	-	-	-	-	+	+	+
Hexane Extract	-	-	-	-	-	-	-	-	-	-

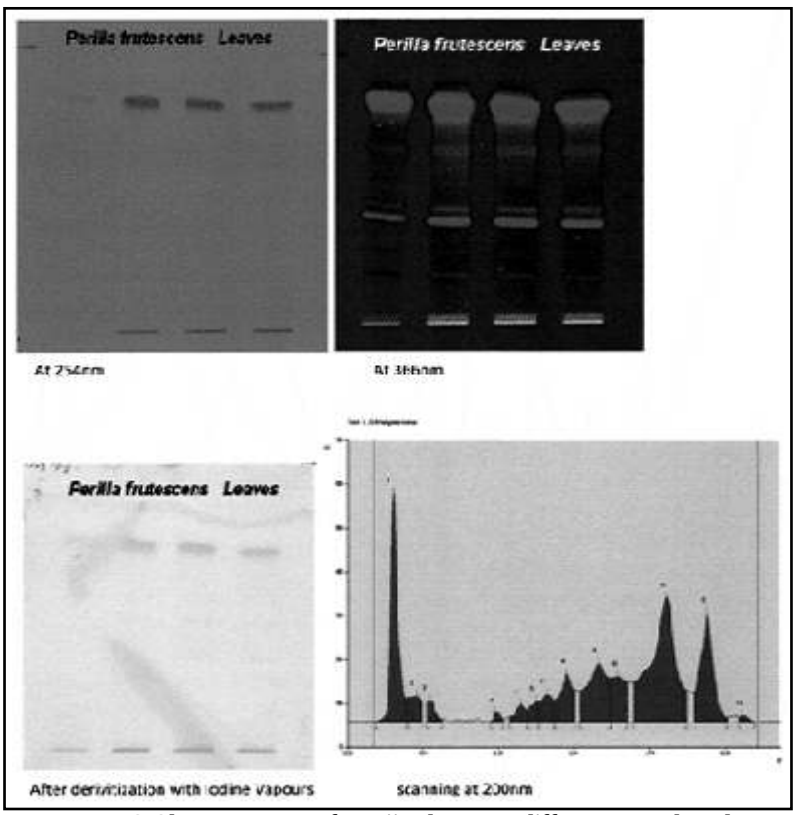


HPTLC Chromatogram of Banjira seed at different wave length

Table – 3. HPTLCScan data of Banjiraseed

Bhangjeera-(seed)20190531-002.cat winCAT

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	0.2 AU	0.03 Rf	12.2 AU	15.13 %	0.07 Rf	1.7 AU	8383.9 AU	1.07 %	unknown *
2	0.18 Rf	1.4 AU	0.21 Rf	15.4 AU	0.63 %	0.22 Rf	5.2 AU	312.0 AU	0.41 %	unknown *
3	0.26 Rf	9.9 AU	0.31 Rf	18.8 AU	0.77 %	0.33 Rf	6.8 AU	646.1 AU	0.85 %	unknown *
4	0.46 Rf	31.4 AU	0.51 Rf	42.9 AU	9.97 %	0.54 Rf	9.1 AU	8318.0 AU	0.97 %	unknown *
5	0.56 Rf	43.7 AU	0.59 Rf	51.8 AU	2.12 %	0.59 Rf	1.5 AU	1548.2 AU	2.04 %	unknown *
6	0.60 Rf	51.0 AU	0.63 Rf	76.2 AU	3.13 %	0.64 Rf	4.7 AU	2950.7 AU	3.89 %	unknown *
7	0.66 Rf	96.0 AU	0.69 Rf	84.3 AU	3.46 %	0.70 Rf	0.9 AU	2429.9 AU	3.20 %	unknown *
8	0.70 Rf	80.9 AU	0.75 Rf	55.6 AU	4.60 %	0.77 Rf	7.9 AU	4991.3 AU	9.77 %	unknown *
9	0.77 Rf	99.7 AU	0.80 Rf	46.3 AU	25.53 %	0.84 Rf	6.2 AU	5427.9 AU	13.53 %	unknown *
10	0.85 Rf	65.5 AU	0.90 Rf	55.6 AU	0.49 %	0.92 Rf	4.3 AU	9789.4 AU	2.88 %	unknown *
11	0.92 Rf	14.7 AU	0.93 Rf	49.3 AU	2.02 %	0.96 Rf	0.4 AU	851.6 AU	1.12 %	unknown *
12	0.96 Rf	0.8 AU	0.99 Rf	27.7 AU	1.14 %	1.00 Rf	0.2 AU	196.3 AU	0.26 %	unknown *



HPTLC Chromatogram of Banjira leaves at different wave length

The methanolic extract of both seeds and leaves shows more number of phytochemicals. So we have chosen the methanolic extract for the chromatographic analysis. The TLC profiling of methanolic extract in the solvent system (chloroform: methanol= 90:10) confirms the presence of different phytochemicals which are present in seeds and leaves. The scanning of peaks at 200nm shows the 12 phytochemical isolated in seeds (table – 3). 13 phytochemical isolated in leaves (table – 4). So, it is concluded by these results both the leaves and seeds of bangjira rich with phytochemicals.

Table – 4. HPTL Scan data of Banjira Leave

Bhangjeera-leaves-20190524-005.cna winCA

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	1.5 AU	0.03 Rf	21.5 AU	30.67 %	0.07 Rf	5.3 AU	0937.4 AU	7.52 %	unknown *
2	0.07 Rf	55.4 AU	0.10 Rf	59.7 AU	3.51 %	0.11 Rf	7.7 AU	2056.2 AU	3.30 %	unknown *
3	0.12 Rf	47.6 AU	0.13 Rf	50.2 AU	2.95 %	0.16 Rf	5.8 AU	1088.2 AU	1.74 %	unknown *
4	0.29 Rf	2.6 AU	0.30 Rf	21.2 AU	1.25 %	0.32 Rf	7.7 AU	401.5 AU	0.64 %	unknown *
5	0.34 Rf	11.8 AU	0.37 Rf	40.9 AU	2.40 %	0.39 Rf	6.6 AU	1139.3 AU	1.82 %	unknown *
6	0.39 Rf	28.9 AU	0.41 Rf	48.2 AU	2.83 %	0.42 Rf	7.5 AU	1018.9 AU	1.63 %	unknown *
7	0.42 Rf	47.5 AU	0.44 Rf	63.4 AU	3.73 %	0.46 Rf	0.6 AU	2167.2 AU	3.47 %	unknown *
8	0.46 Rf	51.2 AU	0.49 Rf	09.5 AU	0.40 %	0.52 Rf	1.0 AU	3859.1 AU	0.34 %	unknown *
9	0.53 Rf	73.2 AU	0.55 Rf	35.0 AU	7.94 %	0.61 Rf	0.3 AU	7223.6 AU	11.57 %	unknown *
10	0.61 Rf	00.6 AU	0.63 Rf	03.8 AU	6.10 %	0.65 Rf	1.0 AU	4076.8 AU	6.53 %	unknown *
11	0.67 Rf	89.1 AU	0.76 Rf	82.7 AU	16.83 %	0.81 Rf	2.6 AU	9070.1 AU	10.55 %	unknown *
12	0.83 Rf	66.6 AU	0.86 Rf	48.6 AU	4.62 %	0.92 Rf	1.3 AU	8910.3 AU	4.27 %	unknown *
13	0.95 Rf	11.6 AU	0.96 Rf	15.3 AU	0.90 %	0.99 Rf	1.1 AU	380.8 AU	0.01 %	unknown *

Conclusion

Phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumer due to the presence of various compound. So the plant bangjira is the rich source of phytochemicals cause of its nutritious and medicinal value.

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Pharmacological Overview on Neem Extract with Experimental Screening as Potential Fungicides

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Abstract-Neem is known for its medicinal properties since immemorial. This has now become the cynosure of modern medicine. In the present manuscript, a brief overview on neem extract (NE) with specific reference to pharmacological importance has been presented. Experimental approach was made regarding isolation, optical characterization and screening of neem extracts against the two test fungi *viz; R. solani* and *S. rolfsii.* Optical spectra reveals well standardized intensity ratio of Soret to Q bands for chlorophyll-a at 1.23, with a large characteristic discrepancy in the content of chlorophyll-b. Food poison method reveal the potential of neem extract towards control of the fungal growth of the both class of fungi in doze dependent manner ranging 50 to 500 ppm. Comparative screening data reveals that the growth of *S rolfsii* was inhibited by 90%. This was followed by over *R solani* wherein 86% of inhibition was observed within 24 hour of exposure of NE under identical experimental conditions. The present study provides an encyclopedic and exploratory view on the pharmacological potential of NE for future applications in modern medicine.

Keywords : NE, *S rolfsii*, *R solani*, and Chlorophyll

Introduction

Neem (*Azadirachta indica*, *Indian Lilac*, *Margosa*, *Dogonyaro*) is the evergreen tree that grows with 10 to 15 meter height and of about 2-3 meter girth in tropical and semi-tropical regions of India, Pakistan and Bangladesh. *Azadirachta* is derived from Persian "Azadirachta" (*azaddhirakt*, Azad- Darakth- E-Hind') that means 'Free tree of India' (Girish and Bhatt, 2008). *The old importance of neem as traditional medicine has now well accepted by modern medicine.* (Subapriya *et.al;* 2005). Nimbiol, quercetin, sitosterol, polyphenolic flavonoids present in neem leaves are known for their potential antimicrobial properties (Alzohairy *et. al;* 2016). Bacterial infections in eyes and ear are well cured through administration of neem extracts (Mahmoud *et. al;* 2010), however, anti-inflammatory effect of *Azadirachta* is low over dexa-methasone (Abu syed *et. al;* 2008). Extracts derived from various parts of neem are well documented for their immunomodulatory, antimicrobial, antidiabetic, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antiviral, antioxidant, antimutagenic and anticarcinogenic, properties (Vaquil *et.al;* 2018, Rahmani *et. al;* 2018).

Around 20-25 % of the world population is affected by fungal infections of skin and nails caused by dermatophytes. This prevails as high as 40 % in tropical areas (Nagabhushan *et al.*, 2013). The nail lesions produced are about 50 % of the whole infections on nails. (D.Iván *et. al;* 2015). Leaf and seed extracts of *neem* were found active against a a series of dermatophytes viz: *T.rubrum, T. violaceaum, M. nanum* and *E. floccosum (Khan et. al; 1987, Natarajanet. al; 2002). Aqeous* extracts of leaf and seed sufficiently destroys *T. rubrum*, *T. mentagrophytes* and *M. nanum (Natarajan*

et.al; 2002). Ethanolic extracts of seed oil and hexane extract of neam leaf were found active against *Candida* inimmuno compromised patients. (Loyed*et. al;*2005).

Antifungal properties of neem extracts are due to presence of triterpenoids (Govindachari *et al.* 1998). Extract of neem seed oil reduce the growth of *P. arachidis* (Govinda chari *et. al;* 1998). Aqueous extract of neem inhibits the growth of *Aniger* (Bohra and Purohit, 2002), *A, flavus*, *A, fumigatus*, *A. niger*, *A. terreus*, *C. albicans* and *M. gypseum* (Mahmood *et. al;* 2011), *A. niger, C. albicans*(Arumugam *et. al;* 2011) *A. oryzae*, *R.stolonifer* and *A. niger*. (Chukwuma *et. al;* 2018).

Material and methods

Starting materials

Neem leaf was collected from nearby locations of Kakori village at Lucknow and dried under ambient conditions over 25 days.PDA was prepared through meshing the boiled slice of peeled potatoes (200 g) followed by admixing the filtrate with dextrose (20 g), agar (20 g) in water (500 ml) under boiling and stirring till 10 min ,and cooling to 45^{0}C. Aqueous neem extract (NE) was prepared through treating dried powder of neem *leaves* (50 g) with autoclaved ultrapure water (300 ml), followed by standing the content over 24hours at 25±1 $^{\circ}$C. Supernatant of content was centrifuged @ 3000 rpm over 30 min, thereafter serially diluted into 50 to 500 ppm and employed for screening against the test fungi (Charchafchi *et. al;* 2007). Extract was characterized through optical spectra in quartz cell in acetone over Genesis 10 Thermospectronic spectrophotometer USA.

Pathogenicity test

The phytopathogenic fungus cultures of *Rhizoctonia solani* (*R.solani)* and *Sclerotium rolfsii (S.rolfsii)* were externally procured were grown on PDA medium. The autoclaved soil (2 kg) filled in plastic pots was infested with inoculum (2g) from top depth of 5 cm soil layer. Soybean (*Bragg*) seeds (10 NOs), surface sterilized with mercuric chloride (0.1%) were sown and kept under observation over 7 days. The expressed symptoms were compared and re-isolation of the organism was done on PDA. Further comparison of slants was made with previously isolated fungus as well as symptoms produced.

Antifungal activity

Antifungal activity of test specimen was conducted through food poison method with reference to carbendiazim as control. Test specimens were aseptically poured near.Test formulations were mixed with PDA (20 ml) in petriplates (90 cm diameter) in laminar flow and left till solidified. Medium was centrally inoculated by placing a 5 mm disc already, cut from the margin of 5 days old culture of the test fungus. The plates were sealed with parafilmtape and incubated at 25$\pm$1ºC. The colony diameter (mm) was measured at the intervals of 24 hrs till the petriplates were fully covered with growth of the fungal mycelium. Relative susceptibility of each organism was determined by clear zone of inhibition of growth around the disc treated with test specimen. Experiments executed in triplicate at different concentration (ppm) of NE ranging 100 to 500. The % inhibition of growth was calculated as [(C-T)/C]X100, where C and T are the colony diameters (mm) of control and test plates respectively.

Results and Discussion

Figure – 1. presents the optical spectrum of NE in acetone. Spectrum reveals two characteristic ranges designated as "red" (Q-) band and "blue" (Soret, S) band (Gouteman,1978, Hoff, 1991). Such bands appears in the spectrum of chlorophyll due to delocalization of π-electrons associated with porphyrin skeleton. The present optical spectrum reveals locations of Q and S bands of chlorophyll at 668 and 430 nm

respectively (Jeffrey *et.al;* 1996). The corresponding bands (S and Q) for chlorophyll b are located at 644 and 458 nm respectively (Hoff 1991). The ratios of absorbance intensities for S to Q bands are 1.23 for Chlorophyll-a, and 2.82 for Chlorophyll-b. The present calculations based on optical spectrum reveals the ratio of intensities of S to Q band for chlorophyll-a as 1.23 which is in agreement with literature data (Hoff, 1991). However, for chlorophyll-b, the intensity ratio of S to Q band was found as 1.15, that is far different from the literature value of 2.82 (Sanja *et. al;* 2016).

Table – 1. presents a comparative account of antifungal activity with reference to concentration and exposure of NE over *R. solani* and *S. rolfsii*. The maximal growth of *R. solani* and *S. rolfsii* was observed at 96 hrs. A remarked inhibition of hyphal growth and abnormal patches of aerial hyphal mass have been observed in the treatment of neem extract at 500 ppm against *R.solani* (Table – 1). Measurement of radial hyphae growth revealed that NE retards the growth of both class of fungi in dose dependent manner. In general, the inhibition of both class of fungi was progressed under steady

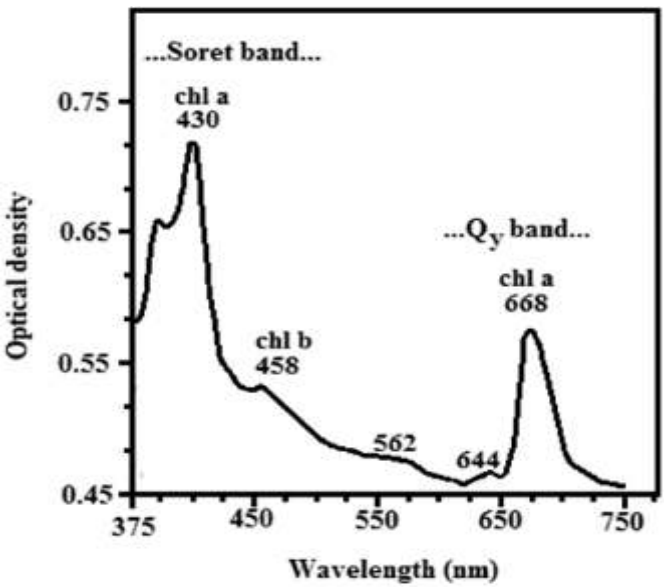

Figure – 1. Optical spectrum of NE in acetone

Table – 1.Time and doze dependent activity of NE against the test fungi

NE (ppm)	Antifungal activity (% inhibition)							
	S. rolfsii				*R.solani*			
	24	**48**	**72**	**96**	**24**	**48**	**72**	**96**
50	43.89	37.17	33.70	32.29	40.00	37.77	36.73	32.85
100	67.98	53.58	47.75	45.83	74.50	55.00	42.10	37.55
200	71.79	61.41	60.56	57.08	76.25	70.44	57.68	39.79
300	73.59	69.23	67.07	59.79	78.00	77.77	71.57	51.53
400	84.10	82.05	76.40	66.14	82.50	80.77	78.94	64.59
500	88.71	85.89	82.58	78.12	90.00	86.66	82.63	81.63

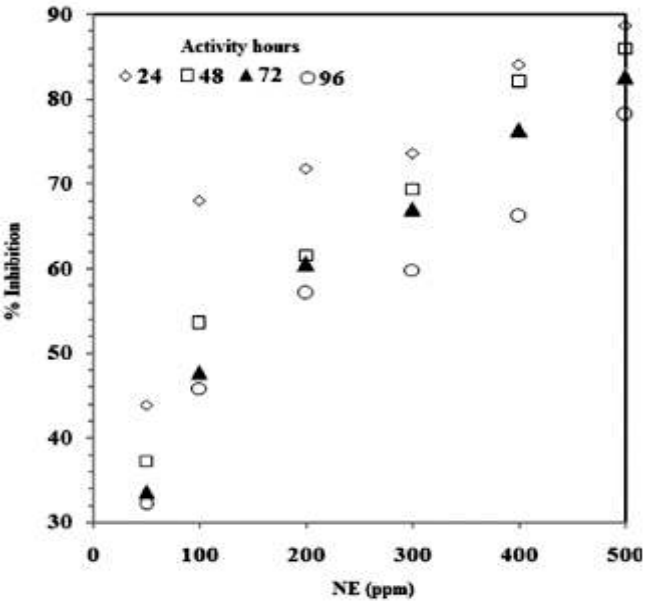

Figure – 2a Effect of NE (ppm) on 5 inhibition of *S. rolfshii*

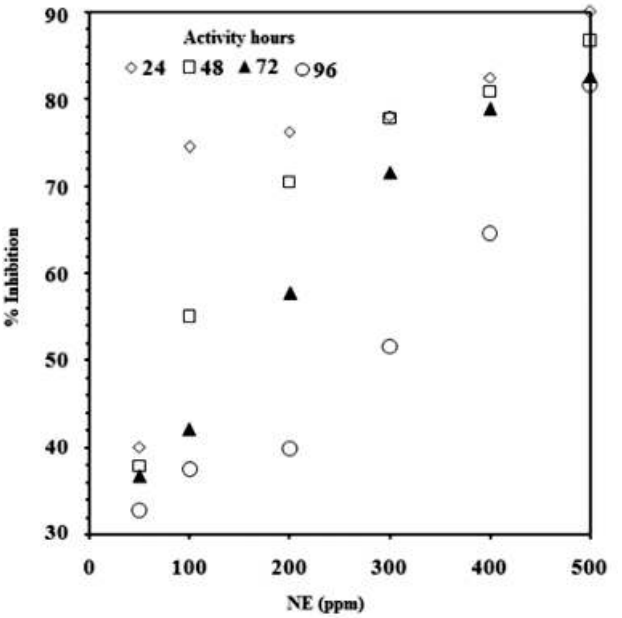

Figure – 2b Effect of NE (ppm) on 5 inhibition of *R solani*

manner with time and concentration of NE. Relatively, NE was found less active towards inhibition of *S rolfsii* over *R solani*. NE at 500 ppm found to impart substantial antifungal activity against *R. solani* by inhibiting 90% of fungal mycelial growth whereas weak antifungal activity of neem extract was observed at 100 and 200 ppm (Figure – 2).

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About Flowers (Shown on the cover page)
Antiobesity Herbs

Commiphora mukul

Scientific classification

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Burseraceae
Genus:	*Commiphora*
Species:	*C. mukul*

Commiphora mukul is sought for its gummy resin which is harvested from the plant's bark through the process of tapping. In India and Pakistan, guggul is cultivated commercially. The resin of *C. mukul*, known as *gum guggulu*, has a fragrance similar to that of myrrh and is commonly used in incense and perfumes. It is the same product that was known in Hebrew, ancient Greek and Latin sources as bdellium.

The gum can be purchased in a loosely packed form called *dhoop* (an incense from India) which is burned over hot coals. This produces a fragrant dense smoke. The burning coals which let out the smoke are then carried around to different rooms and held in all corners for a few seconds. This is said to drive away evil spirits as well as remove the evil eye from the home and its family members. Over a hundred metabolites of various chemical compositions were reported from the leaves, stem, latex, root and fruit samples. High concentrations of quinic acid and myo-inositol were found in fruits and leaves.

The extract of gum guggul called gugulipid guggulipid or guglipid has been used in Unani and Ayurvedic medicine for nearly 3,000 years in India. One chemical ingredient in the extract is the steroid guggul sterone which acts as an antagonist of the farnesoid X receptor once believed to result in decreased cholesterol synthesis in the liver. However, several studies have been published that indicate no overall reduction in total cholesterol occurs using various dosages of guggulsterone and levels of low-density lipoprotein ("bad cholesterol") increased in many people.

Garcinia cambogia

Scientific classification

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Malpighiales
Family:	Clusiaceae
Tribe:	Garcinieae
Genus:	*Garcinia*
Species:	*cambogia*

Garcinia species are evergreen trees and shrubs, dioecious and in several cases apomictic. The fruit is a berry with fleshy endocarp which in several species is delicious. The fruit of most species of *Garcinia* are eaten locally; some species' fruits are highly esteemed in one region but remain unknown just a few hundred kilometres away. The best-known species is *Garcinia cambogia* which is now cultivated throughout Southeast Asia and other tropical countries.

Most species in Garcinia are known for their gumresin, brownish-yellow from xanthonoids

such as mangostin, and used as purgative or cathartic, but most frequently at least in former times as a pigment. The colour term gamboge refers to this pigment.

Extracts of the exocarp of certain species typically *G. gummi-gutta*, but also *G. mangostana* are often contained in appetite suppressants. Their effectiveness at normal consumption levels is unproven while at least one case of severe acidosis caused by long-term consumption of such products has been documented. Further more, they may contain significant amounts of hydroxycitric acid which is somewhat toxic and might even destroy the testicles after prolonged use.

Terminalia chebula

Scientific classification

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Myrtales
Family:	Combretaceae
Genus:	Terminalia
Species:	T. chebula

Terminalia chebula is a medium to large deciduous tree growing to 30 m (98 ft) tall with a trunk up to 1 m (3 ft 3 in) in diameter. The leaves are alternate to subopposite in arrangement, oval, 7–8 cm (2.8–3.1 in) long and 4.5–10 cm (1.8–3.9 in) broad with a 1–3 cm (0.39–1.18 in) petiole. They have an acute tip, cordate at the base, margins entire, glabrous above with a yellowish pubescence below. The fruit is drupe-like, 2–4.5 cm (0.79–1.77 in) long and 1.2–2.5 cm (0.47–0.98 in) broad blackish

with five longitudinal ridges. The dull white to yellow flowers are monoecious and have a strong unpleasant odour. They are borne in terminal spikes or short panicles. The fruits are smooth ellipsoid to ovoid drupes, yellow to orange-brown in colour, with a single angled stone.

A number of glycosides have been isolated from *haritaki*, including the triterpenes arjunglucoside I, arjungenin, and the chebulosides I and II. Other constituents include a coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds including ellagic acid, 2,4-chebulyl-β-D-glucopyranose, chebulinic acid, gallic acid, ethyl gallate, punicalagin, terflavin A, terchebin, luteolin, and tannic acid. Chebulic acid is a phenolic acid compound isolated from the ripe fruits. Luteic acid can be isolated from the bark.

Terminalia chebula also contains terflavin B, a type of tannin while chebulinic acid is found in the fruits. *Terminalia chebula* is the main ingredient in the Ayurvedic formulation *Triphala* which is used for kidney and liver dysfunctions. The dried fruit is also used in Ayurveda as a purported antitussive, cardiotonic, homeostatic, diuretic, and laxative.

Trigonella foenum-graecum

Scientific classification

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Fabales
Family:	Fabaceae
Genus:	Trigonella
Species:	T. foenum-graecum

Fenugreek is believed to have been brought into cultivation in the Near East. It is uncertain which wild strain of the genus, *Trigonella* gave rise to domesticated fenugreek, charred fenugreek seeds have been recovered from Tell Halal, Iraq (carbon dated to 4000 BC), and Bronze Age levels of Lachish and desiccated seeds from the tomb of Tutankhamen. Cato the Elder lists fenugreek with clover and vetch as crops grown to feed cattle.

In one first-century A.D. recipe there was used Romans flavoured wine with fenugreek. In the 1st century AD, in Galilee it was grown as a staple food, as Josephus mentions it in his book, the *Wars of the Jews.* India is a major producer of fenugreek. Rajasthan accounts for over 80% of India's output

Fenugreek is used as aherb (dried or fresh leaves), spice (seeds) and vegetable (fresh leaves, sprouts and microgreens). Sotolon is the chemical responsible for the distinctive maple syrup smell of fenugreek.

Cuboid-shaped, yellow- to amber-coloured fenugreek seeds are frequently encountered in the cuisines of the Indian subcontinent, used in the preparation of pickles, vegetable dishes, dal, and spice mixes such as *panchphoron* and *sambar* powder. They are often roasted to reduce bitterness and enhance flavour.

In traditional medicine, fenugreek is thought to promote digestion, induce labour, and reduce blood sugar levels in diabetics, although the evidence that fenugreek has any therapeutic worth is lacking. In herbalism, fenugreek is thought to increase breast milk supply in nursing mothers.

Forth Coming Events

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   Theme: Current challenges of Pharmaceutical & Biopharmaceutical Industries in regulatory framework
   https://regulatoryaffairs.pharmaceuticalconferences.com/

2. International Conference on Organic and Inorganic Chemistry
   August 08-09, 2019 Amsterdam, Netherlands
   Theme: *Accentuate Innovations and Emerging Novel Research in Organic and Inorganic Chemistry*
   https://organic-chemistry.chemistryconferences.org/

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   August 12-13, 2019 Auckland, New Zealand
   Theme: Innovating Petroleum Resources to Navigate the Future
   https://petrochemistry.global-summit.com/

4. International Conference on Herbals and Traditional Medicine
   August 14-15, 2019 Auckland, New Zealand
   Theme: Unwinding Nature's Recipes for Health
   https://herbal.global-summit.com/

5. International Conference On Pharma and Food (ICPAF)
   29th-30th August, 2019 at Delhi, India.
   http://academicsera.com/Conference2019/India/5/ICPAF/

6. International Conference on Physical and Theoretical Chemistry
   September 02-03, 2019 Zurich, Switzerland
   Theme: Unique Pioneering Research Strategies and Approaches in Physical and Theoretical Chemistry
   https://physicalchemistry.chemistryconferences.org/

7. International Conferences on Medical and Health Science (ICMHS)
   24th - 25th September, 2019 in Pune, India
   http://theires.org/Conference2019/India/4/ICMHS/

8. International Conference on Food Microbiology and Food Safety (ICFMFS)
   28th - 29th September, 2019 in Goa, India .
   http://theires.org/Conference2019/India/5/ICFMFS/

9. International Conference on Structural Biology
   14-16 October, 2019 London, UK
   Theme: An Insight into Every Dimension of Advanced Structural Biology Research
   https://structuralbiology.expertconferences.org/

10. International Conference on Medical and Biosciences (ICMBS)
15th-16th October, 2019, New Delhi , India
http://researchworld.org/Conference2019/India/5/ICMBS/

11. International Conference on Biopharma and Biotherapeutics
October 25-26, 2019 | Copenhagen, Denmark
https://biopharmaceutics.pharmaceuticalconferences.com/

12. World Congress on Analytical and Bioanalytical Chemistry
October 30-31, 2019 London, UK
Theme: Novel Approaches to Analytical and Bioanalytical Techniques
https://analytika.pharmaceuticalconferences.com/

13. Advanced Pharmaceutics and Clinical Research
November 04-05, 2019 | Paris, France
Theme: Collaboration of advanced pharmaceutics and clinical research
https://advancedpharmaceutics.pharmaceuticalconferences.com/

14. World Congress on Bioorganic and Medicinal Chemistry
November 14-15, 2019 Cape Town, South Africa
Theme: Shaping the Future of Bioorganic and Medicinal Chemistry
https://bioorganic-medicinal.chemistryconferences.org/

15. World Polymer Chemistry & Biopolymers Congress
November 18-19, 2019 Rome, Italy
Theme: Building for the Future in Polymer Advances
https://polymerchemistry.insightconferences.com/

16. Cellular and Molecular Mechanism Conference: Health and Disease
November 21-22, 2019 Bali, Indonesia
https://cellularmechanism.conferenceseries.com/

17. Middle East Pharmacy and Pharmaceutical Conference
December 09-10, 2019 Dubai, UAE
Theme: Innovative Researches and Developments in Pharmaceutical Sciences
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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