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Lavendula angustifolia(Lavendula)

Bombax ceiba(semal)

Allium sativum(Garlic)

Tachyspermum ammi(Ajowain)

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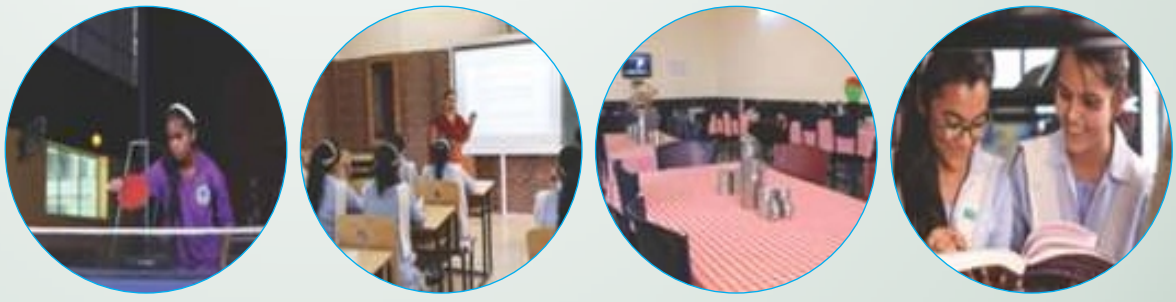

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Editorial

Again the year2021 is witness to the surge of Covid-19 pandemic spreading its tentacles across the globe with new variant of Corona virus giving Hypoxemia/Hypoxia and destroying tissues which is many times fatal and createhavockilling more than 5lakhs peoples till date in India alone.
An additional pandemic of Mucormycosis (black fungus disease) is also spreading very fast. It affects the sinuses, the brain and the lungs and can be life-threatening in diabetic or severely immunocompromised individuals, such as cancer patients or people with HIV/AIDS.
Doctors believe mucormycosis, which has an overall mortality rate of 50%, may be triggered by the use of steroids, a life-saving treatment for severe and critically ill Covid-19 patients.
This second wave of pandemic is more serious and fatal due to a new variant is highly mutated form of the corona virus and it is said that third wave may infect children also. The only way to protect ourselves is to vaccinate the whole population.

Vaccines helps preparing persons immune systems to be ready in advance, giving the immune system a head start on viruses and other invaders. Vaccines train our immune system to make specific antibodies which stay alert to kill infectious diseases that might invade a person. This gives persons immune system the opportunity to respond rapidly and at a higher activity level when exposed to the disease. The individual success of vaccines depends on the stability and consistency of the enemy virus. For example, a measles vaccine works for a lifetime. Influenza, on the other hand, mutates a lot and requires newly formulated vaccines every year.

Medicine is used after ailment and vaccine is used before ailment,for medicines certain test are to be verified it'sin-vitro,in-vivo study its pharmacodynamics and pharmacokinetics,then toxicity on animal,then clinical study which takes time and money.

Herbal medicines from vedas are all clinical based references, because at that time no scientific tools were there. Therefore,we have to ascertain with modern parameters referred herbs in these books and then useit as medicine.

Now a new medicine Virafin from ZydusCadila and 2-DGreceived emergency use approval from the DGCI.Sputnik and PakVac are also coming up.

In the beginning Covid-19 was first treated by hydroxychloroquine which is derived from a herb Cinchona. Herbs are rich in flavonoids, vitamin C, or the carotenoids so can enhance immune function. The flavonoid-rich herbs may also possess mild anti-inflammatory action. Their beneficial effect named as anti-inflammatory and as an immune-stimulant action. It can promote the activity of lymphocytes, increase phagocytosis, and induce interferon production. For example garlic is one of the most remarkable plants that can effect strongly on immune system. Garlic as an immune system booster/resistance booster has been found to exert an immune-potentiating effect by stimulating natural killer cell activity. For example some studies powerfully suggest that garlic is

a promising candidate as an immunomodifier, which preserves the homeostasis of immune functions, because it has a higher concentration of sulphur combinations which are responsible for its therapeutic effects. The chemical components of garlic have also been found for treatment of cancer, diabetes, atherosclerosis, hyperlipidemia and viral diseases.

According to the World Health Organization, healthy foods , hydration and physical exercise are vital. Individuals consuming a well-balanced and rainbow diet(tomatoes, watermelon, pink grapefruit, guava, papaya, cranberries,Orange, carrots, mangos, pumpkins, apricots, broccoli, cabbage,) are healthier with a strong immune system and have a reduced risk of chronic illness, infectious diseases.

Ministry of AYUSH recommends Spices like Haldi (Turmeric), Jeera (Cumin), Dhaniya (Coriander) and Lahsun (Garlic) and herbal tea / decoction (Kadha) made from Tulsi (Basil), Dalchini (Cinnamon), Kalimirch (Black pepper), Shunthi (Dry Ginger) and Munakka (Raisin).All of these herbs are havinga lot of literature scientifically proved and clinically described literature.Still some new herbs are identified for their antimicrobial,antiviralactivity likeTachyspermumammi(ajowain).

This issue of UJPAH is devoted to Ayurvedic immunity booster and antibacterial and antiviral herbs to maintain the immunity and can act as resistance booster in this Covid era of crisis. Achilleamaritima,Rosmarinusofficinalis,Lavendulaangustifoliashowed strong antibacterial activities and having good number of reports as antiviral herbs.

My best wishes to all those scientists, Research scholars, students and teachers who contributed for bringing out this issue and I also express my sincere gratitude to all board members who make this issue a memorable for science fraternity of the Uttarakhand and the people of science at large. Last but not least I express my thanks for all those who have sent messages for this June 2021 issue of the journal.

Dr. S. Farooq
Chief Editor

Contents

Chemical Composition and Antimicrobial Activity of Essential oil from Scales of Moroccan *Juniperus phoenicea*

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Abstract-The essential oil of *Juniperus phoenicea* was obtained by hydrodistillation method using a Clevenger-type apparatus with a yield of 1.9 % and was analyzed by gas chromatography coupled to a mass spectrometer (GC-MS). Twelve volatile compounds were identified representing 99,85% of the total oil composition, while the α-pinene (78,31%), β-Myrcene (11,92%) and limonene (3,96%) were the major compounds. This essential oil was evaluated as an antibacterial and antifungal agent. The result showed that the oil presents a high biological activity as an antibacterial agent against the three tested strains Escherichia coli, Staphylococcus aureus and Pseudomonas aeroginos. It's also active as an antifungal agent against the Candida albicans with a zone inhibition of 28 mm.

Keywords: Medicinal plant, *Juniperus, phoenicea,* Essential oil, Chemical composition, Antimicrobial activity.

Introduction

The history of aromatic and medicinal plants is associated with the evolution of civilizations[1]. Since ancient times and in all parts of the world, these plants have been always occupied an important source of medicine[2].In addition, it was found that their applications are in different areas like cosmetics, perfumes and food[3].The evaluation of phytotherapeutic properties such as an antimicrobial, remains a very interesting task and useful, especially for plants of rare use or less frequent, if not unknown, in medicine and folk medicinal traditions. These plants represent a new source of active compounds[4].

The genus *Juniperus*(Cupressaceae)is an aromatic plant. It has a large number of specie sat about 60[5], increasing rapidly in abundance in arid and semiarid regions, trees hrub ecosystems throughout the Northern Hemisphere[6,7]. They occupy an important place native of the Mediterranean basin[8].This tree reaches commonly 4-8 m height, presents in the form of several species. In Moroccan flora, four species are present as J*uniperus oxycedrus, Juniperus communis, Juniperus phoenicea* and *Juniperus thurifera*[9].The genus *Juniperus* has been widely used in Morocco folk medicine for stomach tonic, diarrhea, rheumatism, and as under protection[10]. The *Juniperus phoenicea* popularly known as "Arâar" is also used as a decoction against rheumatism and diabetes, while dried and powdered fruit can cure skin ulcerations[10]. Its essential oil contains diversity of substances giving them important biological activities such as an antibacterial[11,12], antifungal[11,13], antioxidant[11,14] and cytotoxic against cell lines[12,13].

In continuation of our work on valorization of natural products[15,17], the aim of this study is to extract the essential oil (E.O) from scales of *J. Phoenicea* colletted from the region of Ifran in Morocco using hydro distillation method, and to determine its chemical composition by GCMS and evaluate its antibacterial and antifungal activities.

Material and Methods

Plant material

The cones used in this study were collected from the region of Ifran in the Middle Atlas Mountain during the month of January. The botanical identification was achieved by the

National Scientific Institute (Rabat) where voucher specimen (RAB-104243) was deposited in the herbarium. In the region studied, we have chosen 10 trees. From each tree we took about 2 kg of cones and prepared 10 individual samples. After we made a heterogeneous sample by mixing 500g of each tree cones (about 5 Kg), it was submitted to the air-drying in the shade for two weeks at room temperature and powdered. The moisture level was 12%.

Analysis by gas chromatography-mass spectrometry (GC/MS)

The essential oil of *Juniperus phoenicea* was analyzed by gas chromatography coupled with GC/MS mass spectrometer. This technique is widely used in the qualitative and quantitative analysis of essential oils. The apparatus used is Shimadzu GC-2010 equipped with a BP-5 capillary column (30m x 0.25mm I.D; 0.25mm film thickness); coupled with a Shimadzu QP-2010 Plus mass spectrometer. The carrier gas is helium set at a flow rate of 1ml/min and adjusted at a linear velocity of30 cm/s. The oven was programmed as follows: from 60 to 200°C at 3°C/min, then at 15°C/min to 280°C, then maintained for 10min at this temperature. The injected volume was about 0.1 µl.

Microorganism and culture material

The microbial strains used for the antimicrobial activity test are: Escherichia coli ATCC 25922,Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 and a yeast Candida albicans ATCC 10231.The bacterial strains provided are vivified by a culture of one night, the bacteria are subcultured in a sterile biokar nutrient medium and the albican yeast is subcultured in a YPG medium (yeastextract, peptone, glucose). Biokar steril then is incubated for 18 to 24 hours in an oven under a temperature of 37 °C. The bacterial load is suitable for analysis by the following method: a colony of each bacterial strain is imposed using a sterile loop and incorporated into a sterile tube of physiological saline (NaCl at 0.99%). The density of this solution was compared to the turbidity of the solution to 0.5 McFarland [18],The absorbance of this bacterial suspension at 625 nm is adjusted to have an optical density of 0.08 to 0.12 which corresponds to a concentration bacterial 10^8 CFU / ml, the suspension is diluted by a factor of 2 to have a bacterial concentration of 10^6 CFU / ml. By cons for the yeast strain, agar surface was scraped and introduced into 9 ml of physiological saline, three decimal dilutions are prepared and the latter is used to test the antibacterial activity.

Micro-dilution : Calculation of the minimum inhibitory concentration, to determine the minimum inhibitory concentration of J. phoenicea's E.O, the sterile Elsa microtiter plates are prepared as follows: the first 11 columns are filled with 100 µl of MULER HILTON medium and 50 µl diluted with a factor of (2) of the following emulsion: (since our organic compound is insoluble in water, we solubilized 20 mg of the tested compounds in a volume of 1 ml composed of 0.5 ml of the sterile medium and 0.5 ml of hexane), the microplate wells are then inoculated for each line, a bacterial strain. The test was replicated three times. The plates were incubated for 18 to 24 hours. The reading of the results was defined by the Resazurin staining test, it responded by giving a red color in the presence of bacterial growth.

Results and Discussion

Chemical composition of the essential oil (E.O) of *Juniperus phoenicea*

The essential oil from scales of *Juniperus phoenicea* was obtained by hydrodistillation with a good yield 1.9% compared to previous work[19]. According to the chemical analyses, twelve compounds were identified in the essential oil using a gas chromatography mass GC-MS, representing 99.85% of the total oil (Table-1). Monoterpenes hydrocarbons were the predominant chemical class with the α-Pinene (78.31%) as the major constituent, followed by β-myrcene (11.92%) and limonene (3.96%).

Table-1 Chemical composition of *Juniperus phoenicea* populations.

N°	Identified Compounds	RT	Yield %
1	α-phellandrene	5.688	0.07
2	α-pinene	5.898	**78.31**
3	thujene	6.323	0.14
4	β-phellandrene	7.027	0.22
5	β -pinene	7.152	2.43
6	β -myrcene	7.526	**11.92**
7	3-carene	8.226	1.67
8	limonene	8.871	**3.96**
9	ocimene	9.552	0.29
10	4-carene	11.159	0.56
11	2-methyl - 6-methylene - 7-Octen - 4-ol	15.560	0.16
12	thymol methyl ether	17.881	0.12
Total monoterpenes hydrocarbons			99.85%

Antibacterial activity of Essential oil (E.O)

The in vitro antibacterial activity of this essential oil against three pathogenic strains Gram-positive(Staphylococcus aureus) and Gram-negative (Escherichia coli and Pseudomonas aeroginosa) was assayed using the disc diffusion method by measuring inhibition zone diameter (Figure-1).

The essential oil of *Juniperus phoenicea* tested showed significant antibacterial activity which was extremely active on all tested bacteria compared to those of the antibiotics (Penicilline and Novobiocine), with inhibition zones ranging from 22 to 28 mm (Table- 2).The high antibacterial potential may due to the presence of α-pinene (78.31%)[20]. In essential oil of *Juniperus phoenicea,*

preliminary screening revealed that the EO were effective against all tested bacteria. To accomplish this, additional minimum inhibitory concentration (MIC) assays were performed using the liquid serial dilution method of Mueller Hinton (MH) . MICs varied from 0.937 mg/ml to 0.117 mg/ml. (Table-3).The results proved that E.O of *Juniperus phoenicea* has a good antimicrobial activity for all the three strains tested. Our observations are in consistent with the results obtained by Cosentino et al.[21]. They showed that the minimum inhibitory concentration of the E.O of *Juniperus phoenicea* was 0.9 mg/ml towards the different bacterial strains.

Table -2 Results of antibacterial activities of *J. Phoenicea* Essential Oil (E.O)

	J. phoenicea	**Positif control**		**Negatif control**
	E.O	Penicilline	Novobiocine	Ethanol
Escherichia coli ATCC 25922	22	9	13	-
Staphylococcus aureus. ATCC 25923	28	34	12	-
Pseudomonas aeroginosa. ATCC 27853	24	9	15	-

- :No activity

Table -3 Minimum Inhibitory Concentration of *J. Phoenicea* E.O

	E. Coli	***S. Aureus***	***P. Aeroginosa***
MIC (mg/ml)	0.234	0.937	0.117

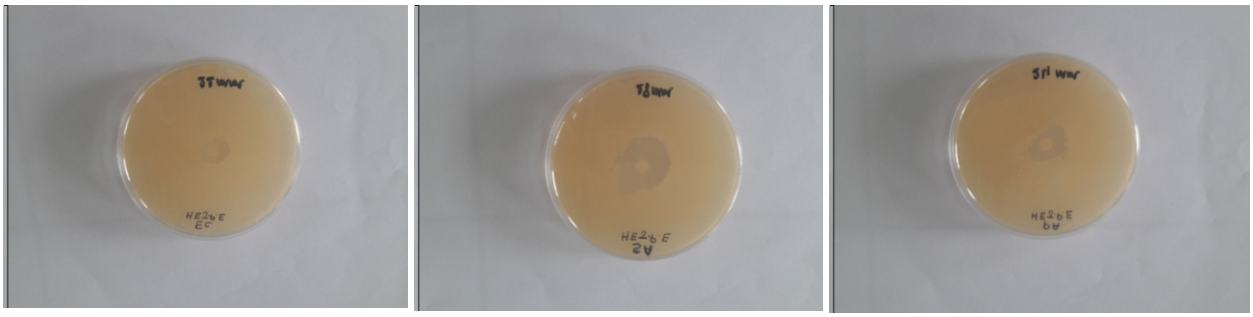

Figure-1 The inhibition zone of the E.O of *J. phoenicea* against the three strains *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

Antifungal activity

The E.O isolated from the scales of *J. phoenicia* were tested for antifungal activity against *C.albicans,* and their fungistatic effects tested with an inhibition diameter of 28 mm compared to the commercial antifungal Metrazol which has more significant action on *C. albicans*(Table-4).The evaluation of the inhibitory effects of E.O allowed us to deduce that they are very active against the tested *C.albicans* yeast. This activity is probably due to the chemical composition of the oil rich in α-pinene, which is also in accordance with the work of Yarelis et al. This indicated that E.O moderately reduce the development of *C. Albicans*[22].

Table-4 Results of antifungal activity of *J. phoenicea* essential oil (E.O)

	J. phoenicea	Control positif	Control negatif
	E.O	Metr azol	Methanol
Candida albicans	28 mm	29 mm	-

- :No activity

Conclusion

The present study provides the chemical composition and antimicrobial activity of the essential oil *J. phoenicea,* the aromatic plant growing in Morocco. The chemical composition of the essential oil extracted from scales of *J. phoenicea* using hydrodistillation method indicate the predominance of α-pinene(78.31%), β-myrcene (11.92%) and limonene (3.96%). The extracted oil on the monoterpenoids is 99.85%. The chemical composition of the essential oil is in good agreement with literature[23].The oil demonstrates a better inhibitory effect against the bacterial and fungal strains study which was deduced from the size variation of the inhibiting zone. This may due to the significant presence of α-pinene in the scale.

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Evaluation of Central Nervous System Acting Effects of Citrus Peel Essential Oils Extracted Using Enzyme Technology on Rodent Models

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Abstract-There is growing evidence of measurable effects of essential oils in animal brains and so more clinical research is required to validate their influence on the human central nervous system. This will enable us to discover essential oil-based drugs for treatment of mental illnesses such as depression, anxiety etc. Several methods have been developed to obtain oil from oil-rich plant materials using aqueous enzymatic methods. By using enzymes to mediate the extraction, it is possible to maintain mild conditions and effect superior extraction. The enzymes such as cellulase, hemicellulase, pectinase and protease are the most favourable enzymes for degrading the cell wall in oilseeds to loosen oil sacs embedded in the seed structures. Reduced equipment costs and energy consumption are also potentially possible, since oil and protein may be recovered simultaneously Therefore, the present study is proposed on quality and quantity enhancement of essential oils from citrus peel waste through enzymatic intervention and evaluates their effect on the central nervous system in Rodent Models. As an attempt to obtain the essential oil through hydro distillation HD and hydro distillation enzyme assisted HDEA was carried out. Several experiments have been conducted to determine the optimal process parameters for both methods, i.e., substrate to solvent ratio, extraction temperature, extraction time, enzyme loading, and incubation time etc. to Obtain essential oil from citrus peel collected from the local market of Dehradun. Total yield and physical characteristics like specific Gravity, viscosity, refractive index, acid, Saponification, iodine no etc. were compared. Both samples of oil CA-1 and CA-2 were further screened for their effect on Central Nervous System on the rodent model. Result indicated HDEA not only improved yield but also has sustainably stimulant effect on the central nervous system as compared to HD.

Keywords: Essential oil, Enzymes, Central nervons system.

Introduction

Essential oils are valuable plant products, generally of a complex composition comprising the volatile principles contained in the plant[1]. The oil droplets being stored in the oil glands or sacs can serve as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth, and stimulating healings. A vast variety of biological and pharmacological activities are associated with them. Experiments in animal models have exhibited the involvement of multiple neurotransmitter systems in the mode of action of essential oils, resulting in physiological effects in the brain. Also, the clinical trial study demonstrated the influence of essential oils in physiological parameters such as blood pressure, heart rate, respiratory rate, brain waves composition, and cortisol serum levels with concomitant psychological effects. Although there is growing evidence of measurable effects of essential oils in animal brains, more clinical research is required to validate their influence on the human central nervous system. This will enable the discovery of essential oil-based drugs for the treatment of mental illnesses such as depression, anxiety etc[2,3]. Generally, methods of extraction

followed for aroma and pigment from plant materials are solvent extraction, hydro-distillation, steam distillation, and supercritical carbon dioxide extraction. Several methods have been developed to obtain oil from oil-rich plant materials using aqueous enzymatic methods. Unlike traditional oil extraction methods, these new bioprocesses are performed without the use of organic solvents. By using enzymes to mediate the extraction, it is possible to maintain mild conditions and effect superior extraction. However, apart from laboratory trials, essential oil extraction using enzymes is largely an unexplored area. There is a great potential for this enzyme-based extraction technology with the selection of appropriate enzymes with optimized operating conditions. Various enzyme combinations are used to loosen the structural integrity of botanical material, thereby enhancing the extraction of the desired flavour and colour components. Recently enzymes have been used for the extraction of flavour and colour from plant materials, as a pre-treatment of the raw material before subjecting the plant material to hydrodistillation/solvent extraction. Deep knowledge of enzymes, their mode of action, conditions for optimum activity, and selection of the right type of enzymes are essential to use them effectively for extraction. The enzymes such as cellulase, hemicellulase, pectinase and protease are the most favourable enzymes for degrading the cell wall in oilseeds to loosen oil sacs embedded in the seed structures. Aqueous processing of oil-bearing materials eliminates the negative environmental impacts due to the emission of organic solvents and does not leave toxic or undesirable solvent residues in the resulting food products. Reduced equipment costs and energy consumption are also potentially possible, since oil and protein may be recovered simultaneously[4,5]. Therefore, the present study is proposed on quality and quantity enhancement of essential oils from citrus peel waste through enzymatic Intervention and evaluation of their effect on the central nervous system in Rodent models. Studies on these aspects are vital and would be very important in understanding enzymatic aided pretreatment role on essential oils yield and quality, this will also help in sustainable utilization of the waste.

Material and Methods

Preparation of substrate

Citrus peels were collected from a local market of Dehradun. Peels were cut into small slice and ground using a blender, followed by oven drying at 60°C for 48 hrs[6]. The ground dried peels were stored in an air-tight container at room temperature. The ground dried peels were stored in an air-tight container at ambient temperature.

Extraction of Essential oil

Hydro-distillation HD(CA-1)

A volume of 300 mL of distilled water was added into the 1-L round bottom flask containing 100 g of ground dried citrus peels. The round bottom flask was connected to the Clevenger-type apparatus. The temperature was set at 100 °C and the HD process was conducted for 3hrs[7]. Once the process was completed, the extract in the trap was collected and the yield was calculated and expressed as percentage in (w/w). The sample designated as CA-1 was stored in the amber airtight sealed vials at −20°C until required for further analysis.

Hydro-distillation with enzyme assisted HDEA (CA-2)

The fresh peels of citrus were cleaned, chopped, and dried and grounded. A total of 100 g of ground-dried citrus peels were mixed with 100 ml of 0.1 M acetate buffer, pH 5.0 in a 250-ml shake flask. A commercial cellulose solution at different enzyme loadings of 10-30

FPU/g-substrate was added into the solvent mixture. The mixture was incubated at 50 ± 2 °C, 200 rpm for 90 min[8]. The sample designated as CA-2 was filtered using a plastic sieve and stored in the amber airtight sealed vials at − 20 °C prior to hydrodistillation procedures.

Determination of yield

The extract in the trap was collected and the yield was calculated and expressed as a percentage (w/w)[9],calculated on moisture free basis. The oil was stored in the amber airtight sealed vials at 0 °C until required for further analysis. (Table -2).

Yield%w/w=Weight of essential oil (g)/Weight of substrate (g)×100%.

Physical Analysis

Essential oil along with aroma and flavours represent a highly complex class of natural products. Quantitative determination and physicochemical analysis of essential oils viz. refractive index, saponification value, acid value, ester value, optical rotation, specific rotation and specific gravity etc. were carried out by following standard methods of Guenther[10]. These methods have proved to be of great value in the essential oil industry because of their simplicity and reproducibility. The details are as being presented in Table -1.

Toxicity Study

Acute Toxicity Study: Five groups(n=5) of male albino mice were used in the acute toxicity study. Animal from all groups was fasted overnight and administered (P.O.) with a single dose (50, 100, 150, 250, 500, 1000, 1500, and 2000 mg/Kg body weight.) of samples CA-1 and CA-2 group of animals which received an equal volume of P.B.S. served as control. The study as performed in accordance with OCED guidelines[11]. Change in the behavior of animals were observed for 72 hours after extract administration For any sign of toxicity and mortality, animal were observed for 14 days.

CNS Activity

The locomotor activity (horizontal activity) can be easily measured using actophotometer which operates on photoelectric cells which are connected in a circuit with a counter. When a beam of light falling on the photocell is at on the animal a count is recorded. An actophotometer could have either a circular or square area in which the animal moves. Both rats and mice may be used for testing in this equipment.

Wistar rats of both sex (male and female) weighing 150-250 gms with a variation of ± 2.0 gm were taken for study. Test rats were kept in individual elastic cages with wire tops, prior to the use for screening. All the animals were fasted for at least 12 hours before use, allowing only access to water. The rats were divided into groups of five animals each. Each rat was weighed individually and was marked to distinguish one from another. The equipment was checked and it was ensured that all the photocell counter was recorded for each group for 10 mins. At the end of counting, each group of rats was removed from the counting chamber. Drugs were given orally as per the following schedule and after 60 minutes they were retested for activity scores for 10 minutes. The dose for drug was 100 mg/kg body weight of rat while the dose for standard reference drug i.e., gaba pantene was 250mg/kg body weight. Animals were divided into 5 groups and received different drugs as control group receiving Tween 80, Standard groups receiving Caffiene and Gaba Pantene and Test groups receiving CA-1 and CA-2.

CNS motor activity was calculated as per the following formula

$$\% \text{ CNS activity} = \frac{\text{Initial no. of counts - Final no. of counts}}{\text{Initial no. of counts}} \times 100$$

Statistically Data

All data were expressed as mean SEM + wherever applicable, the data were analysed statistically by student's t- test, using graph pad instant version 2.05a and one way ANOVA. The level of significance was $p < 0.05$ and n represents five per group.

Results and Discussion

As an attempt to obtain the essential oil through HD and HDEA, several experiments have been conducted to determine the optimal process Parameters for both methods, i.e. substrate to solvent ratio, extraction temperature, extraction time, enzyme loading, and incubation time Total yield of (w/w) and physical characteristics like specific gravity, viscosity, refractive index, acid, saponification, iodine no etc. were compared. Results are summarized in (Table-1). Both samples of oil CA-1 and CA-2 were further screened for their effect on Central Nervous System on rat model using actophotometer and acute toxicity studies. Results are summarized in Table-2.

Table -1 Yield and Physical Characteristics of Essential oils CA-1 and CA-2

Sample	Yield %w/w	Physical Characteristic	Specific gravity	Viscosity (cps)	Refractive index	Total acid number (mg KOH/g oil)	Iodine number (g/100g oil)	Saponification value (mg KOH/g oil)	Peroxide value (mEq O_2/kg oil)
CA-1	3.12±0.50 %	Clear solution with lemon like odour	0.692± 0.02	109±0.5	1.46	7	84	145	7.50
CA-2	5.02±0.50 %	Clear solution with lemon like odour	0.671± 0.01	117±0.45	1.45	5	89	132	7.01

Table -2 Effect of Essential Oil On CNS on Rodent Model

S.No.	Groups	Drugs	Dose	CNS Activity		% CNS Activity
				Loco motor count (Before)	Loco motor count (After)	
1.	B	Caffeine	100 mg/kg	37.6	63.2	68.08
2.	C	Gaba - Pantene	250 mg/kg	83	40.6	51.08
3.	D	CA -1	100 mg/kg Body weight	43	58.2	35.34
4.	E	CA -2	100 mg/kg Body weight	32.9	55.8	**69.60**

Gabba Pantene is CNS depressant and caffeine are CNS stimulant (count before is less and more after in stimulant while reverse in depressant)

As an attempt to obtain the essential oil through HD and HDEA, several experiments have been conducted to determine the optimal process parameters for both methods, i.e. substrate to solvent ratio, extraction temperature, extraction time, enzyme loading, and incubation time. HD with the process parameters of 1:3 (g/ml) of the substrate to solvent ratio, extraction temperature of 100 °C and 180 min of extraction time produced the highest amount of oil from citrus peels with a total yield of 3.12% (w/w) was obtained.

To extract the essential oil from the citrus peels, the HDEA method was conducted by adding cellulase to break down the cell wall of the peels to release out the essential oil. The cellulase loading was varied from 0 to 30 FPU/g and incubated for 60-150 mins. However, the results showed that adding the cellulase and prolonging the incubation time was not able to release out the essential oil. The enzyme loading of 10 FPU/g with an incubation time of 90 min was found to produce the highest yield. The cellulase helped in breaking down the cellular structures to obtain a greater permeability of the cell wall,

Physical characteristics like specific gravity, viscosity, refractive index, acid number, saponification value, iodine no etc were also compared. Results are summarized in (Table-1) Oil obtained by both techniques was found to be a clear solution with lemon-like odor with very little variation in other characteristics.

Both samples of oil CA-1 and CA-2 were further screened for acute toxicity studies and their effect on Central Nervous System on rat model using actophotometer. The results are summarized in (Table-2). Acute toxicity studies revealed that both samples CA-1 and CA-2 did not produce any toxic symptom and mortality when administered orally to mice at a dose of 50, 100, 150, 200, 500, 1000, 1500, 2000 mg/kg body weight in mice.

The aim of biological activity was to study effect of essential oil on the central nervous System. This was done in terms of locomotor activity of rats using actophotometer (activity cage).Most of the central nervous system acting drug influence, the locomotor activities in human beings and animal; the CNS depressant drug such as barbiturates and alcohol reduce motor activity while the stimulants such as caffeine and amphetamines increase the activity. In other words the locomotor activity can be in debt alertness of mental activity. Essential oils exhibit stimulation properties which lie in their structure closely in resemblance with actual hormones.[13,14] The penetration potential of these oils to reach the subcutaneous tissues is one of a key features of these oils used in aromatherapy. The mechanism of their action involves the integration of essential oils into a biological signal of the receptor cells in the nose when inhaled. The signal is transmitted to limbic and hypothalamus parts of the brain via olfactory bulb. These signals cause the brain to release neuro messengers like serotonin, endorphin etc., to link our nervous and other body systems assuring the desired change and to provide a feeling of relief. Serotonin, endorphin, and noradrenaline are released from calming oil, euphoric, and stimulating oil respectively to give expected effect on mind and body. From activity data summarized in Table-2. It is evident that in total both the oils HD and HDEA have exhibited CNS stimulant potential (35.34 and 69.60%) locomotor count respectively as compared to caffeine (68.08% locomotor count) but enzyme assisted extraction technique has sustainably enhanced the effect on central nervous system. Thus, HDEA technique tunes the quality and quantity of the essential oil from citrus peels.

Conclusion

Hydro distillation HD and Hydro distillation enzyme assisted HDEA had successfully produced the essential oil from citrus peels. The essential oil produced through both techniques shows comparable physical characteristics in terms of specific gravity, refractive index, acid number, saponification value. HDEE method

resulted in the rupture of essential oil glands, thus releasing the essential oil in good yield. Both samples of oil CA-1 and CA-2 were further screened for their effect on Central Nervous System on the rodent model. From the present study, it could be concluded that citrus peel which is a waste product can be utilized. The essential oil extracted from them using the HDEA technique not only increased the quantity of oil but also quality in terms of its CNS stimulant potential. A detailed study is required in this direction.

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Inhibiting Effect of Moroccan Medicinal Plants on Crystallization of Oxalo-calcic Calculations in vitro

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Abstract- The aim of this work is to study the inhibitory effect of some Moroccan medicinal plants: parsley, nettle, oregano and corn beard on the crystallization of oxalocalcium urinary stones under experimental conditions which simulate the urinary environment (physiological concentrations in calcium and oxalate, temperature and pH). The experimental tests were followed by the turbidimetric method using UV-Visible Model SP8-400 spectrophotometry, the response of which restores the concentration of calcium oxalate. The results showed that the potassium and magnesium ions which constitute the main elements of these plants compete with the calcium ions in order to combine with the oxalate ions. All the competitive reactions reflecting the affinities of the different ions towards each other contribute to the observed overall inhibition of the crystallization of calcium oxalate.

Keywords: Crystallization, Urinary Calculus, Calcium oxalate, Inhibition, Moroccan Medicinal Plants

Introduction

Urolithiasis is a common pathology affecting nearly 20% of the population in industrialized and developing countries[1-3]. There are Several types of urinary stones whose physicochemical analysis provides information that can effectively contribute to understanding the mechanisms involved in their formation[4,5]. We note there is high frequency of oxalocalcic stones which represent 70 to 85% of urinary stones [6].

Several factors of an endogenous, metabolic[7,8], infectious, anatomical and also nutritional and medicinal nature[9] are at the origin of urolithiasis. Oxalocalcium urinary stones are found in the urine in three distinct crystalline forms[10,11], the predominant form of which is that of calcium oxalate monohydrate (COM), followed by the dihydrate (COD), then the trihydrate form (TOC) very rare.

The essential mechanism of urinary stone formation[12] is dependent on the urine concentrating too much of poorly soluble compounds, which can then precipitate as crystals and then clump together in the form of stones. The mechanism of stone formation passes, schematically, through seven stages: supersaturation, germination or nucleation, crystal growth, aggregation of crystals with formation of particles greater than 100 µm, retention of crystals in the epithelium, the accretion of new crystals and finally crystalline conversion leading to the most thermodynamically stable form[13]. These seven steps can be brought together in two

main phases. First, crystallogenesis which includes the first four stages, followed by calculogenesis. Crystallogenesis is not a pathological phenomenon in itself, it is observed in normal subjects as well as in lithiasis subjects, while calculogenesis is a phenomenon indicating urolithiasis.

The major lithogenic factor, in all chemical varieties of lithiasis, is the supersaturation of urine with solutes entering into the composition of stones. The latter leads to the precipitation of crystals, an obligatory initial step in the formation of stones [16-18]. Indeed, in the case of oxalocalcium lithiasis, the supersaturation results from the increase in the urine concentration of oxalate and / or calcium and possibly leads to the crystallization of calcium oxalate which has been studied[14,15]. The action of the promoter solutes is partially counterbalanced by inhibitors of nucleation and aggregation of crystals, such as citrate, magnesium, potassium ions and protein macromolecules[19,20]. In general, stone formation results from the disruption of the balance between promoter solutes and those acting as inhibitors, capable of opposing one or more steps in the lithogenesis of calcium salts, present in the urine. Some authors[21-23] have been able to show that certain juices with a high content of citrate, potassium and magnesium increase citraturia, in turn increasing the inhibitory power of urine[24-26].

Thus, in Morocco as in many countries, a large number of patients use natural substances in the treatment of urolithiasis. In fact, studies have been carried out on the effect of medicinal plants on the crystallization of urinary calculi, either in cystine form or in oxalocalcic form[27,28]. Citrate inhibits crystallization of calcium oxalate and calcium phosphate by decreasing urinary saturation with calcium salts, hence the importance of hypocitraturia as a factor promoting stone formation. Potassium reduces the excretion of calcium by combining with oxalate, thereby reducing the risk of kidney stone formation. Magnesium increases the solubility of calcium in urine; it seems to have a preventive effect on kidney stones. The potassium-magnesium combination in the form of citrate reduces the recurrence of kidney stones[29].

As part of our investigation on medicinal plants growing in Moroccan flora, we reported here, in vitro, the effect on calcium oxalate crystallization of aqueous extract of four medicinal plants used in Moroccan traditional pharmacopoeia.

Material and Methods

The model adopted to follow the crystallization of calcium oxalate in the supersaturated solution at 37°C and pH = 5.6, is followed by the turbidimetric method. The kinetic by turbidimetry was measured using a Model SP8-400UV-Visible spectrophotometer and the wavelength was set at 620 nm corresponding to the maximum absorption of calcium oxalate[30]. A quartz cell is used to perform the analyzes. Thermostatization at 37°C is maintained in the solution to be analyzed and stirring is ensured using a 5 mm magnetic bar.

The crystallization medium is obtained by mixing two solutions A and B, the constitution of which is as follows respectively: Na_2SO_4, $10H_2O$; $MgSO_4$, $7H_2O$; NH_4Cl; KCl; $CaCl_2$; and $NaH_2PO_4.2H_2O$; Na_2HPO_4,$12H_2O$; NaCl; Na oxalate; The products were of higher analytical purity.

Ultra Violet - Visible molecular absorption spectrophotometry is used to monitor oxalocalcic crystallization in a synthetic urinary medium.

The experimental tests in fact consisted of mixing a volume of a few milliliters of a calcium solution A with the same volume of an oxalic solution B (Table-1), the stirring, the temperature and the pH being kept constant.

Table-1 Chemical composition of the two solutions forming synthetic urine

Solution	Compounds	Concentration (mol/l)
A	$Na_2So_4,10H_2O$	$1.93.10^{-3}$
	$MgSO_4 ,7H_2O$	$5.92.10^{-3}$
	$NH_4 Cl$	$8.67.10^{-2}$
	KCl	0.163
	$CaCl_2$	7.10^{-3}
B	$NaH_2 PO_4 ,2H_2 O$	$1.54.10^{-2}$
	$Na_2 HPO_4 ,12H_2 O$	$1.56.10^{-2}$
	NaCl	0.223
	Na oxalate	2.10^{-3}

The model for the study of inhibitors on oxalocalcium crystallization which we adopted was based on the principle of turbidimetry referring to crystal growth which involves nucleation, growth and aggregation[31,32].

Turbidimetry is an optical method that measures the haze (absorbance) that exists in a solution. The value of absorbance A is determined with respect to a reference value and is expressed by the formula (1)

$$A=\log(\frac{I_O}{I})\text{--------(1)}$$

Where I. represents the light intensity of the incident ray and the intensity transmitted to the photoelectric cell. We define from relation (1) the turbidity of a solution by the formula (2)

$$T=(\frac{1}{l})\times A=(\frac{1}{l})\times\log(\frac{I_O}{I})\ \text{-----(2)}$$

Where l designates the length of the optical path traveled by the light ray in the cell. The general shape of the turbidimetric curves obtained (absorbance as a function of time) is

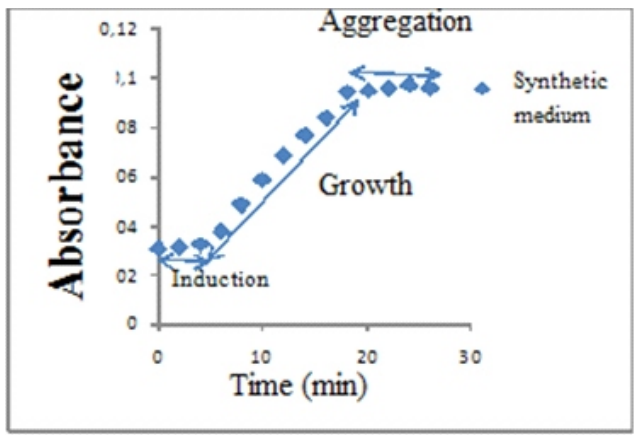

Figure-1 Evolution of calcium oxalate crystallization in the urinary synthetic urinary environment in the absence of plants.

This curve can be divided into three parts:
(i)A latency time during which the absorbance is practically zero thus corresponding to the crystalline nucleation phase and which is characterized by an induction time.
(ii)An ascending phase of strong growth, linear over a large part of time corresponding to crystal growth. It is characterized by a second kinetic parameter which is the turbidimetric slope.
(iii)Finally, a slow decrease passing through a maximum and reflecting the aggregation phase which is of great interest in the study of crystallization.
This model also has the advantage of quantifying the inhibitory power expressed in% relative to a control. It is given by the formula (3)

$$\%I = (1 - \frac{P_{ai}}{P_{si}}) \times 100 \quad \text{------(3)}$$

Where P_{ai} and P_{si} represent respectively the values of the turbidimetric slopes with and without inhibitor.

Plant characterization

The plants studied are: parsley *(Petroselinum sativum)*, nettle *(Urtica dioica)*, oregano *(Origanum vulgare)* and corn beard *(Zea mays)*. The plants materiel were identified by Professor Leila EL GHAZI from the department of biology, Faculty of Sciences, University Hassan II of Casablanca, Morocco, according to the flora of Morocco.
The plants were dried in the open air for 48 hours in the shade. The extracts of the plants studied were prepared by the infusion method with a concentration of 1mg / 1ml (plant / water). The same amounts of extract were added to the mixture of solutions A and B. Four plant extracts (parsley, nettle, oregano and corn barb) were investigated in the synthetic urinary medium.

Mineral content determination

The determination of potassium (K) and magnesium (Mg) contents was carried out using the standard methods [33]. Dried samples powder (0.5g) was ashed in a porcelain crucible at 550°C, in a muffle furnace for 24 hours. Then, 25 ml of aqua regia (HNO_3, HCl, 2/5: 3/5 v) were added and the mixture is heated at reflux for 2 hours at a temperature of 150 ° C. After cooling, 20 ml of distilled water were added, then we filter, we collect the filtrate in a 50 ml flask and make up to the mark with distilled water. The mineral constituents present in samples were analyzed separately using an atomic absorption spectrophotometer (Shimadzu, AA., 7000, Japan). A calibration curve for each of the mineral to be determined, was prepared using the standard solutions, before the readings were obtained.

Results and Discussion

Kinetics of crystallization in aqueous medium, synthetic medium and hypercalcic medium.

The experimental device that we have developed has the advantage of maintaining constant agitation, a fixed temperature at 37 ° C and introduction of the solutions at the same temperature. A volume of 70 ml of solution A is poured into a 250 ml beaker placed in a water bath thermostatically controlled at working temperature with continuous stirring. The same volume of solution B is also poured into a 250 ml beaker and placed in the same thermostated bath with continuous stirring.
The two solutions A and B are mixed under the same experimental conditions and the stopwatch is started by taking a sample in the quartz cuvette with a volume of 1.5 ml and an optical path of 1 cm.
The aqueous medium has the same concentration of calcium and of oxalate as the synthetic medium except that the other constituents are not added; for the hypercalcic medium; it has the same constituents as the synthetic medium except that the concentration of calcium has been doubled.
Absorbance is measured every 2 minutes for an average time of 30 minutes. Thus, we obtained the absorbance variation curve as a function of time, the shape of which is given in Figure-2.

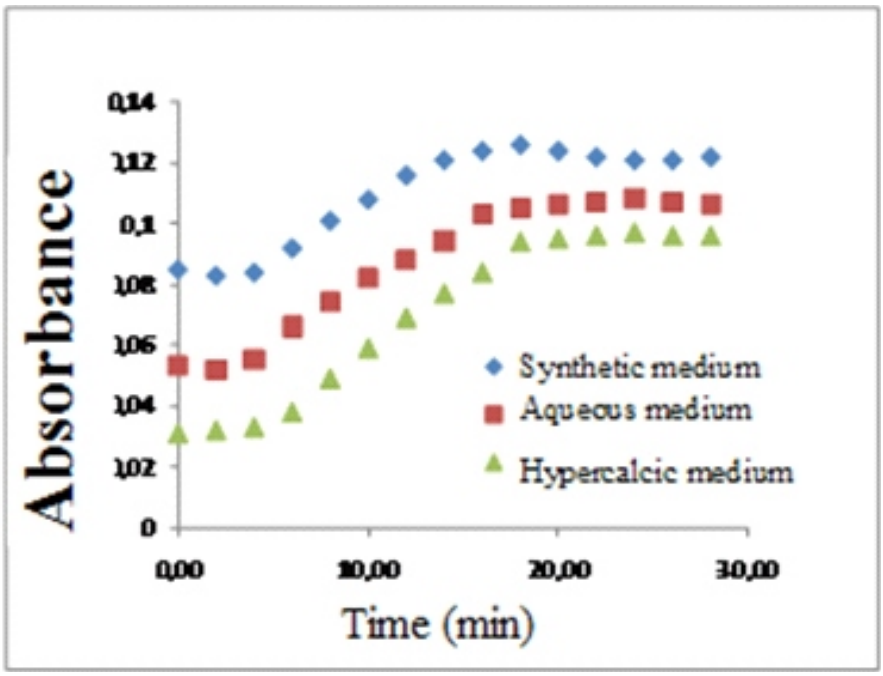

Figure-2 Evolution of calcium oxalate crystallization in the urinary synthetic urinary, aqueous and hypercalcium.

The growth lines for each medium, as well as their slopes are shown in Figure -3.

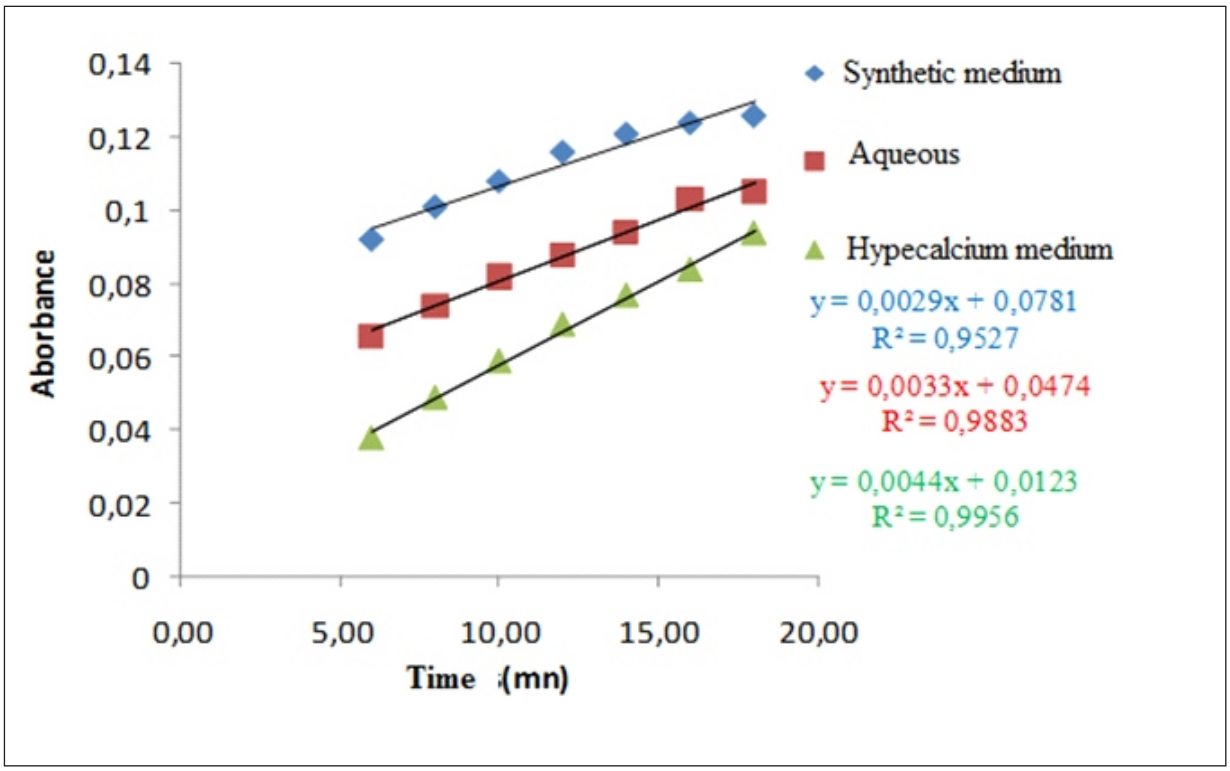

Figure-3 Determination of the crystaline parameters from the crystalline growth rights for the synthetic, aqueous and hypecalcium medium.

We then determined the induction time Ti which corresponds to the time elapsed between the addition of sodium oxalate and the moment when the growth is experimentally measurable.

The curves admit linear ranges characterized by turbidimetric slopes whose correlation coefficients R are greater than 95%.

In Table-2 below, the different crystalline parameters of the three media studied are shown.

Table - 2 crystalline parameters for the synthetic, aqueous and hypercalcic medium

Medium	Ti : Induction time	Tc : Growth time	Ta: Aggregation time	Slope	R^2
Synthetic	4	14	From18	0,0044	99,56
Aqueous	4	14	From 18	0,0033	98,83
Hypercalcic	4	14	From 18	0,0029	95,27

Based on the results obtained it was observed that hypercalcic medium promotes more crystallization of calcium oxalate followed by the aqueous medium and finally the synthetic medium.

This can be explained by the fact that the hypercalcic medium contains more calcium which is a promoter of calcium oxalate crystallization than the other two media.

For the same concentrations of calcium and of oxalate, the synthetic medium exhibits slightly greater inhibition than that of aqueous medium, this is due to the presence of the inhibitory ions potassium and magnesium in the synthetic medium.

Crystallization kinetics in the presence of medicinal plants in a synthetic urinary medium

We tested the plants mentioned above using the turbidimetric model which has a fairly high sensitivity and reliability. The results obtained for this study are presented in Figure-4 and likewise the growth lines corresponding to each plant.

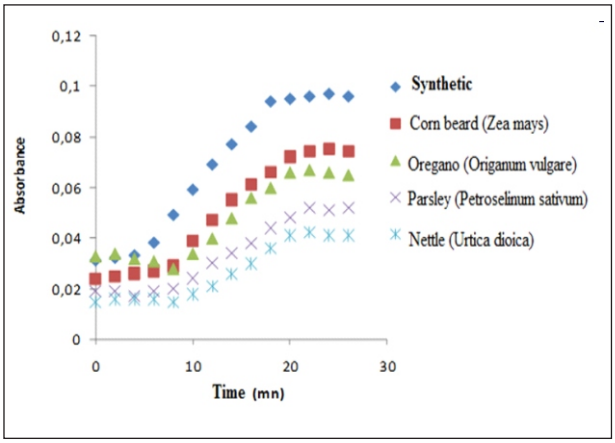

Figure-4 Evolution of the crystallization of calcium oxalate in the synthetic urinary environment in the presence of medicinal plants.

In Table-3 below, the different crystalline parameters of the synthetic medium are shown in the presence of the different plants studied with different concentrations as well as the inhibition rates corresponding to each inhibitor in the synthetic medium.

Table-3

	Concentration mg/ml	Ti : Induction time	Tc : Growth time	Ta: Aggregation time	Slope	R^2	I%
Synthetic	0	4	14	From 18	0,0044	99,38	--
Corn Beard	1	6	14	From 18	0,0040	99,26	9,09
Oregano	1	8	12	From 18	0,0035	99,43	20,45
Parsley	1	8	14	From 18	0,0023	99,44	47,73
Nettle	1	8	14	From 18	0,0019	98,90	56,82

From the results in Table-4 it can be seen that the rate of inhibition increases in the following order: Corn bar (9.09%), Oregano (20.45%), Parsley (47.73%), and finally the nettle (56.82%). To explain this difference in inhibition rates, we thought to find a Chemical composition of inhibitory elements in these plants.

Table-4 below shows the plants used and their chemical composition. The table shows that inhibition rate increases with potassium and magnesium content in each plant.

Table-4 Chemical composition of plants in minerals

	Potassium (mg/100g)	Magnesium mg/100g	Inhibition rate
Corn Beard	287	127	9,09
Oregano	1000	270	20,45
Parsley	1272	372	47,73
Nettle	2196	473	56,82
Solubilities of the oxalates	$K_2C_2O_4$ 39.9 g / 100g water	MgC_2O_4 10.4g/100g water	CaC_2O_4 6.7-10.4 g /100g water

By comparing the solubilities of the oxalates of these elements with that of calcium oxalate, we notice that these solubilities are classified in decreasing order; $K_2C_2O_4$ (39.9 g / 100g of water) followed by MgC_2O_4 (10.4 g / 100g of water) and finally CaC_2O_4 (6.7-10.4 g / 100g of water); therefore the oxalate combines first with potassium, then with magnesium and finally with calcium, which decreases the amount of calcium oxalate formed, which happens in majority of urinary stones.

Conclusion

The results demonstrated the efficacy of the plants studied on the crystallization of oxalocalcium urinary stones, particularly *Urtica dioica* which exhibit a higher inhibitory effect in views of its high potassium and magnesium content. We can conclude that the natural extracts could, therefore be used in the treatment of urolithiasis of patients in traditional pharmacopoeia.

Conflict of interest

The authors declare that they have no competing interests.

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Phytochemical Screening of *Bombaxceiba* Flowers

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Abstract- Traditional knowledge of medicinal plants is showing important and significant values to society. *Bombaxceiba* is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. The present study includes the detailed exploration of phytochemical properties of various extracts of *Bombaxceiba* in an attempt to provide a direction for further research. Flowers of *Bombaxceiba* were air-dried and its coarsely powdered samples were subjected to Soxhlet extraction using diverse solvents (petroleum ether, chloroform , ethanol, ethyl acetate and distilled water)). Freshly prepared extracts were exposed to standard phytochemical analysis for qualitative estimation of phytoconsituents. Phytochemical analysis revealed the presence of several phytochemicals viz., alkaloids, flavonoids, steroids, phenol, tannins, steroid, terpenoids and glycocides. The methanolic extract displayed the presence of highest phytochemical compounds. It may be due to the higher solubility of active components in this solvent as compared to other solvents. The studies justify the use of *Bombaxceiba* in traditional medicines. The investigation further propose that the metabolites present in leaf tissue of *Bombaxceiba* can be potential source of novel natural antibacterial and antioxidant agents which may be of prospective application in food industry as an antioxidant.

Keywords: Phytochemical, *Bombaxceiba*, Antioxidant

Introduction

Since the ancient times, nature has been a huge source of medicinal plants. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine [1,2]. People are now showing interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs which have adverse side effects. Medicinal plants are mostly locally available, relatively cheaper and there is every virtue in exploiting such local and traditional remedies when they have been tested and proven to be non-toxic, safe, inexpensive and culturally acceptable to the community [3]. The medicinal value of plants is mainly due to the presence of some chemical substances known as phytochemicals. These are basically plant metabolites synthesized in all part of plant body by itself and have some definite physiological action on animals [4,5].

Bombaxceiba, commonly known as the Silk cotton tree belongs to the genus Bombax and family Malvaceae it is an important medicinal plant of tropical and sub-tropical India[6,7]. This tree is also found widely in tropical Asia, Africa and Australia[8]. The different parts of this plant have been used in the traditional system of medicines since ancient times[9]. The tree is famous for it's large, showy, six-inch flowers with thick, waxy, red petals that densely clothe leafless branch tips in late winter and early spring[10]. Many parts of the plant (root, stem bark, gum, leaf, flower, fruit, seed and heartwood) are mainly used by various tribal communities and forest dwellers for the treatment of wide variety of ailments[11]. Various activities have been reported in almost all parts of *Bombaxceiba*, some of these are hypertensive, antioxidant, hypoglycemic, antipyretic and hepatoprotective. The plant is used in traditional system of medicine as diuretic, dysenteric, emetic and curing diarrhoea, wounds, acne, skin blemish

and pigmentation, cold and cough [12].
The aim of this work was to determine the specifically phytochemical constituents of the floral extract of *Bombaxceiba*.

Material and Methods

The present study was carried at Post Graduate laboratory of department of Biochemistry and Biotechnology at Sardar Bhagwan Singh University, Balawala, Dehradun, India.

Sample collection and authentication

B. ceiba flowers were collected from campus of Sardar Bhagwan Singh University, Balawala, Dehradun in the month of January 2021 and authenticated from Botanical survey of India (BSI) Dehradun, (Uttarakhand), India.

Prepation of Plant extract

The plant material after collection was washed with distilled water to remove all fibrous and soil debris and then sun dried for 15 days. Dried sample was crushed into powder by electric blender (electric grinder).The fine powder (200gm sample extracted with 800ml of each solvent) was then subjected to Soxhletation by using different solvents in increasing order of polarity (Petroleum ether < Chloroform <Ethanol< Ethyl acetate< Distilled water). Different solvents were used for dissolving different components present in the plant material. The extract was then dried to remove almost all the moisture and solvents and thus the final product was kept in air tight containers and stored at 4°C in the refrigerator for further study.

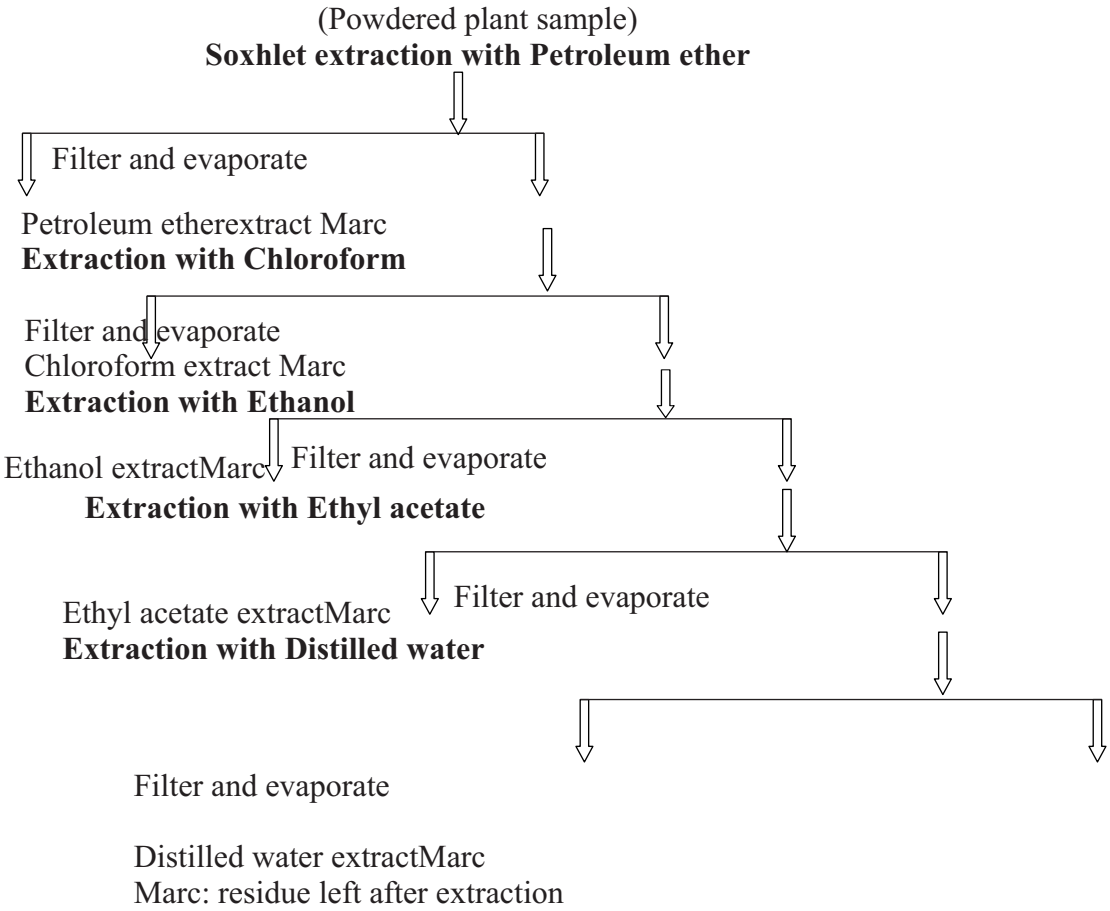

Figure -1 Scheme of *extraction*

Preliminary phytochemical investigation

All the extracts(Petroleum ether, chloroform extact, ethanolic extract, ethyl acetate extract and distilled water extract) obtained were subjected to preliminary phytochemical screening for the detection of various phytochemicals such as alkaloids, flavonoids, carbohydrate, steroids, phenols, tannins, saponins, terpenoid, glycoside, protein and amino acid using following standard methods[13-17].

Detection of Alkaloids -Small portion of the solvent free extract was stirred with a few drops of dil. HCL and filtered. The filtrate was then tested for following colour tests to detect the presence of alkaloid.

a. Mayer's reagent - Test solution with Mayer's reagent (1.36g of mercuric chloride in 60ml distilled water + 5.0g of potassium iodide in 20ml distilled water + 20ml of distilled water) gave cream ppt.

b. Hager's reagent - Test solution with Hager's reagent (saturated aq.solution of picric acid i.e. 1.0% w/w solution of picric acid in hot water) gave yellow ppt.

c. Wagner's reagent - Test solution with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 5ml of water and 100ml distilled water) gave reddish brown ppt.

Detection of Flavonoids

Alkaline reagent test: - Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomecolourless on the addition of dilute acid, indicated the presence of flavonoids.

Test for Carbohydrates

a. Molish's test - To the 2-3 ml of extract, few drops of 95% alpha-napthol solution in alcohol were added. After shaking, conc. H_2SO_4 was added from the sides of the test tube. Appearance of violet ring at the junction of two layers indicated the positive test for reducing sugar.

b. Fehling's test- To1 ml Fehling A and 1 ml Fehling B reagent 1 ml of extract was added and boiled for about 10min. Formation of brick red color precipitate indicated the presence of carbohydrate [18].

c. Benedict's solution test - Equal volume of Benedict's reagent and extract were mixed in test tube. Heated in boiling water bath for 5 min. Appearance of red coloured solution indicates the positive test for reducing sugar.

Test for steroids

a. Liebermann-Burchard Reaction - Mixed 2ml of extract with chloroform. Added 1-2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the sides of test tube. Development of green colour reveals the positive test for steroid moiety.

b. Salkowiski reaction - 2ml of crude extract was dissolved in 2ml of chloroform to this added 2ml of con. H_2SO_4 sidewise, red color ring was produced [19].

Test for phenolic components and tannins

Small quality of test solution dissolved in water and subjected for following test to detect the presence of phenolic compounds and tannins.

a. Dil. $FeCl_3$ solution (5%) test - Test solution with few drops of ferric chloride solution showed intense green color [20-23].

b. Vanillin HCl acid test solution - Test solution with vanillin reagent (1gm vanillin in 10 ml concentrated HCl) gave red color.

Test for saponins- Froth test: 2 ml of crude extract was mixed with 2ml of distilled water in a test tube, the solution was warmed and shaken vigorously; formation of stable foam indicated the presence of saponin.

Test for protein and amino acids

a. Ninhydrin solution test - Heated 3ml of extract and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 min. The development of violet or purple colour showedthe presence of amino acids[24].

b. Biuret test: - To 3ml of aqueous extract added 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour is formed, if proteins are present.

Detection of Glycosides

Extracts will be hydrolyzed with dilute HCl and then filtered. The filtrate obtained will be subjected to the following tests for glycosides.

a. Modified Borntrager's test- Extracts will be treated with 5 % Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was then cooled and extracted with equal amount of benzene. The upper layer was separated and treated with ammonia solution. Formation of Rose Pink colour in the Ammonical layer indicates the presence of glycosides (anthranol, glycosides).

b. Legal test- Extract as treated with sodium nitroprusside in Pyridine and NaOH. Formation of Pink to blood Red colour indicates the presence of glycosides (Cardiac glycosides).

Results and Discussion

Phytochemical Screening

Phytochemical screening of the sequential extract of *Bombaxceiba* revealed the presence of various bioactive components. The test for alkaloid has given positive result whereas saponin and protein test showed negative result for all the four extracts taken under study. Glycosides were present only in ethanol and ethyl acetate extract. Similarly phenolic compounds and tannins were present found in all the extracts except petroleum ether.

The result of phytochemical test is presented in Table -1.

Table- 1 Shows qualitative phytochemical analysis of extracts of *Bombaxceiba*

Phytochemical Test	Petroleum ether Extract	Chloroform Extract	Ethanol Extract	Ethyl acetate Extract	Distilled Water Extract
Test for flavonoids					
Alkaline reagent test	+	+	-	+	-
Test for Alkaloids					
Mayer's test	+	+	-	+	-
Hager's test	-	+	+	-	-
Wager's test	+	+	-	-	+
Test for carbohydrates					
Fehling test	+	-	-	+	+
Molish test	-	+	-	+	
Benedict's test	-	+	-	+	+
Test for phenolic compounds and tannins					
Dil. $Fecl_3$-test	-	+	+	+	+
Vanillin - HCl Test	_	+	+	+	+
Test for steroids					
Salkowiski test	+	+	+	-	-
Test for saponins					
Froth Test	-	-	-	-	-
Test for proteins					
Ninhydrin	-	-	-	-	-
Biuret test	-	-	-	-	-
Test for Terpenoids					
Salkowski test	+	+	+	-	-
Test for Glycosides					
Keller-Killiani test	-	-	+	+	-

(-) A sign indicates absence of constituent in the respective screening test; (+) sign indicates the presence of a constituent in the respective screening test.

These results are in confirmation with earlier studies done for this plant [25,26]. Flavonoids have extensive biological properties that promote human health and help in reduction of risk of diseases due to their antioxidant, anticancer, anti-inflammatory and anti-microbial properties[27]. Tannins are basically cytotoxic agents. They act as free radical scavengers thus can be useful in treatment of various degenerative diseases like cancer, atherosclerosis and aging process[28]. Alkaloids are being used in life saving drugs for some critical disorders like cancer, heart failure, blood pressure due to their wide range of pharmacological activities[29]. Saponins have been considered as bioactive antibacterial agent but also act as anti-tumour agents by inducing apoptosis [30].

Preliminary screening of phytochemicals is a valuable step in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. From the above results, it can be noted that successful extraction of biologically active compounds from plant are largely dependent on the type of solvent used during extraction. In this study, different solvents were used. This study, therefore, validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract.[31].

Conclusion

Herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and mainstream system. India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant based formulations, used in folk medicine and known to rural communities in India. Medicinal plants play a central role not only as traditional medicines, but also as trade commodities.

In the present work phytochemical and antimicrobial investigation of *Bombaxceiba* was performed. Successive solvent extraction was done using soxhlet. Preliminary phytochemical screening of *Bombaxceiba* gave valuable information about the different phytoconstituents present in the plant. It showed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins and amino acids. This will helps the future investigators in regard to the selection of the particular extract for further investigation of isolating the active principle and also will give idea about different phytochemicals, which have been found to possess a wide range of activities in *Bombaxceiba* stem extracts. Further studies on purification qualification and antioxidants potential of the active compounds would be our priority in future studies. Both in vitro and studies are in vivo recommended for their therapeutic application in modern medicine.

Acknowledgment

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Investigation of the Essential Oil of *Ormenis africana* from Morocco: Revision of Chemical Composition and Antibacterial Activity

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Abstract- The essential oil composition from the aerial parts of *Ormenis africana* (Asteraceae), an endemic species from Morocco, has been investigated by GC/MS. A total of 31 compounds were identified, representing 77%. After fractionation by column chromatography, the main compound was isolated and its structure elucidated by NMR spectroscopy. The essential oil was dominated by oxygenated compounds with spathulenol (45.8%) followed by camphor (7.1%), α-cadinol (5.9%) and α-bisabolol (5.9%) as the main compounds. This oil can be classified as spathulenol-type according to its spathulenol content. In vitro the antibacterial activity of the whole essential oil against three Gram positive (*Bacillus cereus, Enterococcus faecalis, Streptococcus C*) bacteria and three Gram negative (*Proteus vulgaris, Escherichia coli*, Pseudomonas aeroginosa) bacteria, showed significant results.

Keywords: Asteraceae, *Ormenis africana*, Essential oil, Spathulenol, Antibacterial activity.

Introduction

Northern Africa or Mediterranean Africa, comprises of Morocco, Algeria and Tunisia with a characteristic bioclimatic zone. In this sense, North African vegetation is a Mediterranean type, totally different in its botanical species and chemical composition with any other tropical African vegetation. In the arid bioclimatic zone, we can distinguish three series: the *Juniperus phoenicea* series, the field sage series with *Artemisia campestris* between the main shrubs and the white sage series. This last series is comprised of a complex group of plants, usually dominated by *Artemisia herba alba*. The main shrub spices are chamaephytes and between them grow *Ormenis africana* [1,2].

Ormenis africana (Jord & Four.) Litard. & Maire or *Chamaemelum africanum* Jord. & Four. (Family Asteraceae, Tribe Inuleae) is a perennial herb perennial herb with yellow flowers. The plant is endemic for North Africa (Morocco, Algeria and Tunisia). In Morocco, it's traditionally used for their spasmolytic properties[3]. The with yellow flowers. The aerial parts of this plant are used for their spasmolytic properties as well as for its stomacal pain[3]. A previous study have been reported on the antioxidant activity of ethanolic extract of inflorescence of *O. africana* found that hydroethanolic extract of this plant contain a considerable levels of antioxidant compound (polyphenols, flavonoids and anthocyanins...) and showed a high antioxidant activity in vitro and in vivo [4].

The chemical composition of the essential oil of *Ormenis africana* has been reported [3]. In the present study, the chemical composition of this specie was rechecked, and the major constituent was analysed by NMR technique after isolation by column chromatography. In addition, essential oils of *Chamaemelum* have been described to possess remarkable biological activities[5a,5b]. In this study, we investigated *in-vitro* the antibacterial activity by the disc diffusion method of the *O. africana* essential oil, against gram-positive and gram-negative microorganisms in comparison with Penicillin G.

Material and Method

Plant material: The plant material sample was collected in May, 2005 from plants growing in Errachidia (south of Morocco) at the flowering stage. Voucher specimens were authenticated by Pr. Ouyahya and deposited in the Department of Botany of the Scientific Institute, Rabat.

Isolation of the essential oil: Essential oil was obtained from the leaves and flowers of *O. africana* by hydrodistillation during five hours in a modified Clevenger type apparatus, yielding 0.8% (v/w) of a yellowish essential oil. This was dried over anhydrous sodium sulphate, diluted at 1% (v/v) in dichloromethane and stored at 4º C until the analysis by GC/MS.

Gas Chromatography-Mass Spectrometry: The GC-MS analysis was carried out with a thermomass spectrometer (model trio 1000); coupled to a thermo gas chromatograph (model 8000) (fusions Instruments). A Hewelt-Packard OV-17 capillary Column (25 m long × 0.25 mm) was employed for the analysis.

The column temperature program was 60 °C for 6 min, with 5 °C increase per min to 150 °C; which was maintained for 10 min. The carrier gas was Helium at a flow rate of 2 ml/min (splitless mode). The detector and Injector temperature were maintained at 250° and 225°C respectively. The quadruple mass spectrometer was scanned over the range 28-400 amu range at 1scan. s^{-1}, with an ionizing voltage of 70 eV, an ionization current of 150 µA.

The individual compounds were identified by MS and their identities was confirmed by comparing their retention indices relatives to C_8-C_{32} *n*-alkanes and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST library and literature [7].

Isolation of the major constituent: The essential oil (500 mg) was subjected to column chromatography over silica gel using n-hexane/diethyl ether (95:5) to give five fractions (F1-F5). Fraction F3 was further chromatographed to yield spathulenol (172 mg).

NMR analysis: The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker ARX 400 (^{1}H 400 MHZ/^{13}C 100 MHz) spectrometer using deuterated chloroform as solvent. The chemical shift values are reported in parts per million with reference to internal TMS.

Antibacterial activity test: The essential oil was tested against 6 bacteria, three Gram-positive: *Bacillus cereus* (IPL 58605), *Enterococcus faecalis* (CIP 103214) and *Streptococcus* C (IPT 2-035), and three Gram-negative: *Proteus vulgaris* (CIP 58605), *Escherichia coli* (CIP 54127) and *Pseudomonas aeruginosa* (CIP A 22). The bacterial strains were supplied by the Pasteur Institute (Casablanca).

Preparation for microorganism culture: Screening of the essential oil was done by agar disc diffusion method[8]. It was performed using an 18 hours culture growth at 37°C. The cultures were adjusted to approximately 10^5 CFU/ml. Five hundred microliters of the suspensions were spread over plates containing Mueller-Hinton agar. Empty sterilised discs (6mm) impregnated with 5 or 10 µl of the essential oil was placed on the surface of the media. The plates were left for 30 min at room temperature to allow the diffusion of the oil, and then they were incubated at 37°C for 24 hours. At the end of the period, the inhibition zone around the disc was measured with a calliper. Standard disc containing Penicillin G was used as reference control. Studies were performed in triplicate.

Results and Discussion

The hydrodistillation of the aerial parts from *Ormenis africana* produced 0.8% (v/w) of a yellowish essential oil. The components of the essential oil by GC-MS analysis are given in Table-1 in order of their elution on HP OV-17 column. Thirty-one constituents were identified representing 77% of the whole oil, which was characterized by oxygenated compounds (27 identified compounds), principally oxygenated sesquiterpenes that represented the most important group with spathulenol (45.82%), -bisabolol (5.92%) and -cadinol (5.87%) as the main compounds. Of the

oxygenated monoterpenes, it is interesting to note the large amount of camphor (7.10%) is followed by cis-chrysanthenol (2.52%), terpinen-4-ol (1.26%) and -terpineol (1.07%). The hydrocarbon fraction represented only by four sesquiterpenes hydrocarbons (-copaene, -caryophyllene, germacrene D and -cadinene) occurred in small amount (2.03% of the total identified essential oil).

These results differ so much with previous studies reported in literature about the same species[3]. Thus, Bellakhdar 1997, reported great differences in the essential oil composition of *O. africana*, characterized by high contents of the oxygenated monoterpene ocimenone (40%) and the sesquiterpene hydrocarbon-copaene (38%), this last compound represent only 0.19% in our study, whereas this main compound reported previously [3], was not found in the essential oil here analyzed. So this report can contribute to a better knowledge of this species.

Table-1 Essential oil composition of *Ormenis africana* (Jord & Fourr.)

Compound	I.R	%
Benzaldéhyde	964	0.03
Yomogi alcohol	999	0.10
1,8-Cinéole	1035	0.12
Cétone artémesia	1062	0.56
Trans-4-thujanol	1071	0.03
Artemesia alcohol	1084	0.13
Sabinene hydrate	1099	0.10
Chrysanthénone	1129	0.30
Trans-pinocarvéol	1139	0.27
Camphor	1149	7.10
Cis-chrysanthénol	1164	2.52
Bornéol	1170	0.92
Terpinen-4-ol	1177	1.26
p-Cymén-8-ol	1189	0.22
α-Terpineol	1192	1.07
Verbenone	1205	0.19
Pipéritone	1253	0.30
Chrysanthényl acetate	1265	0.13
p-Cymén-7-ol	1291	0.34
Carvacrol	1302	0.33
Eugenol	1361	0.17
α-Copaene	1380	0.19
Cis-jasmone	1401	0.14
β-Caryophyllene	1424	0.18
Germacrene D	1486	0.94
δ-Cadinene	1525	0.72
Nerolidol	1563	0.27
Spathulenol	1580	45.82
Caryophyllene oxide	1583	0.80
α-Cadinol	1654	5.87
5-(t-butyl)-4-méthoxy-1,2-dihydrobenzène	1667	2,83
α-Bisabolol	1686	5.92

Components listed in order of elution from a HP OV-17 column.
RI: Kovats indices calculated against C_8-C_{23} n-alkanes on the HP OV-17 column

To remove doubt, a sample of whole essential oil was subjected to column chromatography over silica gel using mixtures of n-hexane/Et_2O of increasing polarity as eluents, the major compound 1 (Figure- 1) was obtained as colourless oil. The mass spectra and ^{1}H and ^{13}C NMR data (Table-2) suggested that 1 is spathulenol[12]. Comparison of the spectroscopical data of 1 with those reported in literature of spathulenol[9] confirmed these assignments.

Although, this plant did not contain santolina alcohol, as identified previously in the oils of *Ormenis multicaulis*[10].

Table-2 Experimental and literature's ^{13}C NMR data for spathulenol (δ in ppm)

Carbon	1	2	3	4	5	6	7	8
Exp	54.4	26.8	41.8	81.0	53.3	29.9	27.5	24.8
Lit[9]	54.3	27.7	41.7	80.9	53.3	29.9	27.4	24.8
Carbon	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Exp	38.9	153.5	20.3	28.7	16.4	26.1	106.3	
Lit[9]	38.8	153.4	20.2	28.6	16.2	26.0	106.2	

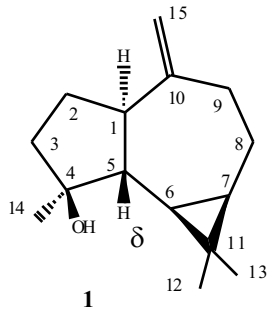

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Figure-1 Structure of purified compound, spathulenol

Table -3 shows in vitro bacteriostatic activity of essential oil from *O. africana* together the inhibition zones formed by standard penicillin G (10 unit) antibiotic discs.

Table-3 Antibacterial activity: Diameter of the inhibition zone[a] of essential oil of *Ormenis africana* and standard antibiotic penicillin G.

	EO *O. Africana*		P10
Microorganisms	5 µl	10 µl	
Bacillus cereus	20	23	13
Streptococcus C	17	21	19
Enterococcus faecalis	16	21	13
Escherichia coli	12	13	16
Proteus vulgaris	11	13	9
Pseudomonas aeroginosa	9	9	9

a: Includes diameter of disc (6mm); P10: penicillin G (10 unit).

Although, at low quantities (5-10µl/disc), this oil exhibited a strong antibacterial activity against the most tested bacteria; in our study, *P. aeruginosa* was resistant at concentration at 5-10µl/disc of *O. africana*. The bacteriostatic properties of the oil are suspected to be associated with the high spathulenol content, which has been previously tested and was found to have significant antibiotic activity [11].

Conclusion

From this study, it can be concluded that essential oil of *Ormenis Africana* possesses a strong antibacterial activity against Gram-positive tested strains, and considerable activity against Gram-negative bacteria, this activity may be due of its high percentage of oxygenated sesquiterpenes, spathulenol (45,8%), α-cadinol (5.87%) and α-bisabolol (5.92%),the structure of the major compound was confirmed by NMR spectroscopy after purification using column chromatography. Our antimicrobial study justified the popular usage of this plant as traditional remedies for some infections.

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Modulatory Effect of Whole Flour and Hydroalcoholic Extract of Finger Millet (*Elusine coracana*) on the Abnormalities Associated with Metabolic Syndrome in Hyperlipidemic Diabetic Rats

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Abstract- The present study was designed to evaluate the effect of whole flour and hydroalcoholic extract of finger millet (*Elusine coracana*) in high fat diet (HFD) and streptozotocin induced metabolic syndrome in rats. The HFD was fed to the rats for a period of 45 days to induce hyperlipidemia. Diabetes was induced by single intraperitoneal injection of streptozotocin (65mg/kg i.p) in 0.1M citrate buffer pH 4.5. Animals with fasting blood sugar level of 250 mg/dl were considered as hyperlipidemic diabetic rats (HDR) and were selected for the study. The HDR were divided into five groups with six animals in each group and one group of normal animals. The HDR received whole flour and hydroalcoholic extract of *Elusine coracana* at a dose of 100, 200 and 400 mg/kg for a period of 21 days. Body weight, body mass index, fasting blood sugar level, lipid profile and the level of oxidative stress was measured in animals after the treatment. All treatments significantly decreased body weight, BMI, fasting blood sugar and also improved lipid profile in HDR as compared to the toxicant control. The treatments significantly reduced the level of lipid peroxidation and improved superoxide dismutase and reduced glutathione in the pancreas of HDR. Whole flour and hydroalcoholic extract of *Elusine coracana* at a dose of 200 and 400 mg/kg caused significant alleviation of the abnormalities of metabolic syndrome in rats.

Keywords: Finger millet, High fat diet, Hyperlipidemic diabetic rats, Metabolic syndrome.

Introduction

Metabolic syndrome (MetS) is a group of metabolic disorders characterized by insulin resistance, hypertension, central obesity and atherogenic dyslipidemia. It is also known as insulin resistance syndrome or syndrome X. It is progressively being recognized as a significant cardiovascular risk factor and a vital risk factor for the expansion of type 2 diabetes mellitus worldwide(Rochlani *et al*.,2017). The diabetes consultation group of the WHO defined MetS Internationally in 1998 as the presence of insulin resistance, obesity,hyperlipidemia and hypertention in a patient (Saklayen MG, 2018). There exists a complex relationship between different factors such as lack of physical activity, stress, over intake of food, environmental and lifestyle factors and MetS. In clinical and epidemiological studies, obesity is highly connected with all cardiovascular risk factors. Adipose tissues are recognised as a source of several molecules like excess of free fatty acids, cytokines, adipokines, increase leptin, fibrinogens and AT2 that are pathogenic and contributes to the progression of MetS. It has been noticed that obese adipose tissues release excess of fatty acids and cytokines that induce insulin resistance(Grundy *et al*., 2004, Anwer *et al*., 2018). Since thousands of years medicinal plants serve as an excellent source of bioactive compounds for the treatment of various diseases including metabolic syndrome due to presence of broad range of phytochemicals with diverse metabolic effects. Now a days, attention have been focused on

plant foods which may be beneficial in Preventing metabolic syndrome and its associated conditions like cardiovascular diseases and diabetes. Plant derived drugs are widely used and believed to be safe, cost effective with fewer side effects. *Eleusine coracana* is an annual plant mostly grown in Asia and Africa and serves as a major meal in Southern India and Ethiopia. It is called Ragi in India and Madua in Hindi (Mall TP and Tripathi SC, 2016). *Eleusine coracana* is believed to be rich in dietary fibres (22% both soluble and insoluble), micronutrients, proteins (such as leucine, isoleucine, lysine, tryptophan etc.), carbohydrates, minerals (calcium, magnesium), fats and polyphenols. It is also said to have a high content of calcium (Erhabor *et al*., 2013). Polyphenols such as gallic acid, tannic acid, vanillic acid, ferulic acid, caffeic acid, and chlorogenic acid are present in seeds (Chethan *et al*.,2008). Various pharmacological studies have been carried out on the millet in order to validate the ethno medicinal claims and set forth detailed pharmacological activities such as antioxidant(Sreeramulu D. *et al*.,2009), anticancer (Singh *et. al*., 2009) antimicrobial, (Mathanghi SK and Sudha K, 2012), wound healing activity, (Hegde *et al*.,2002) antidiabetic (Rajasekarana *et al*., 2004) and hepatoprotective activity (Chethan *et al*., 2008). The present study was planned to determine whether chronic administration of flour of finger millet and hydroalcoholic extract of seeds of finger millet could restore the various coexisting conditions with metabolic syndrome or not. The study was aimed to evaluate and compare the effect of chronic administration of finger millet flour and whole seed extracts of finger millet in metabolic syndrome in hyperlipidemic diabetic rats.

Material and Methods

Drugs and chemicals

Streptozotocin (CDH, India), thiobarbiturtic acid, trichloroacetic acid, sodium carbonate, sodium bicarbonate, EDTA, Tris-hydrocchloride (High Media and Sigma) and DTNB (Thermfisher Scientific Company) were used in the study. Diagnostic kits used for the estimation of biochemical parameters were purchased.

Plant materials

The seeds of *Eleusine coracana* were collected from Chamoli district of Uttarakhand. The plants was identified and authenticated by Department of Botany, Forest Research Institute, Dehradun, Uttarakhand, India.

Extraction of seeds of *Eleusine coracana*

The seeds of *Eleusine coracana* were shade dried at room temperature and grounded to moderately coarse powder. 1kg of coarse powder was defatted with petroleum ether by using soxhlet apparatus. After this the plant material extracted with the mixture of water and methanol in the ratio of 60:40. The extract was collected in beaker or china dish and evaporated in water bath to get dry residue. The residue was weighed and stored in dessicators.

Preliminary phytochemical investigations

The extract of seeds of *Eleusine coracana* was subjected to qualitative phytochemical screening (carbohydrates, alkaloids, cardiac and anthraquinoneglycoside, protein, tannins, flavanoids and saponins) for identification of various phytoconstituents (Kokate CK, 1994).

Pharmacological investigations

Animals

Adult wistar albino rats of either sex (200-250gm) were used for this study. The animals were housed at 24°C ± 2°C and relative humidity 55 ± 5% with 12:12 hour light and dark cycle in standard polypropylene cages. They were provided food and water ad libitum. Before experimental studies the animals were acclimatized. The experimental protocol was approved by the Institutional Animals Ethics Committee of SBSPGI with approval number of IAEC/273CPCSEA/SBS/01/2014-2016 for conducting the experimental studies on animals.

Acute toxicity study

The toxicity studies of both of the plants was already performed by OECD guideline 423. So doses were selected on the basis of available literature on *Eleusine coracana*. Doses selected for pharmacological activity of *Eleusine coracana* was 100, 200 and 300 mg/kg (Lorke D, 1983).

Induction of hyperlipidemia and obesity

Obesity was induced in wistar rats (200 -250 gm) by commercially available edible dalda (vanaspathy) and culinary grade coconut oil obtained from local market. The high fat diet (HFD) was prepared by homogenously mixing dalda and coconut oil the ratio of 3:2w/w (Supriya K *et al*., 2012) and was given to the rats for 45 days. At the regular intervals of 7 days body weight was checked.

Induction of Diabetes

Diabetes was induced in hyperlipidemic rats (350-400 gm, body weight) by single intraperitoneal injection of streptozotocin (65mg/kg i.p) in 0.1M citrate buffer pH 4.5 (Mabhida *et al*., 2019). The control group received equivalent amount of citrate buffer. Animals showing FBS level more than 200 mg/dl were selected for the study.

Treatment Protocol

The Hyperlipidemic diabetic rats were divided into 5 groups and one group of normal rats with each group having 5 animals. The animals received the seed extract as a suspension in 1% CMC using distilled water as a vehicle for a period of 21 days. One group of animals received the pellets of flour of seeds of *Eleusine coracana* along with the High Fat Diet (HFD) for 21 days.

Group 1(Normal control):- Normal animals received 1% CMC in distilled water (1 ml/kg,p.o.).

Group 2 (Toxicant control):- Hyperlipidemic diabetic animals received 1% CMC in distilled water (1 ml/kg, p.o.).

Group 3:- Hyperlipidemic diabetic animals + Pellets of flour of seeds of *Eleusine coracana*

Group 4:- Hyperlipidemic diabetic animals + hydroalcoholic extract of *Eleusine coracana* (100 mg/kg, p.o).

Group 5:- Hyperlipidemic diabetic animals + hydroalcholic extract of *Eleusine coracana* (200 mg/kg,p.o.).

Group 6:- Hyperlipidemic diabetic animals + hydroalcoholic extract of *Eleusine coracana* (400 mg/kg,p.o.).

Before induction and after the treatment period of 21 days body weight was checked and BMI was calculated. Average feed intake was estimated for all the treated animals and physical, biochemical parameters and oxidative stress was estimated. Blood was collected from the retro orbital puncture under mild anaesthesia from the animals and it was then centrifuged at 2500rpm with the help of cooling centrifuge and serum was separated to evaluate various biochemical parameters by using autoanalyser.

Estimation of body weight

After HFD and treatment of plant extracts body weight of all animals in each group were checked and the weight difference was calculated.

Body mass index (BMI)

Obesity is defined by body mass index and further evaluated by both percentage body fat and total body length (Paras Gupta *et.al*.,2011).

BMI= mass(kg)/(height(m)) 2 Or

BMI=mass (lb)/ (height (in)) 2 X 703

Where m and h are the subject's weight and height respectively

Estimation of blood glucose

The blood samples were collected from retro orbital puncture on days 0, 7, and 14 following overnight fasting and blood glucose levels were measured by using GOD-POD kit by Erba, India in an auto-analyser. The method uses a modified Trindercolor reaction (Trinder, 1969).

Estimation of Serum Lipid Profiles

At the end of treatment period, animals were fasted overnight, blood sample was collected by retro orbital puncture under ether anaesthesia and it was then centrifuged at 2500rpm with the help of cooling centrifuge and serum was separated to determine the level of total cholesterol, triglycerides and high density lipoprotein were evaluated by Enzymatic Colorimetric Method (Mirlohi *et al*., 2012) and phosphotungstic Acid Method (Miller *et al*., 1977) respectively, using Bayer Diagnostic Kit, India in an auto-analyser.

Estimation of Lipid peroxidation and superoxide and reduced glutathione

Estimation of lipid peroxidation and superoxide and reduced glutathione was done by from the tissue supernatant by the method Slater and Sawyer, 1971, Mishra *et al*., 1972 and Moron *et al*.,1979 respectively.

Statistical Analysis

The results were expressed as mean ± SEM from six animals. Statistical difference in mean was analyzed using one way ANOVA (Analysis of Variance) followed by Dunnett's 't' test .P values less than 0.05 will be considered as indicative of significance.

Results and Discussion

Preliminary phytochemical identification test of hydroalcoholic extract of seeds of *Eleusine coracana*

Phytochemical studies revealed the presence of alkaloids, carbohydrate (non-reducing sugar), protein, amino acid and tannins in the seed extract of *Eleusine coracana* (Table-1).

Table-1 Phytochemical investigation of hydroalcholic extract of *Eleusine coracana*

S.N	Phytochemical test	Petroleum ether extract of *Eleusine coracana*	Hydroalcoholic extract of *Eleusine coracana*
1	Alkaloid	(+)	(+)
2	Flavanoids	(-)	(-)
3	Carbohydrate	(-)	(-)
4	Test for non -reducing polysaccharides	(-)	(+)
5	Protein	(-)	(+)
6	Tannins	(+)	(+)
7	Amino acids	(-)	(+)
8	Saponins	(-)	(-)
9	Triterpenoids	(-)	(+)
10	Steroids	(+)	(-)

Effect of whole flour and hydroalcoholic extract of seeds of *Eleusine coracana* on the body weight and body mass index in hyperlipidemic diabetic rats

Effect of the treatments on body weight and BMI of animals was recorded on various time intervals as shown in Table-3.2. In normal animals no significant weight change (210± 0.5 g) was observed during various time intervals. In the HFD treated animals (toxicant control) the weight gain and BMI was significant ($p<0.001$) on 21st day when compared to the first day of the treatment. Significant decrease ($p<0.01$) in body weight was observed in groups treated with the flour and hydroalcoholic extract of *Eleusine coracana* at a dose of 200 mg/kg and 400 mg/kg given for a period of 30 days as compared to the toxicant control group. The hydroalcoholic extract at a dose of 400 mg/kg exhibited maximum reduction in body weight among all the treatment groups on day 21st as compared to the body weight of hyperlipidemic diabetic animals on the 1st day of the treatment. In the study, the whole flour and hydroalcoholic extracts of *Eleusine coracana* at a dose of 200 mg/kg and 400 mg/kg caused significant decrease in BMI. However hydroalcoholic extract at a dose of 400 mg/kg caused most significant reduction ($p<0.001$) in BMI in hyperlipidemic diabetic rats as compared to the day 1 of the treatment.

Results suggest that the flour of *Eleusine coracana* protected the animals from the effect of high fat diet and prevented further induction of obesity as indicated by decrease in BMI as comapred to other.

Table-2 Effect of whole flour and hydro alcoholic extracts of seed of *Eleusine coracana* on body weight and body mass index in hyperlipidemic diabetic rats

Treatment	Body weight(gm)	Body mass index(gm/kg/day)
Normal (1ml/kg normal saline, p.o.)	149±3.50	7.89±0.06
HFD+STZ (60 mg/kg, ip. in citrate buffer pH 4.5)	380.6±2.31	15.21±0.8
HFD+STZ+ flour of seeds of *Eleusine coracana* (as food palate)	190.56±5.58	12.66±3.8*
HFD+STZ+ hydroalcoholic extract of seed of *Eleusine coracana*(100 mg/kg, p.o.)	186.78±3.9	15.9±0.4
HFD+STZ+ hydroalcoholic extract seeds of *Eleusine coracana*(200 mg/k5mg/kg, p.o.)	297±4.88	8.59±2.6***
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (400 mg/kg, p.o.)	301.1±6.78	8.07±6.98***

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N=6.Values are expressed as Mean ±SEM. *P< 0.05, **P< 0.01, ***P< 0.001. The results of test groups were compared with the HFD + STZ treated group (positive control).

Index: STZ = Streptozotocin, HFD= high fat diet p.o= per oral, i.p.=intraperitoneally.

Effect of whole flour and hydroalcoholic extract of *Eleusine coracana* on fasting blood sugar level in hyperlipdemic diabetic rats

Feeding flour of seeds of *Eleusine coracana* to the hyperlipidemic diabetic animals showed no improvement in the FSG level at 0 day after induction of diabetes (Table-3). The animals treated with the flour of seeds of *Eleusine coracana* showed decrease in blood glucose level on 7th and 14th day and at 21st day, on 21st day the treatment caused significant decrease in fasting serum glucose (FBG) level in diabetic rat. Results in Table-3 indicate administration of the hydroalcholic extract of seeds of *Eleusine coracana at* dose of 200 mg/kg caused significant ($P<0.001$) decrease in fasting blood sugar on 7th and 14th day after 21 days in hyperlipidemic rats as compaired to day 1 of treatment. Hydroalcoholic extract of seed of *Eleusine coracana* at the lower dose level (100mg/kg)caused mild decrese in FBG on 21st day in hyperlipidemic rats. Results suggest that feeding the whole flour and hydroalcoholic extract of seeds of *Eleusine coracana* for a long period of time prevented induction of diabetes and also lowered the FSG level to normal in diabetic animals.

Table-3 Effect whole flour and hydro alcoholic extracts of seed of *Eleusine coracana* on the level of fasting blood sugar level in hyperlipidemic diabetic rats

Treatment + Groups	0 hour	72 hours	14th day	21st day
Normal (1ml/kg normal saline, p.o.)	112.7±1.56	109.69±0.58	110.23± 0.79	112.21± 1.05
HFD+STZ (60 mg/kg, ip. in citrate buffer pH 4.5)	116.3±1.46	256.38±1.68	240.66± 4.18	225.72± 3.57
HFD+STZ+ flour of seeds of *Eleusine coracana*	113.3±2.32	170.74±1.4	137.48± 0.81**	130.46± 0.77**
HFD+STZ+ hydroalcoholic extract of seed of *Eleusine coracana* (100mg/kg, p.o.)	111.1±1.66	264.68±1.24	182.54± 0.93*	146.94± 0.67**
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (200 mg/kg, p.o.)	112.4±2.05	251.64±1.16	210.92± 1.19	204.98± 1.05
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (400 mg/kg, p.o.)	111.7±2.72	254.51±1.05	157± 1.4**	122.32± 0.97***

The statistical significance of difference between means was calculated by ANNOVA followed by t test for unpaired comparison. N=6. Values are expressed as Mean ±SEM. *P< 0.05, **P< 0.01, ***P< 0.001. Results on 21st day were compared with the results after 72 hours of HFD+STZ administration.

Index: STZ = Streptozotocin, HFD= high fat diet p.o= per oral, i.p.=intraperitoneally.

Effect of whole flour and hydroalcoholic extract of *Eleusine coracana* on lipid profile in hyperlipidemuc diabetic rats

Results in Table-4 showed administration of high fat diet for a period of 45 days caused significant increase in the cholesterol (226.3±8.11 mg/dl) and triglyceride (242.2± 7.07 mg/dl) level and decrease in the blood HDL (18.4±1.06 mg/dl) level as compared to normal animals.

Results indicate that the whole flour and hydroalcoholic extract of seeds of *Eleusine coracana* on lipid profile in hyperlipidemic diabetic rats. Chronic administration of flour of seeds of *Eleusine coracana* for 21 days caused significant reduction ($p<0.01$, $p<0.001$) in thelevel of cholesterol and triglyceride in Hyperlipidemic diabetic rats suggesting flour of seeds of *Eleusine coracana* possess antihyperlipidemic effect. Administration of flour of seeds of *Eleusine coracana* caused significant increase ($p<0.01$) in the level of HDL-cholesterol in hyperlipidemic diabetic animals.

Treatment with the hydroalcoholic extracts of seeds of *Eleusine coracana* at the intermediate and high dose level for 21 days caused significant ($P<0.001$) decrease in the cholesterol and triglyceride level whereas increased the HDL level in hyperlipidemic diabetic rats. Results suggest that treatment of hyperlipidemic diabetic rats with hydroalcoholic extracts of seed of *Eleusine coracana* showed anti hyperlipidemic activity.

Table-4 Effect of whole flour and hydro alcoholic extracts of seed of *Eleusine coracana* on lipid profile in hyperlipidemic diabetic rats

Treatment	HDL(mg/dl)	TG(mg/dl)	Cholesterol (mg/dl)
Normal (1ml/kg normal saline, p.o.)	48.2±2.64	105.7±1.4	68.1±2.51
HFD+STZ (60 mg/kg, ip. in citrate buffer pH 4.5)	18.4±1.06	242.2±7.07	226.3±8.11
HFD+STZ+ flour of seeds of Eleusine coracana(as food palate)	43.3±2.17***	154.7±5.71**	104.4±5.14**
HFD+STZ+ hydroalcoholic extract of seed of *Eleusine coracana* (100mg/kg, p.o.)	35.1±1.57*	195.51±4.22*	168.6±2.66*
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (200 mg/kg, p.o.)	23.2±1.48	180.44±5.61*	203.1±2.5
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (400 mg/kg, p.o.)	44.7±1.05**	138.21±5.55***	119.2±2**

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N=6.Values are expressed as Mean ±SEM. *P< 0.05, **P< 0.01, ***P< 0.001. The results of test groups were compared with the HFD + STZ treated group (positive control).
Index: STZ = Streptozotocin, HFD= high fat diet p.o= per oral, i.p.=intraperitoneally.

Effect of whole flour and hydroalcoholic extract of *Eleusine coracana* on oxidative stress in hyperlipidemic diabetic rats

Table-5 indicates that malondialdehyde level was significantly increased in the hyperlipidemic diabetic rats. Treatment with whole flour and intermediate dose of *Eleusine coracana* showed significant decrease (P<0.01) in the malondialdehyde level in hyperlidemic diabetic rats. Moreover, malondehyde level was more significantly decreased (P < 0.001) in the diabetic rats treated with high dose of *Eleusine coracana*. As indicated in Table -5, the level of superoxide dismutase (SOD) and glutathione (GSH) level was highly decrease in the hyperlipidemic diabetic rats. Treatment with the whole flour and intermediate and high doses of hydroalcoholic extract of *Eleusine coracana* significantly (P<0.001) increased the level of SOD and GSH in hyperlipidemic diabetic rats suggesting antioxidant activity of seeds of *Eleusine coracana*.

Table-5 Effect of whole flour and hydro alcoholic extracts of seed of *Eleusine coracana* on the level of oxidative stress in hyperlipidemic diabetic rats

Treatment	Lipid peroxidation (nmol/l)	Superoxide Dismutase (EU/dl)	Reduced Glutathione (µg of tissue/ml)
Normal (1ml/kg normal saline, p.o.)	44.9 ± 1.7	24.7±1.41	41.4±1.26
HFD+STZ (60 mg/kg, ip. in citrate buffer pH 4.5)	78.8±1.26	11.3±1.11	13.7±1.35
HFD+STZ+ flour of seeds of *Eleusine coracana*(as food palate)	55.7±1.05*	18.3±0.849**	34.2±1.82**
HFD+STZ+ hydroalcoholic extract of seed of *Eleusine coracana* (100mg/kg,p.o.)	57.7±2.07*	16.7±0.561*	20.9±1.56*
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (200 mg/kg, p.o.)	62±1.38	13.1±1.06	16.6±2.55
HFD+STZ+ hydroalcoholic extract of seeds of *Eleusine coracana* (400 mg/kg, p.o.)	50.6±1.77**	19.7±0.671**	27.4±3.21**

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N=6.Values are expressed as Mean ±SEM. *P< 0.05, **P<0.01, ***P< 0.001. The results of test groups were compared with the HFD + STZ treated group (positive control).

Index: STZ = Streptozotocin, HFD= high fat diet p.o= per oral, i.p. =Intraperitoneally.

Discussion

The present study was aimed to evaluate the effect of whole flour and hydroalcoholic extract of seeds of *Eleusine coracana* on metabolic syndrome in hyperlipidemic diabetic rats. Metabolic syndrome is a vital and increasing public-health and clinical challenge worldwide in the wake of urbanization, unsuitable nutrition, surplus energy intake, increasing obesity and sedentary life habits. It is a severe metabolic disorder, characterized with increased in energy intake and decrease in energy output concerning body weight and glucose metabolism. Metabolic syndrome is associated with insulin resistance and type II diabetes mellitus. As metabolic syndrome is associated with alteration of lipid metabolism and increased oxidative stress, so the study was further designed to evaluate the anti hyperlipidemic and antioxidant effect of the isolated compounds on streptozotocin diabetic rats. Phytochemical studies revealed the presence of alkaloids, carbohydrate (non-reducing sugar), proteins and amino acid and tannins in the seed extract of *Eleusine coracana*. The hydroalcoholic extract of seed of *Eleusine coracana* caused significant decrease in the body weight and BMI in hyperlipidemic diabetic rats. However the whole flour of *Eleusine coracana* does not cause any decrease in body weight and BMI in hyperlipidemic diabetic rats. The whole flour and hydroalcoholic extract (intermediate and higher dose) of *Eleusine coracana* significantly reduced fasting serum glucose level and restored lipid profile to normal in hyperlipidemic diabetic rats. The treatment at different dose levels also decreased the level of lipid peroxidation and improved the level of antioxidant enzymes (reduced glutathione, superoxide dismutase and catalase) in hyperlipidemic diabetic rats at different dose level. Presence of various antioxidants vitamins and total phonolic components, may attribute to the antioxidant effect of seeds of *Eleusine coracana* .

Conclusion

From the results presented above it can be concluded that treatment with whole flour and intermediate and higher doses of hydroalcoholic extract of *Eleusine coracana* exert significant antiobesity, hypoglycaemic, hypolipidemic and antioxidant activity. Moreover, the hydroalcohlic extract of seeds of *Eleusine coracana* at high dose showed best responses. Further detailed studies to find out the exact mechanism of these plants and to identify the active phytoconstituents involved in showing positive effect in metabolic syndrome are required.

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Conflict of interest

The authors of the manuscript have no conflict of interest.

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NIL

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Antibacterial Activity of *Rosmarinus officinalis* and *Lavandula angustifolia* Essential Oils Against Selected Poultry Pathogenic Bacteria

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Abstract- Poultry is the host of many species of bacteria and the intestine is the privileged place of their colonization and their persistence. These bacteria are the cause of several cases of food poisoning in humans through the consumption of eggs or soiled chicken meat. In addition, these bacteria develop resistance to antibiotics that are adjusted to poultry feed as growth promoters. Essential oils are considered as important secondary metabolites for plant defense by their antimicrobial and antioxidant properties. These essential oils may be considered as a source of natural antimicrobials for the conservation of poultry food. The aim of our study is to isolate and identify bacterial strains isolated from poultry and to determine the antibacterial and antioxidant activities of *Rosmarinus officinalis* and *Lavandula angustifolia* essential oils on these bacterial strains.Essential oils of Moroccan *Rosmarinus officinalis* and *Lavandula angustifolia* were extracted by hydrodistillation. The identification of their chemical composition are performed by gas chromatography-mass spectrometry. Antimicrobial activity of extracted essential oils against *Staphylococcus* aureus, *Clostridium perfringens*, *Escherichia coli* and *Salmonella entiritidis* was evaluated by aromatogram test and Microdilution in a liquid medium. The identification of strains are performed by several test: Gram staining, Kligler test, Catalase test, test Mobility and Api Gallery 20E. The results show that essential oils tested have a considerable antibacterial activity against all isolated bacterial strains.

Keywords: Poultry feed, Essential oils, Bacterial strains, Antibiotic resistance, Antimicrobial activity.

Introduction

Poultry has been domesticated by humans, it represents a great stake in the food, economic and scientific fields. However, the consumption of eggs or contaminated poultry meat by humans leads to their intoxication[1]. Today, it is clearly established that pathogens isolated from the colonic flora of poultry are linked to gastrointestinal diseases. These are mainly aerobic sporulating Gram-negative or positive bacteria such as *Enterobacteriaceae, Staphylococcus aureus* and *Clostridium perfringens*[2].

In addition, these bacteria are pathogenic by their ability to infect humans and cause serious diseases such as Salmonella which causes salmonellosis[3]. The risk of Salmonella infection is an important factor to consider when preparing poultry.

Furthermore, several strains of Salmonella develop resistance to antibiotics added to poultry feed as growth promoters. This bacterial resistance constitutes a major problem leading researchers to the suppression of antibiotics and to propose new effective strategies of substitution of the latter[4].

Advances in scientific techniques have allowed the identification of components of volatile plant extracts (notably essential oils) that have multiple antimicrobial activities. Essential oils can therefore be a natural substitute for antibiotics[5].

Morocco, through its geographic and climatic diversity benefits from favorable conditions for the development of a rich and varied flora (often endemic) with significant potential in aromatic and medicinal plants (MAP). The exploitation of this rich and diverse floral source has made the country one of the main producing countries of essential oils, the quality of which is in great demand on the international market and more particularly in Europe and the United States of America. The main essential oils produced in Morocco come from the following plants: *Lavandula angustifolia*, *Rosmarinus officinalis*[6].

The medicinal and biological properties of these essential oils obtained from the plants mentioned above are promising[6]. Today, research continues for the antimicrobial activity of these essential oils by testing them on microorganisms that cause several diseases.

However, the widespread use and the benefits described in the literature of these essential oils by the Moroccan population in chicken farming still remain unsupported by scientific studies and hence the interest of our work.

The objective of this work is to test in vitro the antibacterial effect of essential oils obtained from the medicinal plants *Rosmarinus officinalis* and *Lavandula angustifolia*, on various virulent bacteria isolated from poultry.

Material and Methods

Isolation

The isolates comprising of *Staphylococcus aureus, Clostridium perfringens, Escherichia coli,* and *Salmonella entiritidis* were isolated from the stool of broiler chickens picked up from the Casablanca-Settat region (Morocco).

Morphological and biochemical tests of isolates

Bacterial strains were identified to genus level on the basis of colony morphology (appearance, size, margin, shape, elevation), microscopic examination (Gram stain), physiological tests (Kligler test, mannitol mobility, EMB) and biochemical tests (catalase, oxidase,) using API 20E (bioMerieux), adopting standard procedures.

Hydrodistillation by Clevenger

For the extraction of *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils by hydro distillation under optimal operating conditions, a quantity of 100 g of rosemary and lavendulan was added to 1000 ml of distilled water in a 2-litre flask. The set was placed in a balloon heater attached to a refrigerator to ensure condensation of essential oils for 3 hours. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water[7]. The essential oil was collected, dried under magnesium sulfate and stored in sealed vials in the dark at 4^0C, until used [8]. Experiments were conducted twice for each condition.

Chromatographic analysis of essential oils

The separation and identification of the different chemical compounds of the extracted essential oils were performed by gas chromatography coupled with mass spectrometer GCMS-QP2010 Plus.

Evaluation of antibacterial activity

Aromatogram

In this step 100 µl of each bacterial strain in nutrient broth corresponding to 106 UFC/ml were inoculated by flooding in Muller-Hinton agar. Then disks soaked with 10 µl of essential oil were deposed on the agar. After incubation at 37°C for 24 hours, zones of inhibition appeared around these disks[7].

Microdilution test

The determination of the minimum inhibitory concentration (MIC) of the oils is performed according to the protocol described by[9]. The minimum inhibitory concentration (MIC) of each essential oil was determined by the microdilution method on 96-well plates as described by[4].

50µl of Mueller-Hinton broth were added to each microplate well and different concentrations of EO dissolved in 10% DMSO were added to all wells. Then, 50µl of bacterial suspension corresponding to 106 CFU/ml were added to all the wells, then the microplates were incubated for 24h at 37°C. Microbial growth in each well was determined using the Resazurin technique. Resazurin working solution was prepared at a concentration of 0.01% (w/v) in distilled water and sterilized by filtration through a 0.45 mm membrane. 20µl of the Resazurin solution were added to each well, and the microplates were incubated for 2h at 37°C. The growth was indicated by change in colour from purple to pink color.

Antibiogram

Instead of discs soaked with essential oils, antibiotic discs were used. After incubation at 37°C for 24 hours, results were analyzed. The antibiotics used were penicillin P10, Chloramphenicol C30, Gentamycin GM, Tetracycline TE30, Amoxicillin AML, Sulfamides SSS, Ciprofloxacin CIP, Trimethoprim TMP.

Results and Discussion

Chemical composition of *L. angustifolia* and *R. officinalis* essential oils

The chemical composition of the two essential oils; *L.angustifolia* and *R.officinalis*, by gas chromatography mass spectrometry (GC-MS) is presented in Table-1. The results identified 53 components that vary in percentage from one essential oil to another. The essential oil of *L.angustifolia* has major monoterpenic compounds such as 1,8-cineol (61.76%) and α-pinene (17.35%), limonene (4.65%), p-cymene (2.47%) and aromadendrene (2.63%). Previous studies have shown that the chemical composition of the essential oil of *L.angustifolia* cultivated in many regions of the world presents a predominance of monoterpene compounds in most of the cases, but with different percentages[10] which is in agreement with our study. In addition, we observed that α-pinene (24.5%), camphor (22.8%) and 1,8-cineole (22.6%) were the major components of *R.officinalis* essential oil. The same results were obtained from analysis of *R.officinalis* essential oil from the region of Tlemcen - Algeria[11]. However, other studies from Morocco and Tunisia have shown a similar composition but with very high concentrations of α-pinene (37-40%), cineole(58.7-63%) and camphor (41.7-53.8%) and cineole (40.1-55.1%), known as monoterpenes[12,13].

The variations encountered in the chemical composition of essential oils, from quantitative and qualitative point of view, may be due to certain ecological factors, the part of the plant used, the age of the plant and the period of the vegetative cycle or even to genetic factors[14].

Table-1 Percentage compositions of two essential oil.

Compounds	*L.angustifolia*	*R.officinalis*
(E)-α-Ocimene	0.07	0
(z)-α-Ocimene	0.06	0
1,8 -Cineole	61.76	22.6
3-Carene	0	0.19
3-Methylbutanal	0.11	0
3-Octanone	0	0.67
Alloaromadendrene	0.51	0
Aromadendrene	2.63	0
Borneol	0.09	1.77
Bornylacetate	0	1.34
$C_{15}H_{26}O$	0.12	0
Camphene	0.06	7.8
Camphor	0.27	22.8
cis-p-Menth -1(7),8 -dien -2-ol	0.1	0
Epi-Globulol	0.09	0
Geraniol/p -cymen -8-ol	0.18	0
Gerany lacetate	0.19	0
Globulol	0.36	0
Isoamyl isobutyrate	0.07	0
Isoborneol	0	0.06
Isoborny lacetate	0	0.04
Limonene	4.65	4.7
Linalool	0.07	0.47

Linaly lacetate	0	0.02
Myrcene	0.33	1.71
p-Cymene	2.47	2.62
p-Cymenene	0.07	0
Pinocarvone	0.18	0
Sabinene	0	0.01
Terpinen -4-ol	0.48	1.01
Terpinolene	0.09	0.01
trans -Carveol	0.05	0
trans -Pinacarvol	1	0
trans -p-Menth -1(7),8 -dien -2-ol	0.17	0
Tricyclene	0	0.34
Verbenene	0.3	0
α-Copaene	0.03	0
α-Fenchene	0.02	0
α-Gurjuine	0.26	0
α-Humulene	0	0.02
α-Phellandrene	0.24	0.01
α-Pinene	17.35	24.5
α-Terpinene	0.04	0.01
α-Terpineol	1.04	0
α-terpineol	0	1.93
α-Terpinylacetate	1.36	0
α -Caryophyllene	0.07	0

α-Eudesmol	0.05	0
β-Guiene	0.14	0
β-Phellandrene	0.13	0
β-Pinene	0.53	2.96
γ-Terpinene	0.59	0.01
δ-Elemene	0.05	0

Evaluation of antibacterial activity

In disk diffusion assay, the antibacterial activity of essential oils has shown a potential inhibition zones. This last one differs from bacterium to another and from essential oil to another. The results of the experiments on the antibacterial effect of essential oils are presented in (Table-2 and 3). Based on the antibiotic resistance profiles, we determined a range of antibiotics in this study, most isolates were clearly multi drug resistant.

Screening of the antibacterial properties of the two essential oils revealed that these oils had antimicrobial activity against all the strains tested with a slight difference in sensitivity between gram positive and gram negative (Table-3 and 4). In our study the *L.angustifolia* essential oil showed an effect on all bacteria except *Clostridium perfringens*. The diameters of inhibition were between 16 and 30mm. On the other hand, the *R.officinalis* essential oil seem to have less effect on the tested bacterial strains compared to *L.angustifolia* essential oil. *Salmonella entiritidis* seems to be the most sensitive to this essential oil compared to *E. coli,* although they are Gram negative. As reported, this important bioactivity of these essential oils is due to their richness in phenolic derivatives (1,8-cineol, alpha-pinene, camphor)[10,15,16]. It is also reported that the different components of essential oils show different degrees of activity against gram-negative and gram-positive bacteria and this is largely due to the percentage of the phenolic components of essential oils[17]. Other studies revealed no selective antimicrobial activity against gram-positive or gram-negative bacteria [18].

The MIC of essential oils is in agreement with the results obtained in the aromatogram, i.e. the larger the diameter around the disc, the more interesting the MIC. Generally, the MIC values obtained from two essential oil for the strains studied vary between 0.26 and 4.61%. These results correspond to the results obtained by other [11].

Table-2 Resistance of studied strains to antibacterial agents

Strains	**Resistance profile**
Staphylococcus aureus	P,C,SSS,GM,TMP,AML,TE,CIP
Clostridium perfringens	P ,C,SSS,GM,TMP,AML,TE,CIP
Escherichia coli	P, SSS, TMP, AML, TE, CIP
Salmonella enteritidis	AML, P, SSS, TMP

AML : *Amoxicillin 25µg; TE : Tétracycline 30µg ; P : Pénicilline 6 µg ; G : Gentamicine 15 µg ; C : Chloramphénicol 30 µg ; CIP : Ciprofloxacine 5 µg ; SSS : Sulfamide 200 µg ; TMP : Triméthoprime 5µg.*

Table-3 Antibacterial activity (inhibition zone measured in mm) of essential oils against selected strains of bacteria

Strains	*R.officinalis*	*L.angustifolia*	*Mean* ± SD
Clostridium perfringens	-	-	
Escherichia coli	18± 0.05[a]	20 ± 0.0[a]	
Staphylococcus aureus	-	20 ±1.0[c]	
Salmonella entiritidis	30 ± 2.26[a]	33± 0.3[b]	

In the same column with different superscript letters differ significantly ($P < 0.05$).
Diameter of inhibition zone (mm) including disc diameter of 6 mm.

Table-4 Minimum inhibitory concentration (MIC; %) and Minimum bactericidal concentration (MBC; %) of two essential oils against selected strains of bacteria

Microorganisms	***L.angustifolia***		***R.officinalis***	
	MIC	MBC	MIC	MBC
Staphylococcus aureus	0.52	2.08	0.52	-
Clostridium perfringens	4.61	8.33	-	-
Escherichia coli	0.52	4.61	8.33	-
Salmonella entiritidis	1.04	4.61	0.52	8.33

- : not detected.

The ratio MBC/MIC allows to define the bacteriostatic or bactericidal character of an essential oil. When this ratio is less than 4, the essential oil is considered bactericidal [19]. In our study, the MBC/MIC ratios of the different essential oils tested are between 1 and 4 for all the bacterial strains studied. Thus, the essential oil (*L.angustifolia*) seems to have on the one hand a bactericidal activity on *Salmonella entiritidis, Escherichia coli* and *Staphy lococcus aureus* and on the other hand a bacteriostatic activity on *Clostridium perfringens*. It is reported that the antibacterial activity of essential oil is due to phenolic components. However, the compounds present in the highest proportions are not necessarily responsible for the total activity; the involvement of less abundant constituents should also be considered [21] and therefore the activity comes back due to the presence of components such as p-cymene, 1,8-Cineole, linalool, borneol[22,23,24,25]. In addition, the particular structure of the external membrane of this genus, particularly impermeable to hydrophobic molecules, combined with the mechanisms of proton efflux reducing the pH gradient across the cytoplasmic membrane, ultimately leading to cell death [20].

Conclusion

Essential oils are aromatic substances with a complex chemical composition which give them very interesting antibacterial properties. In the present study, we have shown that the essential oils of *Rosmarinus officinalis* and *Lavandula angustifolia* have an effective antibacterial activity against *Staphylococcus aureus, Clostridium perfringens, Escherichia coli* and *Salmonella entiritidis* isolated from poultry in Morocco. This effectiveness is due to high presence of monoterpenoids, since the analysis by gas chromatography coupled with mass spectrometer showed the presence of 1,8-Cineole (61,76) and α-Pinene (17,35), as major components of *Lavandula* essential oil, and 1,8-Cineole (22.6), Camphor (22.8) and α-Pinene (24,5) in essential oil of *Rosmarinus*. These components are responsible for attacking the bacterial wall which mainly destabilizes the cell architecture leading to the degradation of the integrity of the membrane and increased permeability, this disrupts many cellular activities. The studies described here establish the utility of the tested essential oils to open new horizons towards their use as new alternative strategies to the antibiotics used in poultry food. Further exploration are needed to understand the mode of action of these essential oils to inhibit or kill bacteria.

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In Vitro Evaluation of Sun Protection Factor of Plant Extracts By Ultraviolet Spectroscopy Method

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Abstract-Acute and chronic exposure to non-physiological doses of ultraviolet radiation leads to variety of changes of skin ranging from sun-burn, erythema to skin cancer. For skin protection from deleterious effects of sunlight, sunscreen products are used in various forms having chemical and physical filters. However, synthetic sunscreen formulations are reported to cause adverse effects. Thus the present study was planned to evaluate the sun protection factor (SPF) of leaves extract of *Murraya koenigii* for their application as herbal sunscreens. The results suggested that the occurrence of active compounds responsible for ultraviolet absorption can be extracted for use in sunscreens preparations as better, cheaper and safe alternative to harmful chemical sunscreens.

Keywords: UV radiations, SPF, *Murraya koenigii*

Introduction

The harmful effects of solar radiations are caused predominantly by the ultraviolet (UV) region of the electromagnetic spectrum, which consists of UV-A (320400 nm), UV-B (290320 nm), UV-C (100290 nm), and vacuo UV (10100 nm). It has been reported that adverse effects by UV-B radiation on the human skin include erythema (or sunburn), accelerated skin aging, cutaneous degeneration, photosensensitivity, phototoxicity, ectinic elastisis and induction of skin cancer (Maske et al. 2013; Mbanga et al. 2014). UV-A radiations produce immediate tanning effect and darkening of melanin in the epidermis. It also causes premature photoaging, suppression of immunological functions and necrosis of endothelial cells. UV-A radiations reaches the deeper layers of the epidermis, dermis, and generates free radicals. The main destroying factors of UV radiations for skin are oxygenated molecules which are often called free radicals such as; superoxide anions (O^{-}_{2}), hydroxyl radical (OH), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. It is well documented that ultraviolet (UV) light indices immune suppression and oxidative stress, which play an important role in the induction of skin cancers (Mishra, *et al*. 2012).

Sunscreens are chemicals that provide protection against the adverse effects of solar and, in particular, UV radiation. There are several agents available from both synthetic and natural sources with UV-filtering properties. Due to their capability to impact considerable human local and systemic exposure, the UV filters should be risk-free (Nohynek *et al*., 2010). Synthetic UV filters have been found to impose toxic effects on humans. In contrast, herbal botanical sunscreens are safe, widely accepted by consumers and also work in various ways, playing multiple roles in ameliorating the process of carcinogenesis. Phytoconstituents extracted from plants have been recently considered as potential sunscreen resources because of their UV ray absorption capacity in the UV regions and their antioxidant property. Effective botanical antioxidant compounds are widely used in traditional medicine including tocopherols, flavonoids, phenolic acids, nitrogen containing compounds (indoles, alkaloids, amines, and amino acids), and monoterpenes. Green tea polyphenols, *Aloe barbadensis* extract, and aromatic compounds isolated from lichens are examples of natural substances evaluated for their sunscreen properties (Bonina *et al*. 1996). Antioxidants from natural sources may provide new possibilities for the treatment and prevention of UV-mediated diseases. The effectiveness of a sunscreen is usually expressed by sun protection factor (SPF) which is the ratio of UV

energy required to produce a minimal erythemal dose (MED) in protected skin to unprotected skin (Sutar and Chaudhari, 2020).There is an extreme potential in various herbals and thus can be explored for the sun protective characteristics along with being highly rich source of phytoconstituents and antioxidants. Thus considering above factors, the current study was planned to evaluate the sun protection capability of *Murraya koenigii.*

Material and Methods

Sample Preparation

Murraya koenigii leaves were washed with distilled water twice and dried. The samples were then ground separately in a mixer grinder. 20 gm from each were taken separately in a beaker. This powder was weighed and loaded to the Soxhlet apparatus and 200 ml of methanol/water was used as solvent for preparing the alcoholic and aqueous extract respectively. The extraction process was carried out overnight and the extract was then filtered .The filtered extract was suitably diluted with methanol/water, dried and kept at 4˚C for measuring SPF and phytochemical analysis.

Phytochemical Analysis of Plant Extract

Flavonoid Test: 5ml of diluted ammonia solution was added to aqueous filtrate of the plant extract followed by the addition of concentrated H_2SO_4. Formation of yellow color indicated the presence of flavonoids.

Saponins Test: One ml of plant extract was diluted with 20 ml distilled water and the tube was shaken. Formation of foam indicates the presence of saponins.

Alkaloids Test: To one ml of plant extract, 3ml of ammonium solution was added and incubated at 37˚C for few minutes. The tubes were then placed in water bath and then mayer's reagent was added. Formation of cream color precipitation showed the presence of alkaloids.

Terpenoids Test: 2ml of chloroform was added to 5ml of plant extract. Conc. H_2SO_4 (3ml) was then carefully added to form a layer. Reddish brown coloration of the interface indicated the presence of terpenoids.

Carbohydrate Test: 1ml of Fehling's A and Fehling's B were heated in a boiling water bath for 5-10 min with the plant extract. Appearance of reddish orange precipitate shows the carbohydrate presence.

Tannins Test: About 2ml of the plant extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution (5%w/v) were added. The formation of a green precipitate was an indication for the presence of tannins.

Phenol Test: Plant extracts were treated with 3-4 drops of $FeCl_3$ solution. Formation of bluish black colour indicates the presence of phenols.

Steroid Test: A red colour produced in the lower chloroform layer on addition of 2 ml plant extract to 2 ml of chloroform and 2 ml conc. H_2S0_4 indicates the presence of steroids.

Determination of SPF

100 mg of aqueous and methanolic extract were dissolved in 100ml of distilled water. From this 2 ml and 4ml of the extract was withdrawn and diluted to 10 ml with distilled water so as to prepare extract with the final concentration of 200µg/ml and 400µg/ml. Thereafter, the absorbance of these extracts was taken by spectrophotometer from wavelength ranging from 290 to 320 at 5nm. SPF for aqueous and methanolic extract was calculated by the formula given by Mansur Methametical equation below and by utilizing values given by Sutar and Chaudhari, 2020. SPF was calculated three times and then mean value was taken in consideration.

In vitro SPF is calculated by following equation:

$$SPF = CF \times \sum EE \times I \times Abs$$

Where;

(I) - the solar irradiance spectrum,

EE (I) - the erythemal action spectrum,

Abs- absorbance of sunscreen product,

CF-correction factor (=10)

The value of EE x I are constant and predetermined as shown in Table -1

Table -1 Values of EE×I used in the calculation of SPF

Wavelength (nm)	EE*I (Normalised)
290nm	0.0150
295nm	0.0817
300 nm	0.2874
305nm	0.3278
310nm	1.864
315nm	0.0839
320nm	0.0180
TOTAL	1

Results and Discussion

Phytochemical Analysis of Plant Extracts

Phytochemical examination revealed the presence of constituents such as carbohydrates, alkaloids, glycosides, saponins, tannins, flavinoids, phenol and terpenoids (Table -2).

Table -2 Phytochemical Analysis

Constituents	*Murraya koenigii*
Carbohydrates	+
Saponins	++
Alkaloids	+
Terpenoids	-
Flavonoids	++
Phenols	-
Tannin	-
Steroids	++

In a similar study conducted by Sivanantham and Thangaraj (2015) on phytochemical composition of carrot (Daucus carota , the preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, carbohydrate, phenol, flavonoids, coumarin and chlorogenic acid in carrot. This study also confirmed that terpenoid is an active component in carrot. Rashmi et al. (2020) have reported the presence of phytochemicals i.e. alkaloids, carbohydrates, cardiac glycosides, phenol, phylobatannins, tannins, terpinoids, in ethanolic extracts and alkaloids, carbohydrate, cardiac glycosides, phylobatannins, tannins, terpinoids, in methanolic extracts and alkaloids, carbohydrate, phenols, terpinoids, tannins, quinons in aqueous extracts of *Murraya koenigii*.

Determination of SPF

SPF numbers have become a worldwide standard for measuring the effectiveness of photoprotective products. The in vitro SPF determination is one of the useful tools for screening tests during product development, as a supplement base for in vivo studies. Whereas the in vivo test is time consuming and includes various degrees of variability. In order to protect against UV radiations, the formulation should have good SPF number and also the formulation should have wide range of absorbance between 290-400 nm ranges. The SPF numbers of extracts were calculated by applying Mansur mathematical equation in the UV-B region, which is considered to be the region of highest incidence during the day and people are exposed for a longer time (Lefahal *et al.* 2018).

In the present research work aqueous and methanolic extract of Murraya koenigii leaves was subjected for SPF evaluation by UV spectroscopic method. SPF value for sunscreen above 2 is considered as having good sunscreen activity. The calculated values of SPF of aqueous and methanoic extract of *Murraya koenigii* are presented in the Table -3 and 4. SPF value of aqueous extract of Murraya koenigii was 0.221 and 0.466 at concentration of 200µg/ml and 400µg/ml respectively. Methanolic extract of *Murraya koenigii* have SPF value 3.469 and 1.704 at a concentration of 200µg/ml and 400µg/ml respectively.

Table -3 Absorbance and SPF value of methanolic extract of *Murraya koenigii*

S.No	Wave length	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	2.244	0.03366	1.101	0.016515
2	**295**	0.817	2.119	1.731223	1.041	0.850497
3	**300**	0.287	1.983	0.569121	0.976	0.280112
4	**305**	0.327	1.889	0.617703	0.927	0.303129
5	**310**	0.186	1.805	0.33573	0.924	0.171864
6	**315**	0.083	1.803	0.149649	0.832	0.069056
7	**320**	0.018	1.801	0.032418	0.722	0.012996
	Total			**3.469504**		**1.704169**

Table -4 Absorbance and SPF value of aqueous extract of *Murraya koenigii*

S.No	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.123	0.001845	0.321	0.004815
2	**295**	0.817	0.122	0.099674	0.3	0.2451
3	**300**	0.2874	0.143	0.0410982	0.273	0.0784602
4	**305**	0.3278	0.132	0.0432696	0.256	0.033792
5	**310**	0.1864	0.124	0.0231136	0.252	0.0469728
6	**315**	0.0837	0.121	0.0101277	0.233	0.0195021
7	**320**	0.018	0.115	0.00207	0.244	0.004392
	Total			**0.2211981**		**0.4668261**

Methanolic extract of *Murraya koenigii* showed the higher SPF value in comparison to aqueous extract which falls near the range of good sunscreen activity. Thus it can proposed that this plant extract can absorb the ultraviolet radiation since they possesses good sun protection activity against ultraviolet radiations.

Mishra *et al*. (2012) reported the SPF values of Calendula oil in cream formulation to be in the range of 14.84 ± 0.16. Several authors have conducted similar studies and reported SPF values for *Boerhavia diffusa* (3.5397.174), fresh *Aloe vera* gel (0.0995), the aqueous and methanolic extracts of *Zingiber officinale* (1.441.82 and 1.481.99, respectively) (Ashawat and Saraf, 2006;2008; Suva, 2014).

Conclusion

The SPF values of the leave extracts of *Murraya koenigii* were assessed and UV protection capabilities were reported. Further, it has been suggested that active components responsible for ultraviolet absorption can be isolated from these plant extracts. Along with their many beneficial effects and safety, this botanical could thus become good, cheap and easily available formulation ingredients in sunscreen products.

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Preliminary Phytochemical Screening of Leaves of *Ocimum tenuiflorum*

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Abstract-The medicinal plants are useful for healing and curing of human diseases. Over 55% of all modern clinical drugs are of natural product origin. The plant, *Ocimum tenuiflorum* is highly used by the people of whole world specially Indians to cure various disorders because of the presence of phytochemical constituents. *Ocimum tenuiflorum* plant is known to possess anticancer, antispasmodic, antiviral, insecticide, antiseptic, analgesic, anti-inflammatory, antimicrobial, antistress, Immunomodulatory, hypotensive and antioxidant properties. The present study reveals that various secondary metabolites such as glycosides, alkaloids, flavonoids, phenols, terpenoids, tannins, saponins, antraquenone , fats and oils and steroids are present in different leaf extract of the plant.
Keywords: *Ocimum tenuiflorum* , secondary metabolites, clinical drug

Introduction

Medicinal plants are rich source of different types of medicines and produce various bioactive molecules. (*Nostro et al*, 2000). Herbal plant extracts are very useful and are the major sources of medicine which play vital role in control various type of pathogens (Doss, 2009). Plants are vital for the wellbeing of humankind in a variety of ways. Some plant extracts contain antimicrobial properties that can treat different pathogens. Most of the population of whole world relies on medicinal plants and natural products for their primary health care needs.

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds, steroids flavonoids, tannin etc. (*Krishnaiah et al*, 2007)

Ocimum tenuiflorum (synonym *Ocimum sanctum*), commonly known as holy basil or Shyama Tulsi, is an aromatic perennial plant in the family Lamiaceae. Tulsi has been used for thousands of years for its diverse healing properties and is regarded in Ayurveda as the "*Elixir of life*" that promotes longevity. (*Gupta et al*, 2002) Tulsi also known as "The Queen of herbs", the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. The sacred basil, Tulsi, is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic, Naturopathy and Unani system of holistic health and herbal medicine. It is mentioned by Charaka in the Charaka Samhita; an Ayurvedic text (Warrier, 1995). Scientific studies have shown that Krishana Tulsi possess antiinflammatory, analgesic, antipyretic, antidiabetic, hepatoprotective, hypolipidemic, antistress, and immunomodulatory activities. (*Manjeshwar et al*, 2013).

Material and Methods

Collection of Sample: The plant *Ocimum tenuiflorum* were collected from the local plant nursery (Rama Nursery, Muzaffarnagar) and identified by the Professor S. Kumar (Head, Department of Botany, D.A.V.College, Muzaffarnagar. The leaves were cleaned and washed in sterile distilled water and air dried at

room temperature. The dried leaves were powdered using blender.

Preparation of leaf Extracts: 10 gram of powdered leaves of *Ocimum tenuiflorum* were weighed and mixed with 100 ml of six different solvents (methanol, ethanol, acetone, chloroform, diethyl ether and distilled water) in conical flasks and kept in rotatory shaker at 150 rpm for 24 hours. After 24 hours it was filtered with Whatman No.1 filter paper. The filtrates were evaporated in a hot air oven at 50°C until dry. One gram dried extracts were resuspended in 10 ml of Dimethyl Sulphoxide (DMSO) individually. The extracts were stored in sample bottles at 4°C prior to use. (Jyothiprabha and Venkatachalam, 2016; Borah and Biswas, 2018).

Qualitative Detection of Phytochemical Constituents: Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures:-

Screening of Alkaloids (Mayer's Test)

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Screening of Phenol

Ferric chloride test: About 2ml plant extract was taken to water and warmed at 500C. Then 2 ml of 0.3% FeCl3 was added. Formation of green or blue color indicates the presence of phenols.

Screening of glycosides (Keller Kiliani Test)

5ml of each extract was added with 2ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Screening of Terpenoids (Salkowski Test)

5ml of extract was taken in a test tube and 2ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening of Flavonoids (Alkaline Reagent Test)

NaOH test: 2ml of extracts was treated with few drops of 20% sodium hydroxide solution formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

H_2SO_4 test: A fraction of the extract was treated with Conc.H_2SO_4 and observed for the formation of orange color.

Screening of Saponins

Foam test: 2ml of extract was taken in a test tube and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Screening of Steroids

1ml of extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening of Tannins

Lead acetate test: Few drops of 1% lead acetate was added in to 2ml of extract. A yellowish precipitate indicated the presence of tannins.

Ferric chloride test: 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green-black or a blue-black coloration.

Screening of Anthraquinone

5ml of chloroform and 5 ml of ammonia solution was added to 0.2 gm of plant extract. Appearance of pink, red or violet colour indicated the presence of anthraquinone.

Screening of Oils and Fats

A small quantity of crude extract was pressed between two filter papers separately. An oily appearance on filter paper indicated the presence of fixed oil and fats.

Results and Discussion

The preliminary phytochemicals screening of different extracts showed that the most important types of phytochemicals found in the leaves of *Ocimum tenuiflorum* are glycosides,

alkaloids, anthraquinone, flavonoids, phenols, terpenoids, tannins, saponins, fats and oil and steroids positively detected in all the extract during phytochemical confirmation. Results indicated that the leaves extract prepared in D.W., ethanol and methanol have all the important phytochemical and have great potential to act as a source of drug. Results shown in the Table.

Table- Preliminary Phytochemical Screening of leaves of *Ocimum tenuiflorum* in different extracts

S.NO	Phytochemicals	D.W	Diethylether	Ethanol	Acetone	Methanol	Chloroform
1	Tannins	+	+	+	+	+	+
2	Flavonoids	+	-	+	+	+	+
3	Saponin	+	+	+	-	+	-
4	Glycosides	+	+	+	-	+	+
5	Terpenoids	+	+	+	+	+	+
6	Steroids	+	-	+	+	+	+
7	Phenols	+	+	+	+	+	+
8	Anthraquinone	+	-	+	+	+	-
9	Alkaloids	+	+	+	+	+	+
10	Fats and oil	+	+	-	-	-	+

'+' indicates presence of compound; '-'indicates absence of compound

The herbs, plant extract and their phytoconstituents have been reported for anti-inflammatory, anti diarrheal, antimicrobial, antioxidant and insecticidal activities (Chouhan and Singh, 2011).Alkaloids have important biological property like cytotoxicity and are used in allopathic systems (Trease and Evans, 2005). The glycosides are useful in lowering blood pressure. (Nyarko and Addy, 1990). Terpenoids are used in the treatment of cough, asthma and hay fever. (*Amin et al*, 2013). Saponin have been extensively used as detergents and pesticides, in addition to their industrial applications as foaming and surface active agents and also beneficial health effects (*Shi et al*, 2004). Phenols and tannins acts as antioxidants (*Han et al*, 2005).

Conclusion

The present study concluded that the leaves of medicinal plant, *Ocimum tenuiflorum* are rich source of the secondary metabolites and have the potential to act as a source of useful drugs because of the presence of various phytochemical contents. These contents are useful and improve the health of human being.

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Plant Mediated Synthesis of Zinc Oxide Nanoparticles and Evaluation of Its Anti - microbial and Anti - oxidant Activities

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Abstract-In this research paper we have reported the single pot synthesis of zinc oxide nanoparticles (ZnONPs) for the first time by utilisation of leaves extract of Himalayan medicinal plant *Artemisia roxburghiana*. The principle of green chemistry was utilised at maximum possible level to make the synthesis not only environmentally compatible but also cost effective. The obtained nanoparticles are of good shape and size as confirmed by the instrumental techniques such as Powder XRD, HR-TEM, HR-SEM and FT-IR. The average size of the synthesized nanoparticles was between 26 to 35 nm. These nanoparticles then screened for the anti - microbial assay in which it has shown positive activity against *E. coli*, *A. Tereus* and *C. falcatum*. The second application of the synthesized nanoparticles is estimation of anti - oxidant activity against the DPPH. The IC_{50} value of the nanoparticles is formed to be 53 in EtoH while that of the standard, ascorbic acid was 26 in the same solvent.

Keywords:*Artemisia roxburghiana*, *Asteraceae*, ZnONPs, Antimicrobial activity and Antioxidant activity

Introduction

Material synthesis by green chemistry route has advantage over traditional methods of synthesis as it produces lesser environmental degradation. Now a days, environmental challenges are intrinsic part of every consideration for the developments from all sphere of mankind walk. Further the term "Sustainable development" is coined for it and came into consideration of human activity. In the synthesis of materials, it is equally important to have deep concern about the environmental health. As we deal with chemicals, sometime very hazardous one, the chances of polluting the nature with hazardous chemicals are high. Although, the materials synthesized that rely purely on chemicals have some disadvantage due to not only for their environmental impact but also for their cost at the same time, green synthesis or plant mediated synthesis is free from all these shortcomings. The materials made in this way should be of highly useful objectives.

The plant mediated synthesis of the nanomaterials is gaining interest in the researchers as it is not only environment friendly but also cost effective. There are several regions on planet which are rich in the plant diversity but due to inconvenience, such plants have not been utilised much for the synthesis of the nanomaterials. One such region of the planet earth has unique place on the global map by having the tallest mountain ranges with the ecosystem. The plants of Garhwal region of the great Himalaya have been in use since very long time by local inhabitants. Several diseases have been cured simply by using decoction of the plants in that ecosystems. There is a list of flora that has its root only in the Himalayan region due to its unique geographical condition.

Apart from this, there are several plants that may have cosmopolitan origin but they differ from one another in their phytoconstituents due to the geographical conditions. One such plant is *Artemisia roxburghiana*, which belongs to family *Asteraceae or compositae* and is perennial, pubescent undershrub, fairly common on open dry localities or terraces of crop fields, most abundantly found in submontane to montane zones of Garhwal Himalaya upto the altitude of 1100 ft. The plant *Artemisia roxburghiana* is rich with phytoconstituents, Artimisin as indicated by the isolation work performed on it. The plant *Artemisia roxburghiana* is in use by local inhabitants as antipyretic or tonic and also used to cure skin and cold related body ailments.The unique research work that has been reported in this paper is the development of the nanoparticles that can have applications from medicinal to core physical.

In this paper, we are reporting the utilisation of *Artemisia roxburghiana* leaves extract for the synthesis of the zinc oxide nanoparticles. The zinc oxide being building material is present in abundance and has been highly utilised in the several applications i.e. electric insulators etc. The plant mediated synthesis of the nanomaterials is not only compatible with the coagulation method but also is one of the most important methods for the synthesis of the nanomaterials at micro to tonnage level. The higher aspect ratio[1,2] of the nanoparticles made it very important and interesting material for different applications. Synthesis of nanoparticles by advanced techniques such as laser ablation method[3,4], chemical reduction[5] method and green chemistry routes[6,7]make it a versatile materials that has more than one synthetic methods. Green chemistry provides an opportunity for the better utilisation of the raw materials with minimum or no waste production at all. The green chemistry route is one of the most important method for the synthesis of, not only nanoparticles but also other chemically derived products. The plant or micro-organism mediated green synthesis of metallic nanoparticles has some advantages over traditional chemical methods as it is cost effective due to availability of plant materials, Further the ease associated with the synthesis. The environmental compatibility is another factor because the solvents used in the green synthesis of metallic nanoparticles does not harm environment so badly as traditional solvents such as benzene. Several metallic nanoparticles have been synthesized and well documented. The silver[8], gold[9], palladium[10] copper[11] and various other metallic nanoparticles have been synthesized and screened for different properties for example magnetic[12], electric[13], biological[14] and in sensing[15]. Zinc in free or combined form is actively involved in many natural or manmade processes i.e. in trace amount, it is present in human body and in different concentration it has been utilised to make circuits, batteries, solar cells etc.

There are large number of infectious diseases that must be checked before its outbreak. A large number of scientists are interested in the development of nanomaterials that can be used to counter such type of diseases. The high surface area to volume ratio of nanoparticles are one of the factors that make it hope against many pathogens. ZnO nanoparticles provide a promising antibacterial and antifungal activity against many pathogens.

The present paper deals with synthesis and characterisation of zinc oxide nanoparticles from the leaves extract of *Artemisia roxburghiana*. The synthesized ZnO nanoparticles were characterised by several techniques such as FT-IR, Powder XRD, HR-SEM and HR-TEM. This confirms that the synthesised nanoparticles are in the range of 46 nm to 50 nm. These nanoparticles were screened for biological activities against common pathogens.

Material and Methods

Synthesis of nanoparticles

Chemicals Used: Zinc acetate pentahydrates ($Zn(CH_3COO)_2.5H_2O$) and sodium hydroxide (NaOH) flakes were the chemicals used, purchased from Sigma-Aldrich, India and Millipore water was the medium of synthesis.

Collection of Plant Materials: The selected plant, *Artemisia roxburghiana* is highly abundant in Himalayan region and still

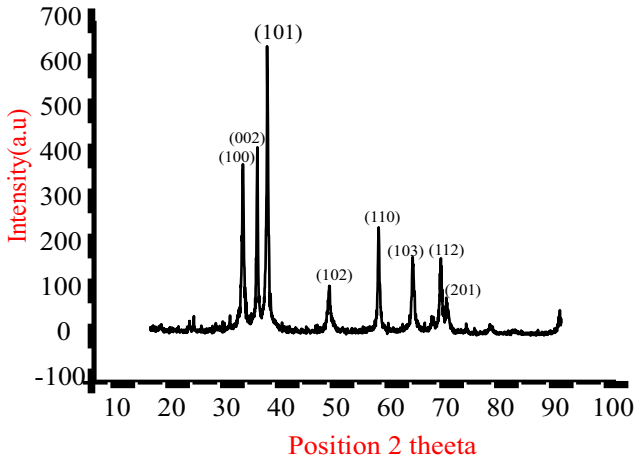
in traditional uses in different region of the Garhwal Himalaya. The plant material used for study were collected from Ghasiaya Mahadev, Srinagar, Garhwal, Uttarakhand, India at the altitude of 1100 meter and identified by Taxonomist, Department of Botany, HNB Garhwal University, where the Voucher Specimen (GUH6391) was deposited .

Experimental The well washed and dried leaves of *Artemisia roxburghiana* dipped in 100 ml double distilled water in the Erlenmeyer flask, the Erlenmeyer flask was put in the heating mantle for 10 min at 60^0C. The colour change from transparent to light brown takes place during extraction, the extract is allowed to cool at room temperature. Then it was filtered off twice a time with Whattman filter paper. The filtrate was stored in refrigerator for further experimentation.

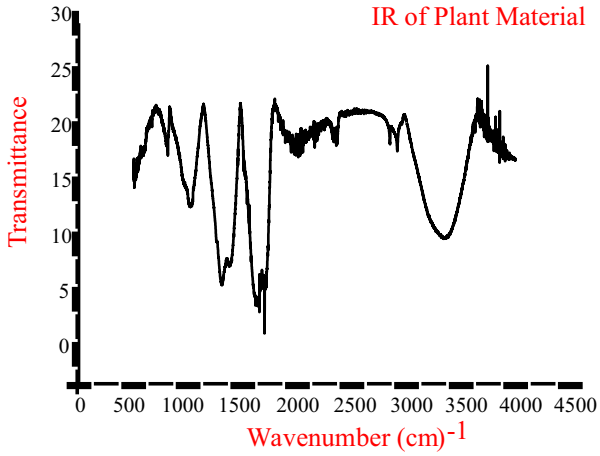
Synthesis of Nanoparticles[16-18] 1 molar solution of NaOH (sodium hydroxide) was prepared by the dissolving it in deionised water (50 ml). 2 mM solution of zinc acetate was also prepared in 500 ml beaker. The beaker containing zinc acetate solution was kept on the magnetic stirrer at the temperature of 120^0C in which plant extract was added drop by drop and the pH of the solution was maintained around 10 and monitored by pH meter. After adding whole plant material the solution was removed from magnetic stirrer and allowed to cool at room temperature. A precipitate of pale yellow colour was formed. The synthesized nanoparticles then centrifuged at 5000 rpm for 20 minutes . After the recovery of sample it was washed twice a time with the deionised water. The synthesized nanoparticles was kept in oven for 48 hours for the complete conversion of $Zn(OH)_2$ (zinc hydroxide) into ZnO (zinc oxide.

Characterisation The synthesized ZnO nanoparticles were characterised by several techniques such as FT-IR, Powder XRD, HR-SEM and HR-TEM

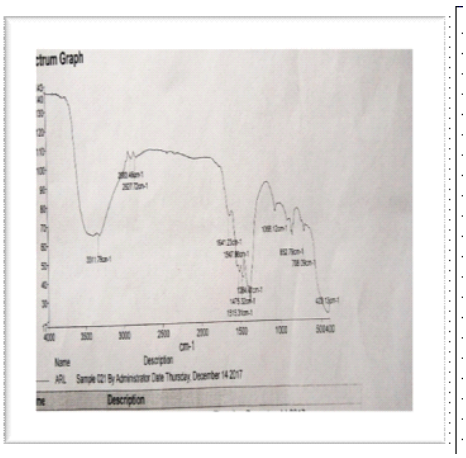
FT-IR The FT-IR of the plant extract and synthesized nanoparticle was carried out by FT-IR spectrophotometer (Agilent Technologies Model: L1600312 spectrum TWOLITA/ZnSe) for determination of functional group present in the plant material and synthesized nanoparticles. It has been found that the synthesized NPs were encapsulated by the compounds which have hydroxyl group at 3500 cm-1, N-H bond peak at 3400 cm-1, C-H bond peak at 2840 cm-1 and a 1717 cm-1 peak for carbonyl bond (C=O). All the mentioned functional groups are also present in the plant extract from which the encapsulating compounds are derived. Apart from those common peaks, there are some additional peaks in the plant extract such as peak for the carbon-carbon triple bond at 2167 cm-1. The change in the absorption pattern from plant material to synthesized zinc oxide nanoparticles indicate that the formation nanomaterials has taken place. So it can be concluded that nearly all functional groups present in the plant have been transferred to synthesized NPs.Powder XRDFor the crystallographic study the powder XRD was done by using PANalytical XPERT-PRO D3663 diffractometer. The average crystallite size of synthesized nanoparticles are of 23.2 nm with hexagonal crystal system. The synthesized nanoparticles have value of coefficient value of a=b=3.2417 and c=5.1876. The diffraction planes of synthesized nanoparticles are indexed as (100), (002), (101),(102),(110),(103)(121) and (201) which indicate the Wurtzite structure of synthesized ZN-NPs. The XRD data has been obtained from the International Diffraction Data Card with reference code 01-079-0205.



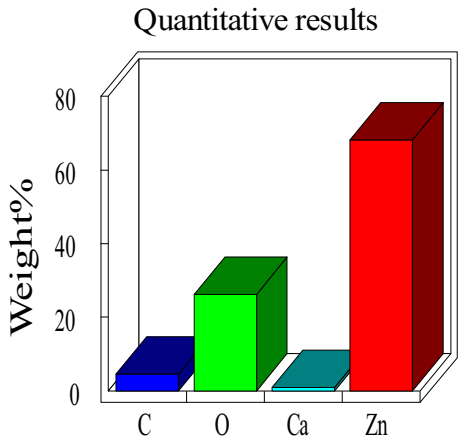
Graph-1 XRD peaks of ZnO-NPs



Graph-2 IR spectra of the plant material



Graph-3 IR spectra of ZnO-NPs



Bar Graph-1 Elemental composition

SEM analysis The scanning electron micrography and EDX data has revelded that the synthesized nanoparticles are spherical in nature with smooth sucface. The EDX (Energy Dispersive X-Ray) data has shown that the synthesized nanoparticles have zinc 68.25% by weight, further calcium is also present by 0.95% as may come from the plant materials, as it has been well established that the calcium pectate functions as adhesive for cell and various other metabolic functions.

TEM analysis The Transmission Electron Microscopy of synthesized nanoparticles was carried out by JEOL JEM 1400 at IIT Delhi. It has been found that the synthesized nanoparticles are of average size of 45 nm with almost spherical in nanture and highly aglomerated. Due agglomeration the size of synthesized nanoparticles comes out to be more than the real value. The size of synthesized nanoparticles was estimated with the help of softwares digimiser and Image.

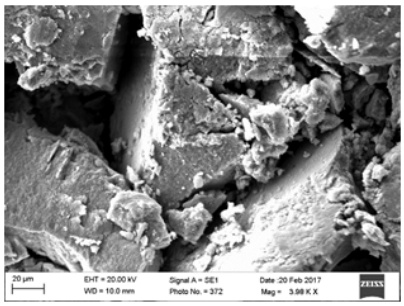
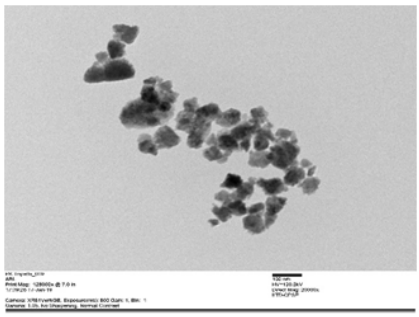
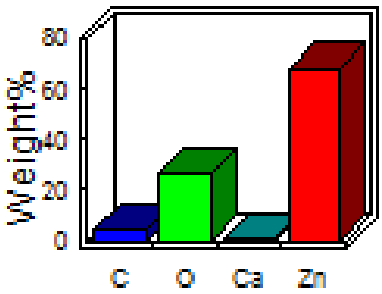


Image-1 SEM images of ZnO-NPs



Images-2 TEM image of ZnO-NPs

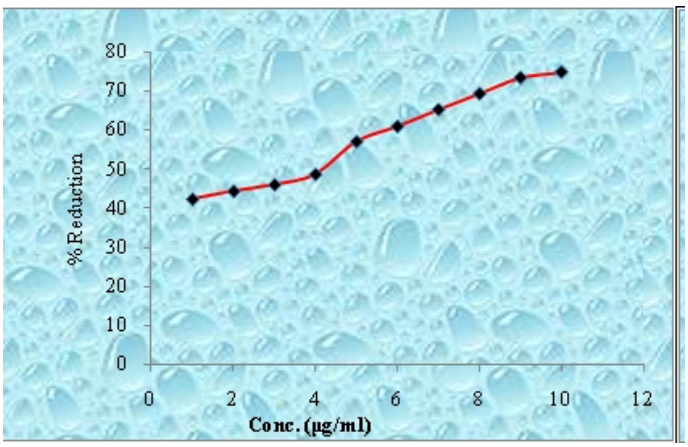


Quantitative results

Biological activity: The antioxidant activity of the synthesized nanomaterials was performed through DPPH methods. DPPH method is widely used to determine the free radical scavanging activity of natural compound or nanomaterials. This method is based on the detrmination of the scavanging ability of antioxidant materials towards stable radicals[19,20].

Table-1 Antioxidant activity

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.282	42.44	53
2.	20	0.272	44.48	
3.	30	0.264	46.12	
4.	40	0.251	48.77	
5.	50	0.210	57.14	
6.	60	0.191	61.02	
7.	70	0.170	65.30	
8.	80	0.150	69.38	
9.	90	0.130	73.46	
10.	100	0.123	74.89	



Graph-4 Antioxidant activity of ZnO-NPs

Antimicrobial activity[21,22] The synthesized nanoparticle were screened against two gram positive (*Staphylococcus aureus and Bacillus cereus*), two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal (*Aspergillus terreus* and *Cyrtomium falcatum*). The methodology of screening was Petriplates containing 20ml Muller Hinton medium (for bacterial species) and 20ml Sabouraud Dextrose agar (for fungal isolates) were seeded with 24hr culture of bacterial and fungal strains respectively. Wells were cut and 20 µl of the sample diluted in distilled water for different concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibiotic activity was assayed by measuring the diameter of the inhibition zone formed around the well. Oxytetracyclin (800ppm) disc was used as a positive control for bacterial strains while Fluconazole (1000ppm) was used as positive control for fungal strain.

Table-2 The antibacterial activity of ZnO-NPs against different pathogens

	Zone of Inhibition (cm)			
Pathogens Name	**Nanoparticles**			**Oxytetracyclin (800) ppm**
	25 mg/ml	**50 mg/ml**	**100 mg/ ml**	**+ve control**
E. coli	Nil	1.1	1.2	4.2
S. aureus	Nil	Nil	Nil	3.9
B. cereus	Nil	Nil	Nil	2.7
P. aeruginosa	Nil	Nil	Nil	2.6
Aspergillus terreus	0.7	1.0	1.2	1.4
C. falcatum	Nil	Nil	0.8	1.5

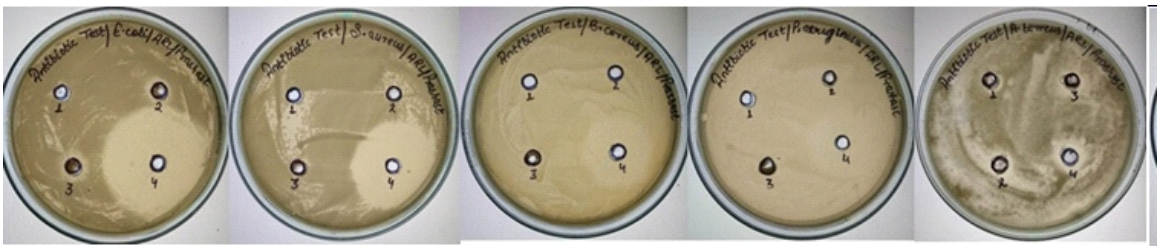


Image (a) Image (b) Image (c) Image (d) Image (e)

The images from (a) to (e) represent the MIC of the synthesized nanoparticles: image (a) E.coli (b) S. aureus (c) B. cereus (d) P. aeruginosa and (e) A. terreus

Conclusion

It has been found that the synthesized ZnO-NPs have activity against gram negative bacteria *E. coli*, fungal pathogens, *Aspergillus terreus* and *Cyrtomium falcatum* at different concentration. Further the antioxidant activity of the synthesized ZnO-NPs were found to have IC_{50} value equal to 53 which shows it has mild activity against radicals.

Acknowledgments

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Design Development and Characterization of Mannosylated Carbon Nanotubes

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Abstract - The proposed study was aimed at developing and exploring the efficiency of novel mannosylated MWCNTs. For selective drug delivery to alveolar macrophages, Paclitaxel was selected for incorporation into mannosylated MWCNTs based on its anticancer activity. Hopefully, this delivery system could be safely administered through i.v. route. We expect that this approach will improve management of drug therapy in cancer patients by delivering the drug at controlled rate for prolonged period of time at a desired site. The drug was found to be white to off-white, crystalline powder that was similar in physical appearance as mentioned in I.P. 1996. Melting point of Paclitaxel was near to reported value. Solubility of the drug in different solvents at ambient temperature depicted its solubility in methanol, ethanol, acetone, dimethylsulphoxide and chloroform, while insoluble in distilled and PBS (pH 7.4). Drug entrapment efficiency of Paclitaxel loaded mannosylated MWCNTs was found to be increased.

Keywords: Paclitaxel, Mannosylated MWCNTs, Anticancer, Solubility

Introduction

Cancer that being in the skin or in tissues that line or cover internal organs, there are number of subtype of carcinoma including adeno-carcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma:Cancer that begins in bone cartilage, fat, muscles, or blood bessels, connective tissues or supportive tissues. Cancer that starts blood forming tissues such as the bone marrow and cause a large number of abnormal blood cells to be produced and entered in the blood. Cancer that begin in the cell of immune system and Cancer that begins in the tissues of brain and spinalcord.

The term lung cancer is used for tumors arising from the respiratory epithelium (bronchi, bronchioles, and alveoli). Mesotheliomas, lymphomas, and stromal tumors (sarcomas) are distinct from epithelial lung cancer. Four major cell types make up 88% of all primary lung neoplasms according to the World Health Organization classification. These are squamous or epidermoid carcinoma, small cell (also called oat cell) carcinoma, adenocarcinoma (including bronchioloalveolar), and large cellcarcinoma. The remainder include undifferentiated carcinomas, carcinoids, bronchial gland tumors (including adenoid cystic carcinomas and mucoepidermoid tumors), and rarer tumor types (Minna *et al*., 2002).

All histologic types of lung cancer are due to smoking. However, lung cancer can also occur in individuals who have never smoked. By far the most common form of lung cancer arising in lifetime nonsmokers, in women, and in young patients (<45 years) is adenocarcinoma. However, in nonsmokers with adenocarcinoma involving the lung, the possibility of other primary sites should be considered. SCLC has scant cytoplasm, small hyperchromatic nuclei with fine chromatin pattern and indistinct nucleoli with diffuse sheets of cells. SCLC comprises about 10%-15% of lung cancers. This type of lung cancer is the most aggressive

strongly related to cigarette smokingwith only 1% of these tumors occurring in non-smokers. SCLCs metastasize rapidly to many sites within the body and are most often discovered after they have spread extensively.

NSCLC is the most common lung cancer, accounting for about 85%-90% of all cases. This has three main types designated by the type of cells found in the tumor. It has abundant cytoplasm, pleomorphic nuclei with coarse chromatin pattern, prominent nucleoli, and glandular or squamous architecture. It doesn't grow and spread as fast as small cell lung cancer, and it's treated differently. There are several types of non-small cell lung cancer.(Chu *et al*.,2006)

Carbon nanotubes (CNTs) have established much recent interest as new entities as a novel drug delivery system for experimental disease diagnosis and treatment because of their unique properties to provide a hollow core appropriate for storing guest molecules(Bianco A; 2004 and Bianco et el. 2005). Important properties of CNTs, making them a famous tool more than other nanocarriers, are greater stability, biocompatibility, nonimmunogenicity, ease of size alteration and high drug-loading potential (Martin & Kohli 2003). Internal and external surfaces of CNTs can be modified on an individual basis as required and a variety of functional groups can be generated on their surface in support of further conjugation with targeting ligands as well as drug molecules. The CNTs can be also degraded within human brain tissue by myeloperoxidase (MPO) and hydrogen peroxide (H_2O_2) (Sajid et al., 2016).

Each type of non-small cell lung cancer has different kinds of cancer cells. The cancer cells of each type grow and spread in different ways. The types of non-small cell lung cancer are named for the kinds of cells found in the cancer and how the cells look under a microscope: Carbon nanomaterials, including carbon nanohorns (CNHs), graphenes (GRs), carbon nanorods (CNRs), polyhydroxy fullerenes (PHF) and CNTs, represent safe and efficacious carrier systems for drug delivery and drug targeting because of their unique physicochemical properties. CNTs were first discovered by Roger Bacon in 1960, and were described fully by Sumio Iijima. CNTs are now the focus of many studies exploring their applications in drug delivery and drug targeting, as well as cosmetic products.

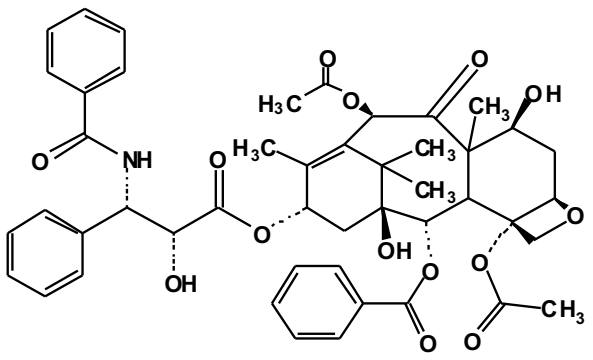
CNTs are ultra-light-weight, tubular, hollow monolithic structures, with a high surface:aspect ratio (length/diameter), rich functional surface chemistry and high drug-loading capacity. They are also biocompatible, nonimmunogenic, and photoluminescent, making them attractive nanocarriers for drug delivery and imaging. CNTs do not require any type of fluorescent labeling for detection because they can be detected directly because of their electron emission properties. CNTs are available as SWCNTs, DWCNTs, TWCNTs and MWCNTs, with cylindrical graphitic layers .

Each type of non-small cell lung cancer has different kinds of cancer cells. The cancer cells of each type grow and spread in different ways.The types of non-small cell lung cancer are named for the kinds of cells found in the cancer and how the cells look under a microscope

Material and Methods

Drug profile

Paclitaxel



Preparation and Characterization:

The present work was aimed to prepare and evaluate mannose conjugated carbon nanotubes for the site-specific delivery of an

anticancer drug i.e. Paclitaxel for effective management of lung cancer.

Purification of pristine MWCNTs

Purification of the MWCNTs was carried out using a modified literature procedure [Liu et al; 1998]. The MWCNTs (300 mg) were added to a mixture of 98% H_2SO_4 and 65% HNO_3 (V:V¼ 3:1, 200 mL) and exposed to sonic irradiation at 0`C for 24 h. The MWCNTs were thoroughly washed with ultrapure water and filtered through a micro-porous filtration membrane (F 0.22 mm). They were redispersed in HNO_3 (2.6 M, 200 mL) and refluxed for 24 h, collected by filtration and washed with ultrapure water to neutrality. The product was then dried under vacuum at 50 `C for 24 h.

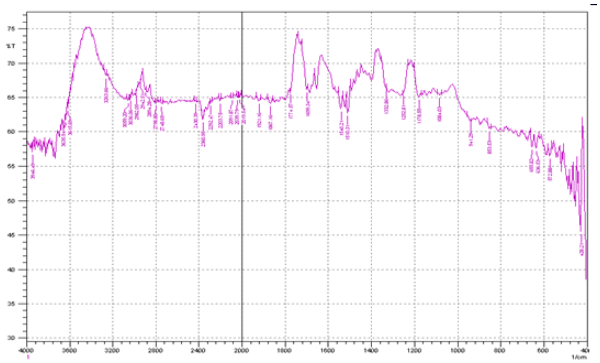


Figure-1 IR of Raw MWCNTs

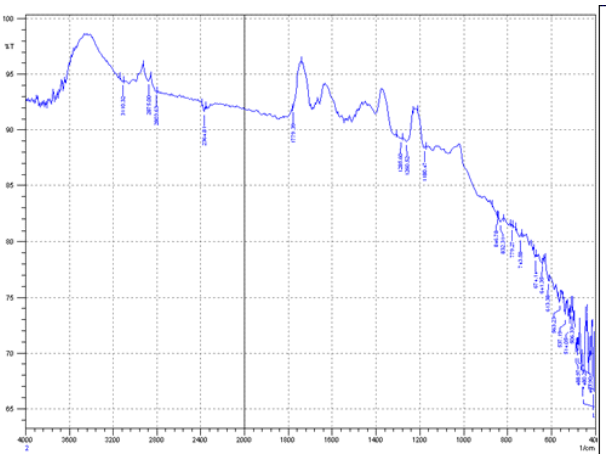


Figure- 2 IR of purified MWCNTs

Preparation of ALG-MWCNTs

MWCNTs (50 mg) were sonicated in sodium ALG solution (100 mg in 0.1 M aqueous NaCl, 100 mL) for 20 min and then stirred at room temperature for 16 h. The modified MWCNTs were collected and washed with ultrapure water by ultracentrifugation to remove unbound ALG, then collected and dried at room temperature to obtain ALG-MWCNTs..

Preparation of CHI/ALG-MWCNTs

The ALG-MWCNTs (40 mg) were sonicated for 20 min and then a CHI solution (6,0 mg in 0.1 M aqueous NaCl and 0.02 M acetic acid, 60 mL) were added. The mixture was stirred for 16 h at room temperature to give the product following ultracentrifugation, washing and drying as described above. (Xiaoke *et al*; 2009)

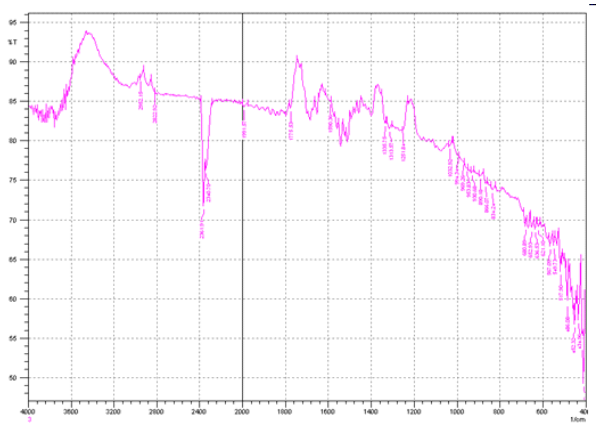


Figure- 3 IR of CHI/ALG-MWCNTs

Preparation of MAN-CHI/ALG-MWCNTs

Mannose (CDH, India) coating was done by ring opening reaction followed by reaction of aldehyde group of mannose with free amine group provided by chitosen on the surface of MWCNTs in sodium acetate buffer (pH 4.0). This led to the formation of Schiff's base (-N=CH-), which may then get reduced to secondary amine (-NHCH-) and remain in equilibrium with Schiff's base. The uncoated mannose and other impurities were removed by dialysis using dialysis membrane (MWCO 1500 Da). The IR spectrum of mannose and mannosylated MWCNTs were taken.

Broad intense O-H stretch and C-O stretch of mannose around 3416.0cm-1and1094.5cm-1respectively and N-H deformation of secondary amine at1412.9 cm-1 confirmed the Schiff base formation and some amine formation via linkage between -CHO of mannose and NH termination of CNTs.

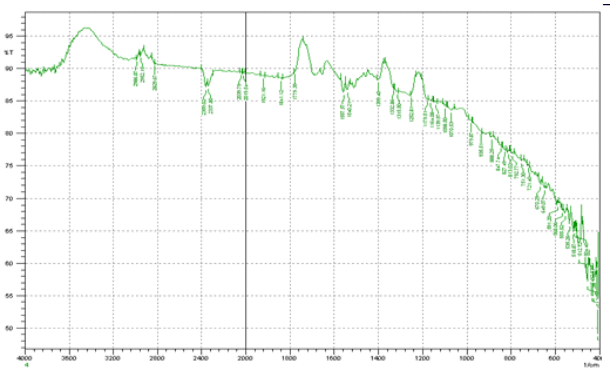


Figure- 4 IR of MAN-CHI/ALG-MWCNTs

D-mannose (8 μM), 28.8 mg in 20 ml was dissolved in sodium acetate buffer (pH 4.0; 0.1 M) and added to Alginate chitosen modified MWCNTs, followed by heating at 60±0.5°C for an h. The mixture was continuously stirred in a magnetic stirrer (Remi, Mumbai, India) at ambient Temperature for 72 h to ensure the completion of reaction. Mannosylated MWCNTs were purified through a dialysis membrane (MWCO 1214 KDa) against triple distilled deionized water for 12 h to remove unreacted mannose along with other impurities, followed by lyophilization (Hetro Drywinner, Germany).

Paclitaxel loading onto the nanotubes

A solution of 25 mg (0.59 m mol) of taxol and 45 mg (7.6 m mol) of succinic anhydride in 50 mL of pyridine was evaporated to dryness in vacuo. The residue was treated with 83 mL of water, stirred for 20 min, and filtered. The precipitate was dissolved in acetone, water was slowly added, and the tine crystals were collected.

PTX conjugation

PTX was modified by succinicanhydride (CDH) according to the literature, written above adding a carboxyl acid group on the molecule. MWCNTs (5mg/mL,) Functionalization were reacted with 30mmol/L of the modified PTX (dissolved in DMSO) in the presence of 5 mmol/L 1-ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC; Otto) and 5mmol/L N-hydroxy sulfo succinimide (Sulfo-NHS, HIMEDIA). The solution was supplemented with PBS at pH 7.4. After 6-h reaction, the resulting MWCNT-PTX was purified to remove unconjugated PTX by filtration through 5-kDa MWCO filters and extensive washing.

Characterization of functionalized MWCNTs

Shape and surface morphology

Transmission electron microscope (TEM) photomicrographs were taken at suitable magnification using electron microscopy (TEM) at AIIMS, New Delhi.

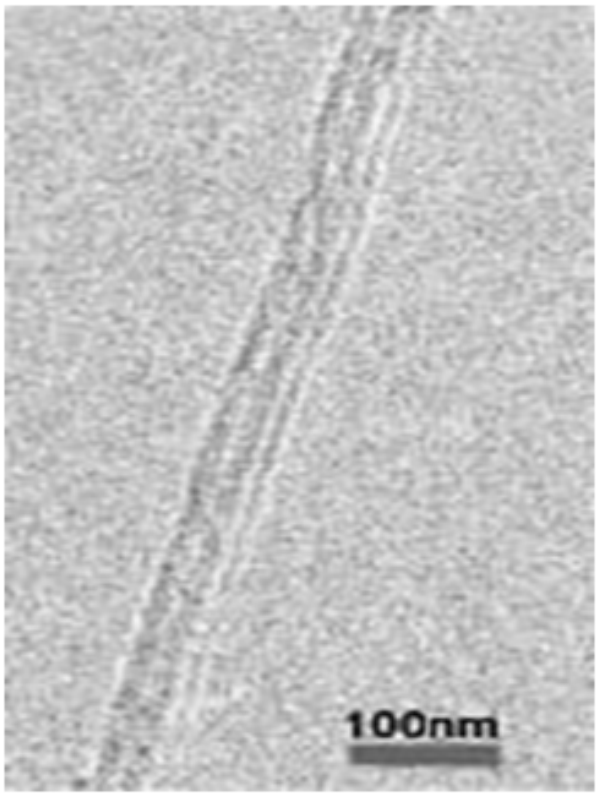


Figure- 5 Purified MWCNTs

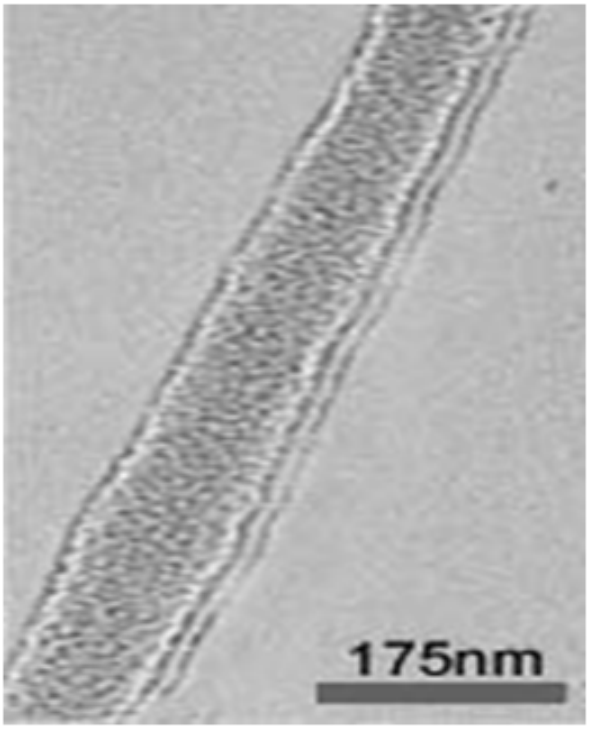


Figure- 6 Alginate Chitosen Coated MWCNTs

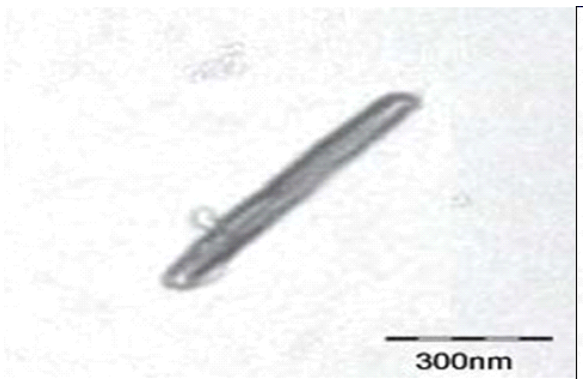


Figure- 7 MAN-CHI/ALG- MWCNTs

The structures of the modified MWCNTs were investigated by TEM. Figure shows the purified nanotubes, after cutting and cleaning, which appear to be smooth and without impurities, indicating that metal particles and amorphous carbon have been completely removed. The cut and purified MWCNTs are generally short(< 500 nm), well separated, and only form small bundles. After coating with ALG and CHI, the polysaccharide chains can be observed on the sidewalls of MWCNTs (Figure-5 and 6). Moreover, the double layered polysaccharide present in CHI/ALG-MWCNTs is thicker than the purified MWCNTs. The side walls of MWCNTs are poorly defined, presumably due to the thick bilayer of bound CHI/ALG polysaccharides.

Surface charge measurement

The zeta potential of the carbon nanotubes was determined by laser Doppler anemometry using a Malvern Zetasizer also called Doppler Electrophoretic Light Scatter Analyzer. The instrument is a laser-based multiple angle particle electrophoresis analyzer. Using Doppler frequency shifts in the dynamic light scattering from particles, the instrument measures the electrophoretic mobility (or zeta potential) distribution together with the hydrodynamic size of particles (size range 10 to 30 μm) in liquid suspensions by photon correlation spectroscopy measurements.

Estimation of drug entrapment

Drug entrapment of the Paclitaxel in mannosylated MWCNTs was determined by dispersing the known molar concentration of Paclitaxel loaded MWCNTs in PBS: METHANOL in ratio of 60:40 This solution was extensively dialyzed with magnetic stirring (50 rpm; Remi, Mumbai, India) in cellulose dialysis bag (MWCO 1000 Da, Sigma, Germany) against PBS : METHANOL under strict sink condition for 10 min to remove any unentrapped drug from the formulation. One mL aliquot was withdrawn and diluted ten times in a volumetric flask with PBS: METHANOL. Absorbance was measured spectrophotmetrically (Schimadzu, 1601 Japan) at 274 nm to indirectly estimate the amount of drug entrapped within the system. The dialyzed formulation was then lyophilized and further characterized.

The amount of drug entrapped in Paclitaxel loaded MWCNTs was also determined by employing the similar procedure as reported for Paclitaxel loaded mannosylated MWCNTs. The percentage drug entrapped is recorded in Table -1.

Table-1 Characterization

	Dispersity	Zeta potential mv	% Entrapment efficiency
PTX/MWCNTs	_ _ _	-16.03 ± 0.14	76.24±0.23
PTX-MAN-CHI/ALG-MWCNTs	++++	-22.32±1.3	81.23±0.17

In vitro drug release study

The in vitro drug release of paclitaxel from mannosylated CNTs formulation was determined using dialysis tube. The definite amount (5ml) of prepared CNTs formulations, free from any unentrapped drug were separately placed into the dialysis tube (MWCO 10 kDa, Hi Media, India), tied at both the ends placed in a beaker containing 50 ml of methanol : phosphate buffer (pH 7.4) 40:60 ratio. The beaker was placed over a magnetic stirrer and the temperature was maintained at 37±1°C throughout the procedure. Samples were withdrawn periodically and after each withdrawal of sample, same volume of PBSwas added to the receptor compartment so as to maintain a constant volume throughout the study. The samples were spectrophotometrically (UV-1601Shimadzu, Japan) analyzed, so as to quantify the Paclitaxel concentration (Table -2 and Figure -8).

Table-2 for % Cumulative drug release

Time (h)	% Cumulative drug release PTX/MWCNTs	% Cumulative drug release PTX/MAN-CHI/ALG-MWCNTs
1	0.90±0.6	1.21±1.5
2	2.41±0.9	3.09±0.7
4	3.98±1.1	4.29±0.8
8	12.39±2.1	13.09±1.7
24	24.83±2.1	28.20±0.8
48	34.8±1.7	37.25±1.11
72	46.05±2.1	50.07±0.7
96	60.65±1.1	69.40±1.9
120	71.3±1.2	81.18±1.8

S.D. ± Mean (n=3)

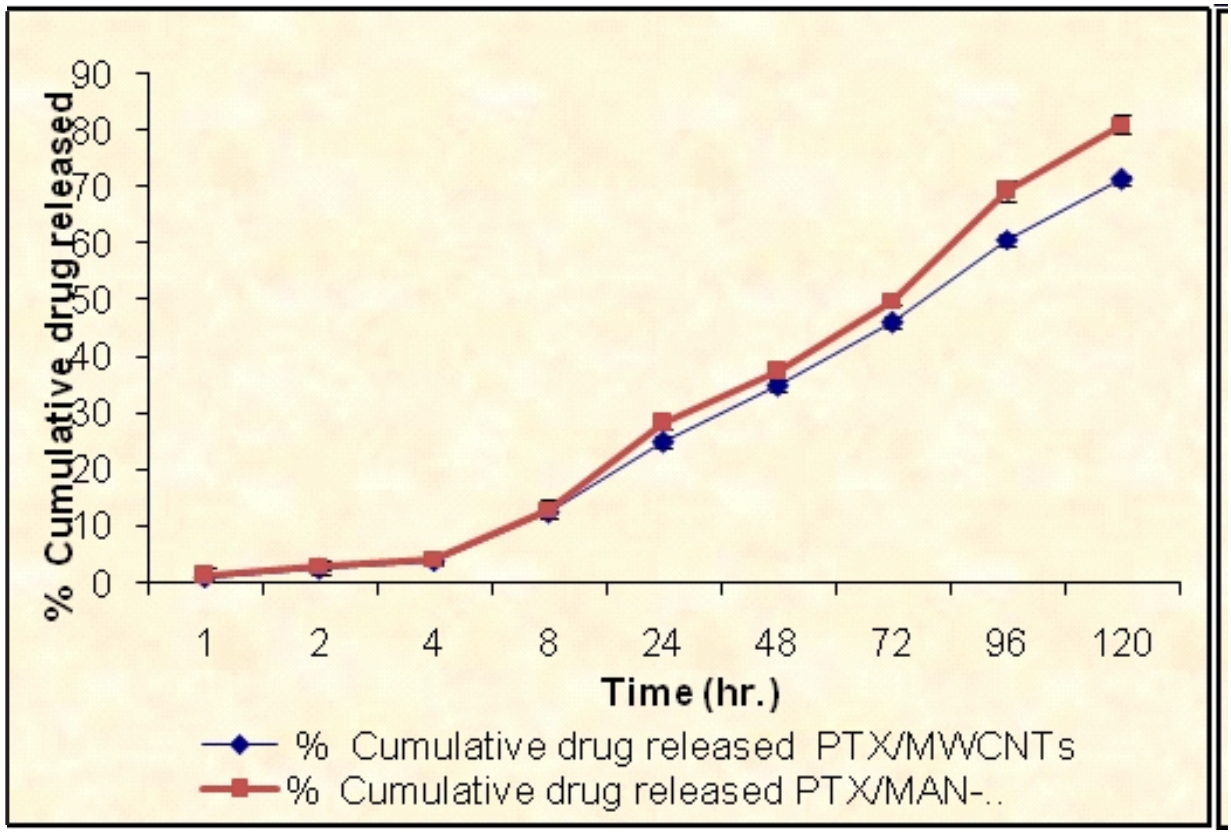


Figure-8 Graph of % Cumulative drug release of PTX/MWCNTs and PTX/MAN-CHI/ALG-MWCNTs

Ex-vivo studies

After preliminary in vitro testing of the formulations for the drug entrapment release rate and stability, the carrier was intended for delivery of drug to animals by parenteral route. However, prior to in vivo studies, the formulations should be evaluated for their efficacy ex-vivo by checking for various interactions. Hence the ex-vivo studies were taken up prior to direct in-vivo efficacy studies in animals.

Cytotoxicity and cell uptake studies

MTT Cytotoxicity assay

Cytotoxicity of paclitaxel loaded MWCNTs and paclitaxel loaded mannosylated carbon nanotubes was determined by measuring the inhibition of cell growth using a tetrazolium dye (MTT) assay. A549 (lung epithelial cancer cell line) was purchased from National Center for Cell Sciences (NCCS), Pune and cultured in RPMI-1640 medium supplemented with 10% Fetal Calf Serum (FCS) and 2 mM glutamine in a humidified incubator at 37±1°C and 5% CO2 atmosphere. A549 cells were seeded at 2X105 cells/mL in 96 well plates. The cells were treated with paclitaxel loaded plain MWCNTs and paclitaxel loaded mannosylated MWCNTs. The formulations were added to each well plate with cell line in a decreasing concentration (10.0 µM to 0.01 µM) simultaneously and incubated for 48 h. Cell viability was analyzed by using the Elisa plate reader at 228 nm. (Yoo et al., 2004)

The drug concentration that reduced the viability of cells was determined by plotting duplicate data points over a concentration range and manually calculating values were obtained using regression analysis.

Table-3 MTTcytotoxicity assay of paclitaxel loaded mannosylated MWCNTs

Paclitaxel Concentration (µ M)	Survival Fraction		
	Free paclitaxel	Paclitaxel loaded plain MWCNTs	Paclitaxel loaded mannosylated MWCNTs
10	45.4	41.3	34.0
20	42.0	39.3	32.9
30	39.8	35.4	30.8
40	36.6	37.5	26.2

S.D.± Mean(n=3)

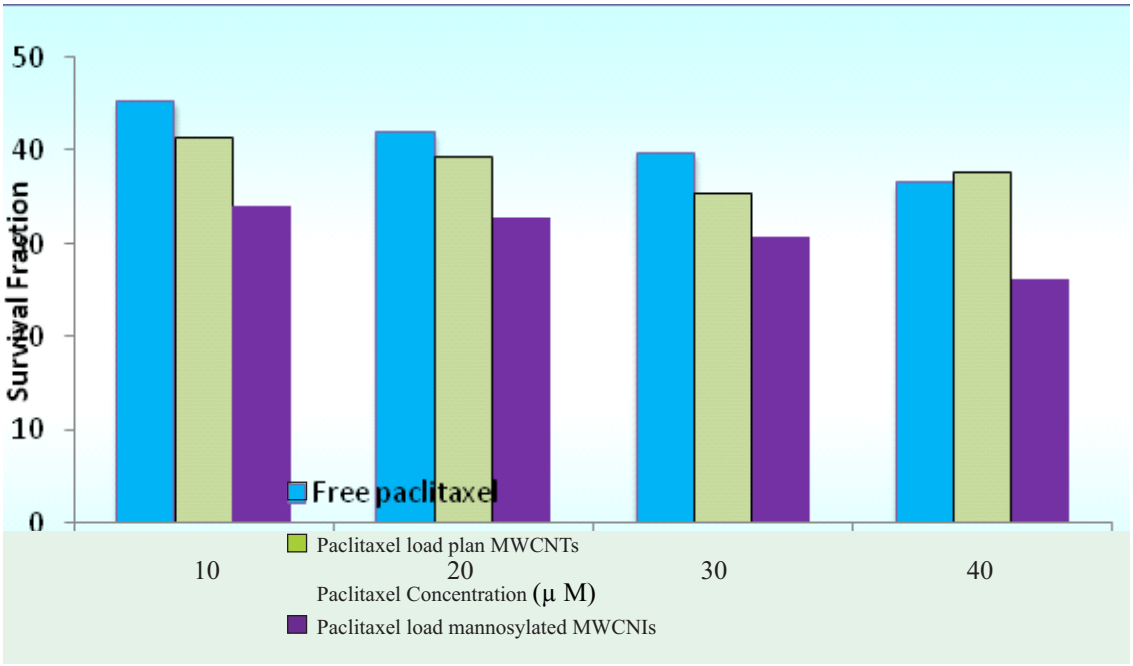


Figure- 9 MTTcytotoxicity assay of PTX loaded mannosylated MWCNTs.

In-vivo studies

The in vivo studies are very important in evaluating the therapeutic efficacy of designed delivery system because in vivo performance is the preliminary step for clinical evaluation of a drug. There are many ways to assess the in vivo performance i.e. measuring the intact drug or metabolite level in the blood or urine, assessing the physiological and biological response in laboratory animals, or by measuring tissue or organ distribution of drug. It is difficult to predict the behavior of a dosage form on the basis of in vitro studies. Hence in vivo studies in animals or human volunteers are very important before the product is introduced into the market. The significant part of this study was to evaluate in vivo performance of optimized formulations with respect to their capability to deliver the drug precisely to the desired site. The animal studies were conducted with the permission of Institutional Animal Ethical Committee. On the basis of in

vitro evaluation of formulations paclitaxel loaded mannosylated CNTs evaluation.

Standard curve of paclitaxel in blood serum

Blood sample was collected through cardiac puncture in a centrifuge tube which contains heparin (anticoagulant) and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected, then added 2ml of 0.4% ortho phosphoric acid and was deprotenized with equal amount of acetonitrile for half an hour to precipitate proteins. The precipitated proteins were separated by centrifugation at 5000rpm for 10min and supernatant was collected and filtered through 0.45µm membrane filter. Mixture of acetonitrile: methanol: phosphate buffer (38:22:40 v/v/v) was used as the mobile phase. Serum with an appropriate volume of a known amount of drug at a concentration range 1000ng 15000ng/ml of serum and filtered. The filtrate (25µl) was injected into a reverse phase C18 150 X 46 mm HPLC column and the eluents were monitored at 228 nm with a flow rate 0.8 ml / min. The peak area of drugs were recorded, the regression of plasma serum concentration of the drug over its peak areas were calculated using the least square method of analysis. The retention time of Paclitaxel was recorded at 7.8 min.

Table-4 Calibration curve of Paclitaxel in blood serum at λ_{max} 228 nm

Concentration (ng/ml)	Peak Area (Observed)	Peak Area (Regressed)	Equation of line:
1000	18380	14216	y = 20.552x - 4721.4 r^2=0.998
2000	31650	34046	
3000	51659	54776	
4000	73576	77506	
5000	101867	98236	
10000	206233	200886	
15000	300952	305536	

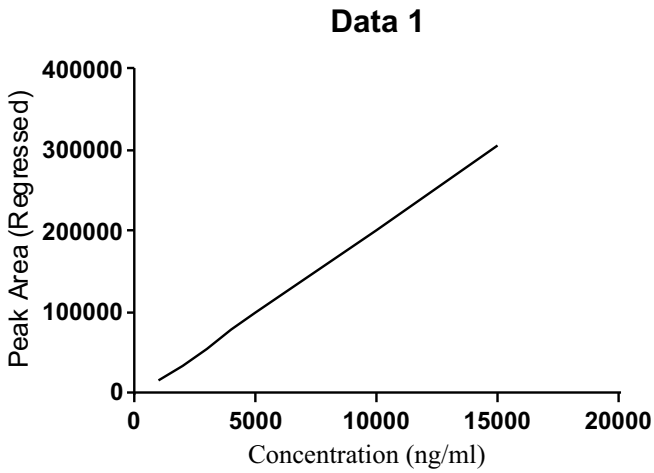


Figure-10 Calibration curve of Paclitaxel in blood serum at λ_{max} 228 nm

Selection of animal

Albino rats of Sprague dawley strain (Hwang et. al., 2008) were chosen for the present study because they offer several advantages as animal model for bio-distribution studies. They are relatively cheap, readily available and easily maintained in laboratory settings. Moreover, their blood volume is large enough to allow frequent blood sampling. This permits Full characterization and determination of the pharmacokinetic profile of the drug.

Biodistribution study in serum and various tissues of rats

The rats were divided into 4 groups, in which first three groups had three animals each and fourth group had only one animal, which served as control. First group was treated with plain paclitaxel solution through i.v. Route. The Paclitaxel loaded plain MWCNTs and Paclitaxel loaded mannosylated MWCNTs dispersion was administered through tail vein in a dose equivalent to Paclitaxel to the animals of second and third group. The animals of fourth group did not receive any drug, which served as control. The blood of animals was collected from retro orbital plexus of the eye in a centrifuge tube containing heparin sodium (anticoagulant) and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected and acetontrile was added to precipitate the proteins. The precipitated proteins were settled by centrifugation at 5000 rpm for 10 minutes and supernatant was collected. One ml of collected supernatant was filtered through 0.45 µM membrane filter and Paclitaxel concentration was determined by HPLC method.

After1, 8 and 24 hours, the rats were sacrificed and the organs i.e. lungs, liver, and spleen were excised and homogenized in dichloromethane: isooctane mixture. The extracts were separated by centrifugation at 1200 rpm for 10 minutes. The organic extract was dissolved in mobile phase i.e.0.05 M Water and acetonitrile (60:40). One ml of this was filtered through 0.45-µm-membrane filter and concentration was determined by HPLC method. The amount of Paclitaxel present in the organs of fourth group animal was determined by simultaneous estimation method using HPLC in Table-5 and Figure-11.

Table-5 Biodistribution of MWCNTs formulations of albino rats.

Time (hrs)	Formulation	Percentage dose recovered		
		Lungs	Liver	Spleen
2	Plain Paclitaxel solution	8.54 ±0.69	15.78±0.96	5.21 ±0.24
	Paclitaxel loaded MWCNTs	19.73±0.94	21.42±0.84	8.31 ±0.32
	Paclitaxel loaded mannosylated MWCNTs	40.53±0.63	21.39±0.95	10.15 ±1.51
8	Plain Paclitaxel solution	5.31±0.94	3.61±0.84	2.85±0.71
	Paclitaxel loaded MWCNTs	10.63±1.32	10.95 ±0.69	2.49±0.39
	Paclitaxel loaded mannosylated MWCNTs	20.17±0.73	24.98±0.87	1.51±0.84
24	Plain Paclitaxel solution	1.07±0.63	2.42±0.95	1.96±0.56
	Paclitaxel loaded MWCNTs	2.18±0.74	3.83±0.26	2.85±0.94
	Paclitaxel loaded mannosylated MWCNTs	4.63±1.32	4.95 ±0.69	10.49±0.39

Mean SD where n= 3

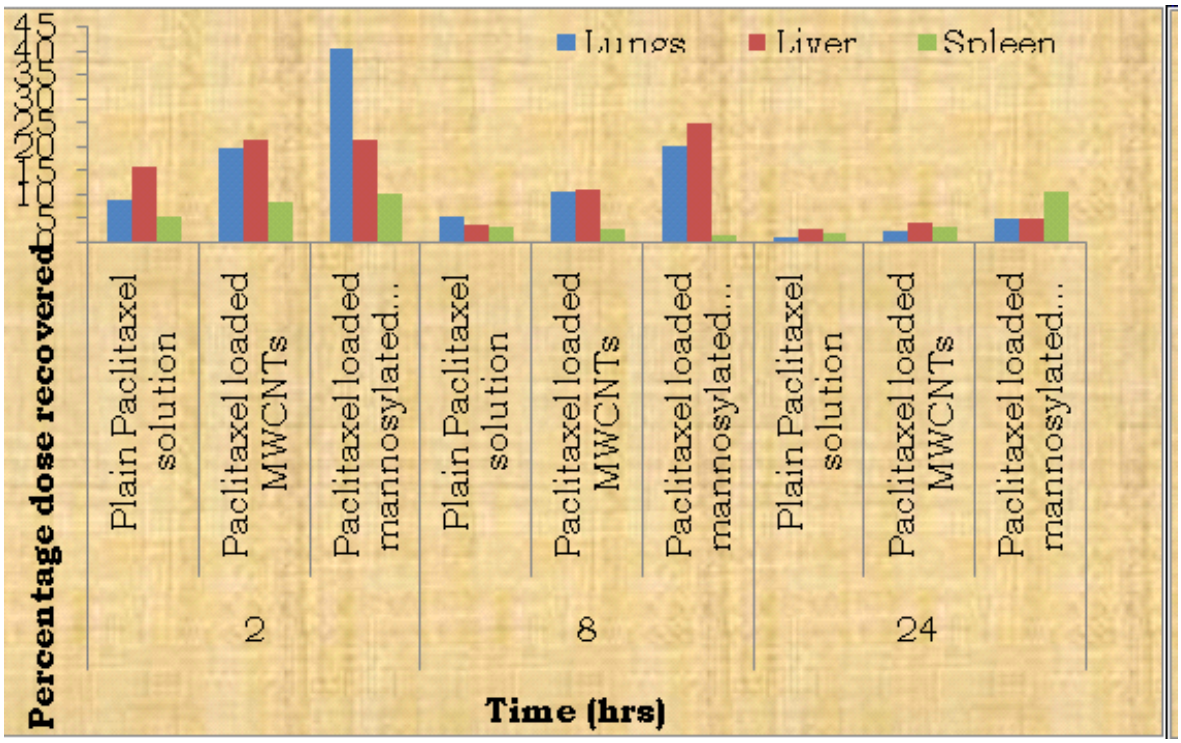


Figure -11 Distribution of paclitaxel in various organs of albino rats after i.v administration of drug

Estimation of paclitaxel in serum

The blood of animals was collected by cardiac puncture in a centrifuge tube containing heparin sodium (anticoagulant) and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected and acetontrile was added to precipitate the proteins. The precipitated proteins were settled by centrifugation at 5000 rpm for 10 minutes and supernatant was collected. One ml of collected supernatant was filtered through 0.45-µm-membrane filter. Same procedure was followed for unconjugated and conjugated CNTs administered rats and Paclitaxel concentration was determined by HPLC method. The observations are shown in Table-6 and Figure-12 .

Table-6 *In vivo* drug plasma concentration time profile of various MWCNTs formulations in albino rats

Time (hrs)	% Dose recovered on administration of		
	Plain PACLITAXEL	PACLITAXEL loaded MWCNTs	PACLITAXEL loaded mannosylated MWCNTs
2	48.17±1.91	28.16±0.82	15.06±0.41
8	2.13±1.19	6.05±0.58	13.38±0.55
24	00	1.30±0.68	7.77±0.61

S.D.±Mean(n=3)

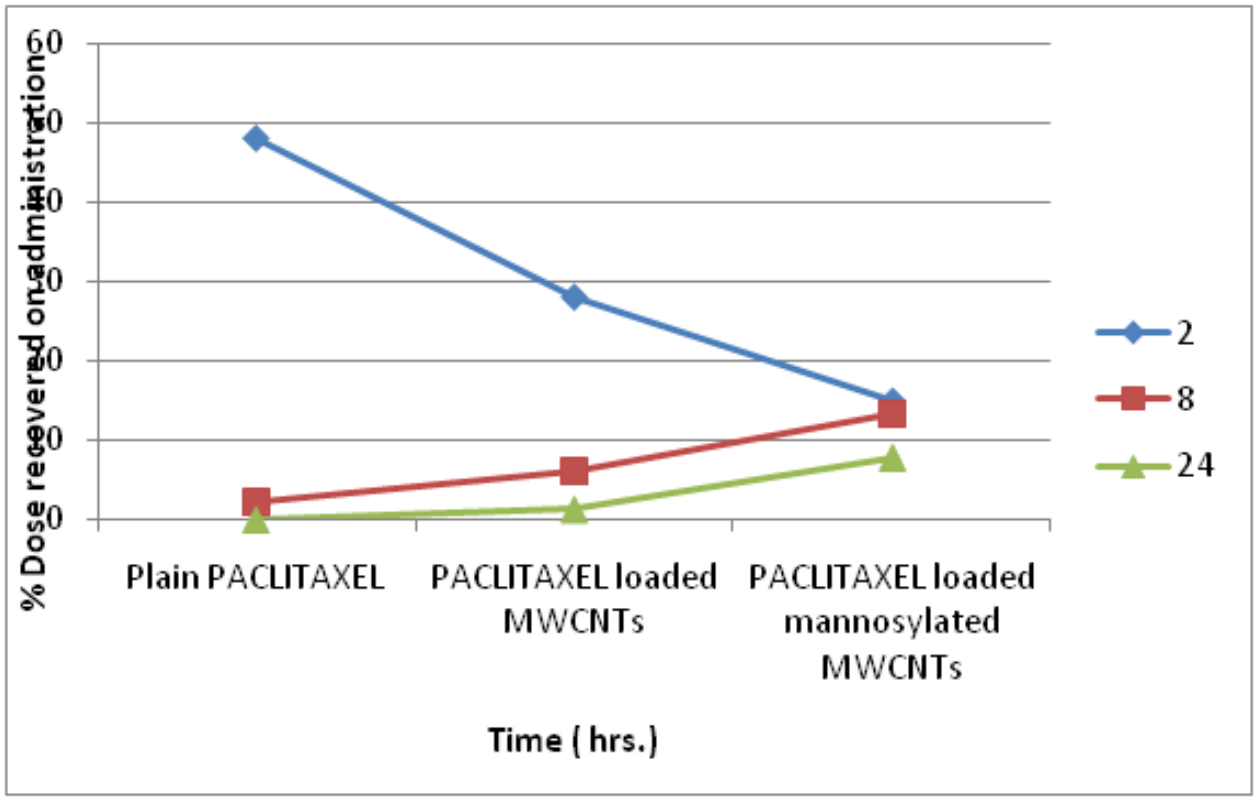


Figure-12 *In-Vivo* drug plasma concentration time profile

Results and Discussion

The purified MWCNTs formulation was optimized to obtain drug entrapment with maximum efficiency. Alginate and chitosen employed in the funtionalization of CNTs was first subjected to optimization. The prepared formulation was further characterized on the basis of Shape morphology of the particles which was studied by TEM analysis and zeta potential studies were carried out by using Laser Light Scattering technique (Malvern Instrument).

Drug entrapment was determined by dialysis using a dialysis membrane and was found to be 81.23±0.17 %. Keeping all optimization parameters under consideration, entrapment was found to be optimum in the selected formulation. IR spectroscopy was performed by KBr pellet method to assess the presence of different functional groups over their surface. The pristine MWCNTs showed a peak at 3387 cm-1that can be ascribed to broad OH stretching owing to bound moisture. Peaks at 2955.04, 2893.32, 2337.80 and1651.12 cm_1suggesting the CNTs backbone and other undefined peaks present in spectrum may be attributed to the presence of amorphous carbon, catalytic and metallic impurities. The IR spectrum of purified MWCNTs exhibits peaks at 2355.4, 1631.9, 1020.6 and 3443.8 cm_1that could be ascribed to the stretching of MWCNTs backbone, C¼Cstretching, OH in plane bending and broad OH stretching, respectively. Mannosylation was carried out by coupling amine group present on the surface of functionalized CNTs. Broad intense O-H stretch and C-O stretch of mannosea round 3410.26 cm-1 and 1058.99 cm-1 respectively and N-H deformation of secondary amine at 1419.66 cm-1 confirmed the schiff's base formation and some amine formation in the linkage between aldehyde of mannose and amine termination groups of MWCNTs.

A comparison was made between drug entrapment, and in-vitro drug release. Particle size of paclitaxel loaded plain CNTs formulation was smaller than paclitaxel loaded mannosylated MWCNTs. This variation may be due to the coating of mannoseon the surface of CNTs.

The drug entrapment efficiency of paclitaxel loaded mannosylated CNTs was more as compared to paclitaxel loaded plain CNTs.

In vitro drug release study of both the formulations was carried out using dialysis tube. Formulation PTX / MAN- CHI/ALG-MWCNTs showed a % cumulative drug release of 81.18±1.8 % while PTX /MWCNTs CNTs released up to 71.3±1.2 % upto 120hours in PBS (pH 7.4). Experiments performed in xeno graft models clearly suggest a dose dependent cytotoxicity response i.e. decrease in cell survival fraction with increasing concentration of drug. It was observed that PTX loaded mannosylated MWCNTs exhibited significantly higher cytotoxicity in comparision to PTX loaded plain MWCNTs. Further PTX solution was found to be least cytotoxic. Mannosylated MWCNTs were most cytotoxic and exhibited greater cellular uptake to the greater extent. This may be attributed to aligand-receptor interaction activity which possibly might have promoted their augmented internalization. PTX loaded plain CNTs were slightly less cytotoxic and exhibited less cellular uptake than mannosylated CNTs. A reason may be lack of ligand receptor interaction and the formulation entered the cell only by passive diffusion via EPR effect.

Experiment performed in xenograft model of A549 tumor bearing cells exhibited a linear relationship between fluorescence and drug concentration with about 60% cellular entry of drug (in the formulation) in the concentration range of 100 ng/ml - 300ng/ml and about 50 % of cellular entry offer the same concentration range of 100 ng/ml-300 ng/ml.

Increased cytotoxicity was observed in case of the mannosylated formulation. It may be due to the fact that the cytotoxicity of CNTs is not dependent on the core, but is most strongly influenced by the nature of CNTs surface. The mannosylated CNTs at a drug concentration of 40μM showed significant cytotoxicity in A549 cell line which may possibly due to attachment of mannose on the surface resulting receptor mediated endocytic uptake.

Bio-distribution studies

In order to determine the fate of paclitaxel loaded mannosylated MWCNTs in vivo, its bio distribution to various organs was investigated. The concentration of paclitaxel in the body depends upon its release, bio distribution, metabolism and excretion from the body. In vivo fates of MWCNTs suggest that uptake of MWCNTs by cancer cells will occur and due to its sustained drug delivery, drug molecule will be available for longer period of time.

In case of free drug maximum dose 48.17±1.91 % was recovered in serum after 2 hour moreover in case of paclitaxel loaded plain MWCNTs and paclitaxel loaded mannosylated CNTs maximum dose recovered in serum was 28.16±0.82 % and15.06±0.41 % after 2 hour, respectively. These results clearly indicated a drastic reduction in serum concentration of free drug in MWCNTs formulation and may be accounted for the fact that the most of the drug present in blood was entrapped in the MWCNTs.

Maximum amount of drug accumulated in various organs following i.v. administration of free drug viz.15.780.96 % in liver (2 hr), 5.21 0.24 % in spleen (2 hr), and 8.54 0.69 % in tumor (2 hr), which were found to be declined constantly.

Encapsulation of drug into MWCNTs was found to be reducing its accumulation in liver, spleen and kidney significantly. Tumor uptake was increased as compared to plain drug solution following the i.v. administration of

paclitaxel loaded MWCNTs. The maximum accumulation in different organs were 21.390.95 % in liver (2hr), 10.15 1.51% in spleen (2hr), and 40.530.63 % in tumor (2 hr). Moreover, the enhance permeability ion and retention effect exerted by MWCNTs promoted their entry to the tumor tissues and reduced access to non tumor tissues.

In case of paclitaxel loaded mannosylated CNTs, uptake of MWCNTs by tumor cells was enhanced due to receptor mediated endocytosis of the CNTs. It was found that after 2 hour, the percent dose recovered from these organs were 21.390.95 % in liver (2hr), 10.15 1.51% in spleen (2 hr), and 40.530.63 % in tumor (2 hr). Besides, the uptake of mannosylated MWCNTs was greater in tumor when compared to plain MWCNTs because of receptor mediated endocytosis. In case of plain CNTs the formulation entered only via passive diffusion.

The above data suggested that although the kidney is a crucial organ for the clearance of bioactives, the mannosylation possibly helped to bypass renal elimination and is finally eliminated after the formulation yielded to metabolism in the liver. The drug levels in liver were increased in case of unconjugated CNTs possibly because of uptake of unconjugated CNTs by mono-nuclear phagocytic system. However the drug level in tumor was also increased in case of uncoated MWCNTs because of indirect action of enhanced residence time of the drug loaded in unconjugated CNTs formulation in the body that promotes distribution of drug to various body organs.

The proposed study was aimed at developing and exploring the efficiency of novel mannosylated MWCNTs. For selective drug delivery to alveolar macrophages paclitaxel was selected for incorporation into mannosylated MWCNTs based on its anticancer activity. Hopefully this delivery system could be safely administered through i.v. route. We expect that this approach will improve management of drug therapy in cancer patients by delivering the drug at controlled rate for prolonged period of time at a desired site.

The drug was found to be white to off-white, crystalline powder that was similar in physical appearance as mentioned in I.P. 1996. Melting point of Paclitaxel was near to reported value. Solubility of the drug in different solvents at room temperature (25°C) depicted its solubility in methanol, ethanol, acetone, dimethylsulphoxide and chloroform, while insoluble in distilled and PBS (pH 7.4).

The drug was identified for any impurities by chemical tests, UV scanning and IR spectroscopy. The absorption maxima of the drug Paclitaxel in methanol was measured in a Shimadzu (1800, Japan) UV/Visible spectrophotometer and was found to be 228 nm. Infrared spectrum of Paclitaxel confirmed the presence of different functional groups which were identical to the spectra of reference drug given in reference. The standard curves of Paclitaxel were prepared in different media and the absorbance data obtained was subjected to linear regression. The equation of line obtained were Y = 0.0298X - 0.0009 and the correlation coefficients were found to be 0.9938 for standard curve of drug in PBS (pH 7.4) which are close to 1.0 indicating good linearity.

Preparation of plain MWCNTs PTX was carried out employing pi pi, interection method which involves the rapid stirring of MWCNTs with paclitaxel solution for 60 minutes . Zeta potential studies were carried out by using Laser Light Scattering technique (Malvern Instrument).Drug loading in the Paclitaxel loaded plain MWCNTs was confirmed by IR spectroscopy as it is showing peaks i.e. C=O stretching at 1073.3cm^{-1} and. Strong N-H bending at 3245.7 cm^{-1} also confirmed the presence of amino group for attaching mannose.

Conclusion

Mannosylation was carried out by coupling amine group present on the surface of CNTs. Broad intense O-H stretch and C-O stretch of mannose around 3509.8 cm^{-1} and 1073.3cm^{-1} respectively and N-H deformation of secondary amine at 3245.7cm^{-1}confirmed the schiff's base formation and some amine formation in the linkage between aldehyde of mannose and amine termination groups of MWCNTs. Paclitaxel loaded mannosylated MWCNTs were further subjected to characterization of various parameters i.e. surface morphology, and surface charge. The drug entrapment efficiency of Paclitaxel loaded mannosylated MWCNTs was determined to be 81.23±0.17%. Drug entrapment efficiency of Paclitaxel loaded mannosylated MWCNTs was found to be increased.

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About Flowers (Shown on the Cover Page)

***Lavendula angustifolia*-Lavendula**

Scientific classification

Kingdom : Plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Eudicots
Clade : Asterids
Order : Lamiales
Family : Lamiaceae
Subfamily : Nepetoideae
Tribe : Ocimeae
Genus : Lavandula
L.

Lavandula is a genus of 47 known species of flowering plants in the mint family, Lamiaceae. It is native to the Old World and is found in Cape Verde and the Canary Islands, and from Europe across to northern and eastern Africa, the Mediterranean, southwest Asia to southeast India.The flower and the oil of lavender are used to make medicine.
Lavender oil is believed to have antiseptic and anti-inflammatory properties, which can help to heal minor burns and bug bites. Research suggests that it may be useful for treating anxiety, insomnia, depression, and restlessness.
Lavender is commonly used for anxiety, stress, and insomnia. It is also used for depression, dementia, pain after surgery, and many other conditions, but there is no good scientific evidence to support many of these uses.
Lavender is used for restlessness and nervousness. It is also used for a variety of digestive complaints including meteorism (abdominal swelling from gas in the intestinal or peritoneal cavity), loss of appetite, vomiting, nausea, intestinal gas (flatulence), and upset stomach.
Some people use lavender for painful conditions including migraine headaches, toothaches, sprains, nerve pain, sores, and joint pain. It is also used for acne and cancer, and to promote menstruation.
In foods and beverages, lavender is used as a flavor component.

In manufacturing, lavender is used in pharmaceutical products and as a fragrance ingredient in soaps, cosmetics, perfumes, potpourri, and decorations.
Lavender is applied to the skin for hair loss (alopecia areata) and pain, and to repel mosquitoes and other insects.
Some people add lavender to bathwater to treat circulation disorders and improve mental well-being. By inhalation, lavender is used as aromatherapy for insomnia, pain, and agitation related to dementia.
In foods and beverages, lavender is used as a flavour component.

***Bombaxceiba*-Semal**

Scientific classification

Kingdom	: Plantae
Clade	: Tracheophytes
Clade	: Angiosperms
Clade	: Eudicots
Clade	: Rosids
Order	: Malvales
Family	:Malvaceae
Genus	:Bombax
Species	:B. Ceiba
	Binomial name
	Bombaxceiba L.

Bombaxceiba belonging to family Bombacaceae also known as silk cotton tree and commonly called semal. Bombaxceiba found in Northern Australia, India, Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java and Sumatra Bombaxceiba has many significant medicinal values. The tree is a powerful, fast-growing lightdemander. It thrives especially in valleys, on sandy loams that are deep and in regions with annual rainfall of 50 to 460cm .Bombaxceiba found in India is a tall deciduous tree with characteristic woody thorns on the bark of the tree . This tree produces huge, crimson, ornithophilous flowers. The flowers have a firm perianth with rigid filaments and well protected ovaries. Nearly all parts of Bombaxceiba, are known to have different medicinal properties which is proved by ethno botanists in many surveys and in the traditional medicine system such as Ayurvedic. Bark has hard-sharp conicles and grey-brown or silver-grey colored. The leaves are broad, spreading, glabrous, lanceolate with having 3-7 leaflets. The seeds of plant are shiny, black or brown, embodied in wool viz long and white, irregularly shaped obovoid, oily and shiny with thick, silky hair. Gum of tree is light brown to translucent locally known as “KATIRA. In Bombaxceiba presence of xanthones, flavonoids, quinines, triterpenes, sterols, hydrocarbons, fatty acids and their esters is distinguished phytochemically and for its various pharmacological activities such as, cytotoxicicity, antioxidant, hypotensive, antiangiogenic, anti-inflammatory, anti-diabetic, and anti-microbial .

Decoction of the bark is given orally to combat fever; diabetics should take decoction of the heartwood; bark juice is given to reduce stomachache. Product 'Acne-n-Pimple Cream' is prepared from Bombax along with other plants to treat pimples and skin eruptions.

***Allium sativum*-Garlic**

Scientific classification

Kingdom	:Plantae
Clade	:Tracheophytes
Clade	:Angiosperms
Clade	:Monocots
Order	:Asparagales
Family	:Amaryllidaceae
Subfamily	:Allioideae
Genus	:Allium
Species	:A. sativum

Binomial name
Allium sativum
L.[1]

Allium sativum is a perennial flowering plant growing from a bulb. It has a tall, erect flowering stem that grows up to 1 m (3 ft). The leaf blade is flat, linear, solid, and approximately 1.252.5 cm (0.51.0 in) wide, with an acute apex. The plant may produce pink to purple flowers from July to September in the Northern Hemisphere. The bulb is odoriferous and contains outer layers of thin sheathing leaves surrounding an inner sheath that encloses the clove. Often the bulb contains 10 to 20 cloves that are asymmetric in shape, except for those closest to the center.Garlic is easy to grow and can be grown year-round in mild climates. While sexual propagation of garlic is possible, nearly all of the garlic in cultivation is propagated asexually, by planting individual cloves in the ground. In colder climates, cloves are best planted about six weeks before the soil freezes. The goal is to have the bulbs produce only roots and no shoots above the ground. Harvest is in late spring or early summer.Currently, garlic is widely used for several conditions linked to the blood system and heart, including atherosclerosis (hardening of the arteries), high cholesterol, heart attack, coronary heart disease, and hypertension.Garlic is also used today by some people for the prevention of lung cancer, prostate cancer, breast cancer, stomach cancer, rectal cancer, and colon cancer.Organo-sulfur compounds found in garlic have been identified as effective in destroying the cells in glioblastomasTrusted Source, a type of deadly brain tumor.

Scientists at the Medical University of South Carolina reported in the journal Cancer that three pure organo-sulfur compounds from garlic DAS, DADS, and DATS "demonstrated efficacy in eradicating brain cancer cells, but DATS proved to be the most effective." Diallylsulfide, a compound in garlic, was 100 times more effective than two popular antibiotics in fighting the Campylobacter bacterium, according to a study published in the Journal of Antimicrobial Chemotherapy.

The Campylobacter bacterium is one of the most common causes of intestinal infections.

Hydrogen sulfide gas has been shown to protect the heart from damage.

However, it is a volatile compound and difficult to deliver as therapy.

Because of this, the scientists decided to focus on diallyltrisulfide, a garlic oil component, as a safer way to deliver the benefits of hydrogen sulfide to the heart.In another study, published in the Journal of Agricultural and Food Chemistry, scientists found that garlic oil may help protect diabetes patients from cardiomyopathy. Cardiomyopathy is the leading cause of death among diabetes patients. It is a chronic disease of the myocardium (heart muscle), which is abnormally thickened, enlarged, and/or stiffened.

The team fed diabetic laboratory rats either garlic oil or corn oil. Those fed garlic oil experienced significantly more changes associated with protection against heart damage, compared with the animals that were fed corn oil. Researchers at Ankara University investigated the effects of garlic extract supplementation on the blood lipid (fat) profile of patients with high blood cholesterol. Their study was published in the Journal of Nutritional Biochemistry.

***Tachyspermum ammi*(Ajowain)**

Scientific classification

Kingdom	:Plantae
Clade	:Tracheophytes
Clade	:Angiosperms
Clade	:Eudicots
Clade	:Asterids
Order	:Apiales
Family	:Apiaceae
Genus	:Trachyspermum
Species	:T. ammi

Binomial name
Trachyspermumammi
(L.) Sprague ex Turrill

Ajwain's small, oval-shaped, seed-like fruits are pale brown schizocarps, which resemble the seeds of other plants in the family Apiaceae such as caraway, cumin and fennel. They have a bitter and pungent taste, with a flavor similar to anise and oregano. They smell almost exactly like thyme because they also contain thymol, but they are more aromatic and less subtle in taste, as well as being somewhat bitter and pungent. Even a small number of fruits tends to dominate the flavor of a dish.

There is little high-quality clinical evidence that ajwain has anti-disease properties in humans. Ajwain is sold as a dietary supplement in capsules, liquids, or powders. An extract of bishop's weed is manufactured as a prescription drug called methoxsalen provided as a skin cream or oral capsule to treat psoriasis, repigmentation from vitiligo, or skin disorders of cutaneous T-cell lymphoma. Because methoxsalen has numerous interactions with disease-specific drugs, it is prescribed to people only by experienced physicians.

Ajwain is used in traditional medicine practices, such as Ayurveda, in herbal blends in the belief it can treat various disorders.There is no evidence or regulatory approval that oral use of ajwain in herbal blends is effective or safe.

Ajwain with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Its seeds are used in small quantities for flavouring numerous foods, as preservatives, in medicine and for the manufacture of essential oil in perfumery13 . In Indian system of medicine, ajwain is administered as a stomach disorders, a paste of crushed fruits is applied externally for relieving colic pains; and a hot and dry fomentation of the fruits applied on chest for asthma . T. ammi has been shown to possess Antimicrobial ,Hypolipidaemic, Digestive stimulant , Antihypertensive, Hepatoprotective, Antispasmodic, Bronchodilating , Antilithiasis, diuretic, Abortifacient, galactogogic , Antiplatelet-Aggregatory , Antiinflammatory, Antitussive effects, Antifilarial , Gestroprotective, Nematicidal, Anthelmintic, Detoxification of aflatoxins , Ameliorative effect . Therapeutic uses of T. ammi fruits include; stomachic, carminative and expectorant, antiseptic and amoebiasis, antimicrobial seeds soaked in lemon juice with Prunusamygdalus (Badam) given in amenhorroea; it is also used as Antipyretic, febrifugal and in the treatment of typhoid fever .

Forth Coming Events

17th European Organic Chemistry Congress, London, London, United Kingdom
Website http://https://organicchemistry.chemistryconferences.org/
Date: July 26,27, 2021

International Conference On Bioinformatics, Biomedicine, Biotechnology And Computational Biology, Manaus, Brazil
Website http://eurasiaweb.com/Conference/25184/EUIC3BCB/
Date: Aug 02,03, 2021

World Congress On Industrial Biotechnology (WCIB), Salvador, Brazil
Website http://conferencefora.org/Conference/25743/WCIB/
Date: Aug 10,11, 2021

14th International Conference On Pharmaceutics And Drug Safety, London, United Kingdom
Website http://drugsafety.pharmaceuticalconferences.com
Date: Aug 06,07, 2021

10th Edition Of International Conference On World Pharmaceutical Sciences & Drug Delivery, London, United Kingdom
Website https://worldpharmaconference.euroscicon.com/registration
Date: Aug 26,27, 2021

International Conference On Pharmaceutics And Drug Delivery Systems, Rome, Italy
Website https://www.genvioconferences.com/pharmaceutics
Date: Sept 15,16, 2021

International Conference On Pharmaceutics And Drug Safety, vienna, south east, Austria
Website https://www.longdom.com/drugsafety
Date: Sept 24,25, 2021

International Conference On Cell And Tissue Science, Mississauga, Canada
Website http://conferencefora.org/Conference/26654/ICCTS/
Date: Oct 01,02, 2021

International Conference On Fungal Infections And Treatments, New York, USA, United States of America
Website https://www.longdom.com/fungalinfections
Date: Nov 01,02, 2021

International Conference On Biological And Medical Sciences (ICBMS), Oxford, Oxford, United Kingdom
Website http://arsss.org/Conference/14209/ICBMS/
Date: Dec 18, 2021

1190th International Conference On Chemical And Biochemical Engineering (ICCBE), Oxford, UK
Website http://iserd.co/Conference2021/UK/7/ICCBE/
Date: Dec 19, 20, 2021

1210th International Conference On Medical, Biological And Pharmaceutical Sciences (ICMBPS) , Hamburg, Germany
Website http://iastem.org/Conference2022/Germany/1/ICMBPS/
Date: Jan 03, 04, 2022

1252nd International Conference On Natural Science And Environment, Boston, United States of America
Website http://theiier.org/Conference2022/USA/1/ICNSE/
Date: Jan 16, 17, 2022

1199th International Conference On Science, Health And Medicine, Denver, United States of America
Website http://iser.co/Conference2022/USA/1/ICSHM/
Date: Jan 17, 18, 2022

International Conference On Recent Advances In Medical, Medicine And Health Sciences, Bali, Bali, Indonesia
Website http://wrfer.org/Conference/19296/ICRAMMHS/
Date: Jan 27, 2022

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Council Initiative for promotion of reverse pharmacology in Ayurvedic drug development

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

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

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

Dr. Rajendra Dobhal
Director General

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  - *in-vivo* studies (CPCSEA Approved Animal House Facility)
  - *in-vitro* studies (with Cell Line Based Investigation Facility)
  - Molecular Biology & Microbiology
  - Phytochemistry & Pharmacognosy
  - Team of Professionally Competent Scientific Writers

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