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Non- antibiotic property of Indian Herbs

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Anantamool

Punica granatum
Anar

Sesamum Indicum
Til

Trachyspermum ammi
Ajowain

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Editorial

Uttarakhand has been rapidly transforming itself into an economic powerhouse. Herbs and Herbal industry can play a pivotal role and UJPAH support this initiative. As research work is first developing step towards its success we together will do this great development of scientific research work. Definitely Indian economy will be a contribution in a humble way.

Herbs are nature's gift to all living being on earth and Hydroponics has proved plants can grow without soil. People are enjoying this technique. Anyways this is a scientific evaluation and scientific development of nature's bounties.

Any how one more factor that cannot be ignored when you talk about science today that is "Artificial Intelligence (AI)" right from literature documentation and identification, Physical, chemical, therapeutically evaluation, drug designing, Pharmacodynamics, Pharmacokinetic, Toxicology and Pharmacogenomics etc. Artificial Intelligence (AI) also canhelp youtoday in paper evaluation to check the claims of plagiarism.

I hope the deliberations of this seminar will be very useful for all participants and my sincere thanks to all who have contributed their valuable research for publication and make this issue a success. I offer my best wishes to all those scientist, research scholars, students and teachers who contributed for bringing out this issue and also express my sincere gratitude to all Board members who made this issue a memorable for scientific fraternity of Uttarakhand.

Dr. S. Farooq
Chief Editor

Contents

In- Vitro antioxidant activity of *Primula denticulata* aerial parts extracts

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Abstract- In this study, we identify the anti-oxidant impact of ethanolic and aqueous extract of aerial parts of *Primula denticulata* and to evaluate the anti-oxidant effect of ethanolic and aqueous extract and compare to it with ascorbic acid and BHT by DPPH Radical Scavenging Assay methods because there is no scientific data available on the anti-oxidant activity of plant. The current investigation also showed that *Primula denticulata* aerial portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may have enormous potential for use as a treatment for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence.

Key words: Oxidative Stress, Anti-oxidants, *Primula Denticulata*, DPPH, Ascorbic acid, BHT.

Introduction

The generation of reactive oxygen species (ROS) and the intracellular ability to eliminate ROS are out of balance, which leads to oxidative stress. It causes excessive damage to all biomolecules, including lipids, proteins, DNA, and RNA[1]. This damage can set off the onset of numerous illnesses, including cancer, oxygen toxicity, ageing, atherosclerosis, lipofuscinosis, and liver injury[2,3]. Anti-oxidants are substances that prevent oxidation or oxidative damage caused by free radicals. As a Results, they can potentially neutralize reactive oxygen species or free radicals. The presence of these phytochemicals in plant products has also led to recent investigations revealing them as possible antioxidants against a range of diseases caused by free radicals[4]. The antioxidant activity of plants is caused by these phytochemicals, which interact with other organisms in the environment to prevent the growth of bacteria or fungi. Because these compounds inhibit infections and have little toxicity to host cells, they are thought to provide the foundation for the development of new antimicrobial medications[5].

Primula denticulata, a member of the Primulaceae family, is a rumbustious, easily growing species that is native to the

Himalayan meadows and lightwoods. Another name for it is drumstick primula. The ideal conditions for *Primula denticulata* growth are partially shaded areas with summertime soil that doesn't dry up. Primrose species are utilized as hepatoprotective, antitumor, diuretic, anti-inflammatory, and bactericidal medicines due to its antioxidant activity[6]. Denticin, denticulatin, and flavonoids are among the triterpene glycosides found in Primula denticulata[7]. The study aimed to explore the anti-oxidant impact of ethanolic and aqueous extract and to evaluate the anti-oxidant effect of ethanolic and aqueous extract by DPPH Radical Scavenging Assay methods because there is no scientific data available on the anti-oxidant activity of the plant.

Material and Methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and Butylated Hydroxy Toluene (BHT) were purchased from Shaila enterprise. Analytical grade reagents were utilized in all other cases.

Plant material collection and authentication

Healthy and disease free plants of *Primula denticulata* were collected in August month from the Mussoorie region. The healthy plant species were randomly collected by hand- picking and later identified number is 114999.

Preparation of plant extract

The shade-dried plant/selected parts of plant material was powdered and extracted by (percolation method) soaking in solvent, it also known as cold extraction or in Soxhlet extractor with successive solvents from non-polar to polar. The extract was concentrated to dry under reduced pressure and controlled temperature (40-50ºC). On concentration, yielded respective solvent extracts. The extracts were preserved in a refrigerator and used for phytochemical test to know the class of chemicals present and used for biological screening (Anti-diabetic, wound healing and antioxidant activity). Extract found to be active were further used to isolate the active constituents present in it.

Successive solvent extraction

About 500g of the air-dried powder of the plant's material was extracted successively with petroleum ether (65-85°C), benzene, chloroform, acetone and ethanol (70% v/v) in a Soxhlet apparatus. Each time before extracting with next solvent, marc was air dried below 50°C. Finally, marc was macerated with water (with small quantity of chloroform) for 24 hrs. to obtain an aqueous extract. The aqueous extract was filtered, the solvent was reduced by evaporation and the extracts were weight correctly. Finally, the extractive value (%) was calculated of air dried drug by suitable method.

In-vitro Antioxidant activity

The antioxidant activity of plant extracts was determined by 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) free radical scavenging assay**.** All the assays were carried out in triplicate and average values were considered.

DPPH Radical Scavenging Assay

The free radical scavenging capacity of the ethanolic and aqueous extracts of Tricosanthes tricuspidata roots, primula denticulata arial parts, determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% ethanol. Ethanolic and

aqueous extracts of given plants were mixed with 95% ethanol and water respectively to prepare the stock solution (10 mg/100ml). From this stock solution 1ml., 2ml. & 3ml., of solution were taken in three-three test tubes respectively & by serial dilution with the same solvent, the final volume of each test tube was made up to 10 ml. whose concentration was then 10 µg/ml., 20 µg/ml., 30 µg/ml. respectively for all extracts. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes and after 10 min, the absorbance was taken at 517 nm. using a spectrophotometer (Double beam UV–visible spectrophotometer). Ascorbic acid and Butylated Hydroxy Toluene (BHT) were used as reference standards and dissolved in distilled water to make the stock solution with the same concentration. A control sample was prepared containing the same volume without any extract and reference standards. % scavenging of the DPPH free radical was measured using the following equation.

% DPPH radical-scavenging = (Absorbance of control - Absorbance of test Sample) X 100 (Absorbance Of control)

Results

Table-1 Preliminary phytochemical investigation of *Primula denticulata*

S.No.	Compound	Test	DP		
			CE	**EE**	**WE**
01	Alkaloid	Hager's	-	+	+
		Dragendroff's	-	-	+
		Wagners test	-	+	+
02	Flavonoid	Fecl3	-	-	+
		Zn-HCL	-	+	-
		Alkaline	-	-	-
03	Triterpenoids	Salwkowski	-	-	-
		Libbermann- Burchards	-	-	-
04	Carbohydrate	Molish's test	-	-	+
		Barfoed test	-	+	+
		Anthrone test	-	-	+
05	Protein	Millon's test	-	-	-
06	Saponis	Froth test	-	+	+

Phytochemical screening

Preliminary phytochemical screening of *Primula denticulata* aerial extract showed that the plant is rich in various active ingredients (secondary plant metabolites). The Results of the phytochemical screening

revealed strong to moderate presence of alkaloids flavonoids saponins and carbohydrates (Table-1).

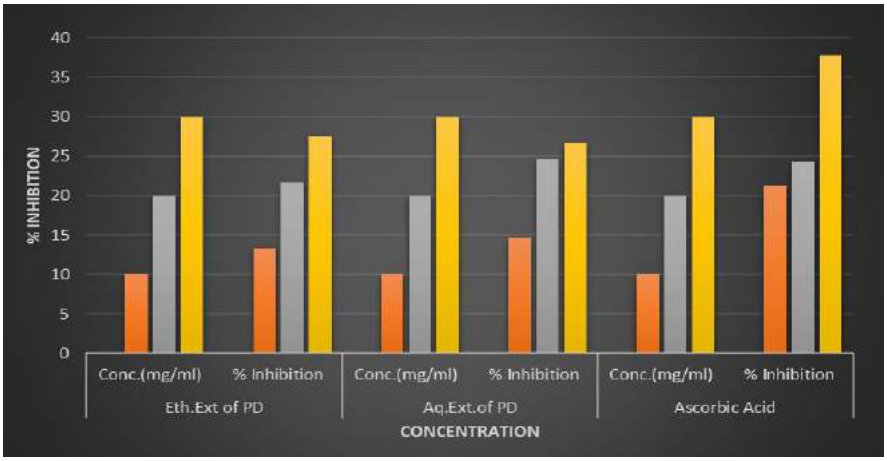
In-Vitro Antioxidant Activity

DPPH radical scavenging activity

DPPH radical scavenging potential of *Primula denticulata* extract at different concentrations investigated in the present study was determined together with standard antioxidant (ascorbic acid Butylated Hydroxy Toluene (BHT) at the same concentrations. *Primula denticulata* extracts (ethanolic and aqueous extracts) showed significant scavenging effect on DPPH free radical in concentration dependent manner. When compared with standard antioxidants used in the experiment, the extract showed relatively lower DPPH free radical scavenging potential.

Table-2 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula denticulata* by DPPH method.

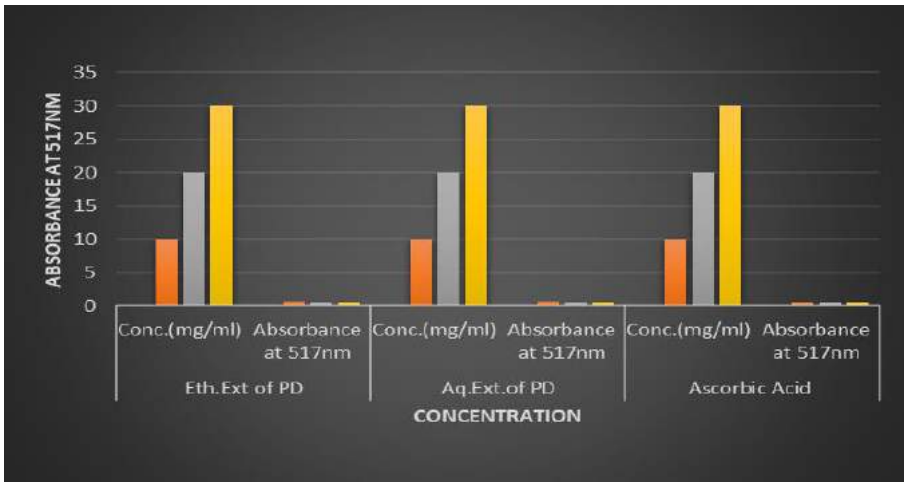
S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		Ascorbic Acid	
	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
01	10	13.34	10	14.69	10	21.28
02	20	21.73	20	24.58	20	24.28
03	30	27.43	30	26.68	30	37.78



Graphical representation showing the % inhibition of DPPH radicals by extracts of *P. denticulata*

Table-3 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula denticulata* by DPPH method.

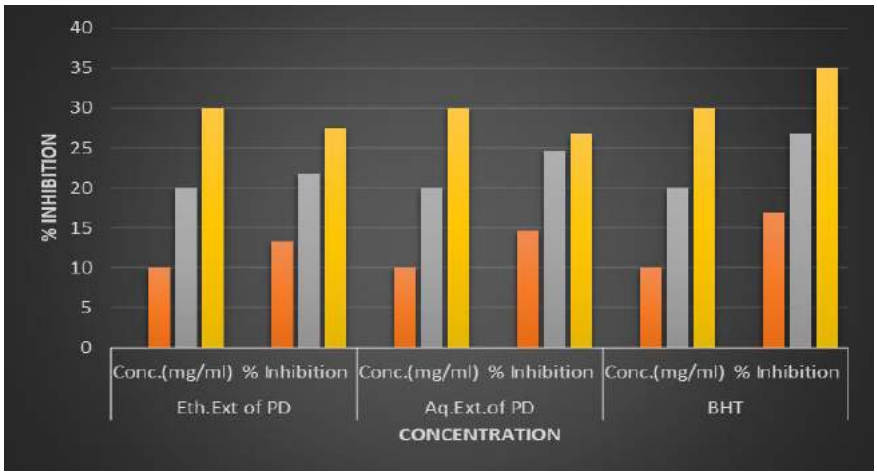
S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		Ascorbic Acid	
	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm
01	10	0.578	10	0.569	10	0.525
02	20	0.522	20	0.503	20	0.505
03	30	0.484	30	0.489	30	0.415



Graphical representation showing the absorbance of DPPH radicals by extracts of *P. denticulata*

Table-4 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula denticulata* by DPPH method.

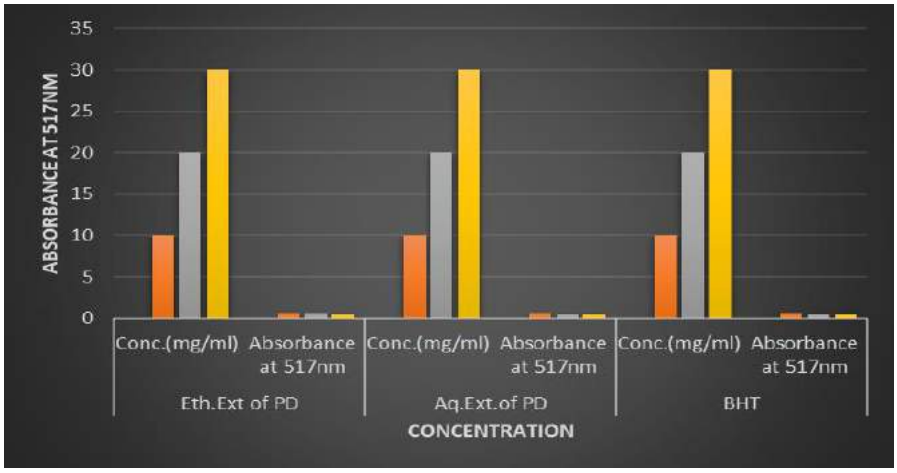
S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		BHT	
	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
01	10	13.34	10	14.69	10	16.94
02	20	21.73	20	24.58	20	26.68
03	30	27.43	30	26.68	30	34.93



Graphical representation showing the % inhibition of DPPH radicals by extracts of *P. denticulata*

Table-5 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula Denticulata* by DPPH method.

S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		BHT	
	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm
01	10	0.578	10	0.569	10	0.554
02	20	0.522	20	0.503	20	0.489
03	30	0.484	30	0.489	30	0.434



Graphical representation showing the absorbance of DPPH radicals by extracts of *P. denticulat*

Discussion

At normal temperature, DPPH is a purple stable free radical with a distinctive absorbance at 517 nm. An antioxidant called 1,1-diphenyl-2-picrylhydrazine readily stifles the nitrogen free radical of DPPH. Purple color decolorization is stoichiometric and depends on the number of electrons acquired[8]. Aerial extracts from Primula denticulata demonstrated a substantial, concentration-dependent scavenging activity on the free radical DPPH. In contrast to the conventional antioxidants employed in the study, the extract exhibited comparatively reduced ability to scavenge free radical's DPPH. As a Results, in vulnerable biological and food systems, *Primula denticulata* leaf extracts may be able to stop reactive radical species from causing damage to biomolecules like DNA, proteins, polyunsaturated fatty acids (PUFA), and carbohydrates. The highly reactive species known as hydroxyl radical (HO•) is produced in biological systems and targets DNA nucleotides, breaking DNA strands and causing cancer and mutagenesis. By removing hydrogen atoms from membrane lipids' polyunsaturated fatty acids, it starts the lipid peroxidation process. It has the ability to harm practically all of the molecules in living cell [9]. The aerial parts extract of *Primula denticulata* demonstrated the capacity to neutralize free radicals produced, and it also demonstrated concentration-dependent hydroxyl radical scavenging that was equivalent to that of the reference standard (Ascorbic acid and BHT) at the same dosages[10].

Conclusion

The current investigation also showed that *Primula denticulata* aerial portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may have enormous potential for use as a treatment for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence. More research is required to produce innovative antioxidant medications.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Spatial and temporal pattern of zooplankton assemblage in response to Limnological factors of river, Yamuna

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Abstract-Zooplankton plays a key role in maintaining aquatic ecosystem quality and is closely related with the community structure. During the present investigation, the zooplankton samples were collected from the three different sampling stations of River Yamuna for a period of one year from April 2012 to March 2013. The physico-chemical parameters of water such as temperature, transparency, velocity, turbidity, conductivity, pH, total alkalinity, total hardness, calcium, magnesium, dissolved oxygen, biological oxygen demand, phosphate and nitrate. The temperature ranged from 17.0±2.59 ºC to 18.58±2.90ºC and pH showed slightly alkaline nature. Sampling station, I registered a maximum dissolved oxygen concentration of 11.41±0.775 mg/l and minimum nutrient concentration. A total of 29 genera of zooplankton belonged to four different taxonomic groups were identified. Among these 10 genera belonged to protozoa, 11 genera belonged to rotifera, 6 genera belonged to copepod and 2 genera belonged to ostracoda. The zooplankton density was highest at sampling site I with maximum number of individuals belonging to all the four group. Rotifera formed the dominant group followed protozoa, copepoda and ostracoda. *Daphnia, Asplanchna* and *Paramecium* was reported with highest number of individuals during the entire study period. The total zooplanktonic richness generally indicated favorable environmental conditions and these were characterized by tolerant of widely fluctuating environmental conditions. Pearson correlation coefficient and Shannon- Weiner diversity index (H') was calculated.

Key words: Aquatic, Community, Shannon-Weiner, Taxonomic, Yamuna, Zooplankton

Introduction

Rivers are important zones for biodiversity but they are exposed to widely fluctuating environmental conditions with periodic changes in inundation, temperature and water quality. Furthermore, they are often exposed to high levels of human

disturbances. Despite these stresses, rivers often support valuable fisheries, provide excellent natural resources for different activities and contributing to regional aquatic diversity. The hydro-zoological communities are particularly important component of river ecosystem. Assessing the diversity of these communities by estimating species abundances/occurrences in valued rivers is one way by which aquatic populations can be compared between sites and be used to help monitor ecosystem quality. Within a particular river, the species composition of aquatic communities is closely linked with seasonal and hydrologic cycles. Many species of zooplankton and aquatic invertebrates can however tolerate changing conditions by both physiological and life cycles adaptations. Rivers are very important ecosystems because they are ecologically and biologically rich and zooplankton is a critical ecological component (Gliwicz, 1999; Hart, 2004). The spatial and temporal dynamics of zooplankton communities are influenced by a variety of physical, chemical and biological factors, whose relative role in structuring zooplankton assemblages and controlling seasonal dynamics may vary within or between biological systems (Hunter and Price, 1992). In general, both biotic and abiotic factors define the microhabitat boundaries for zooplankton by partitioning the environment and minimizing competitive interactions. Further, these are highly susceptible to physical and chemical changes in their environment due to their small size and permeable integument (Nogrady et al. 1993). The various factors that influence the dynamics of natural zooplankton populations are: temperature, oxygen concentration, light intensity and pH. The physical, chemical and biological conditions prevailing in river Yamuna have been studied by Rai (1974 b), De Zwart (1991) and Kaur (1996), Prasad and Singh (2003), Khanna and Fouzia Ishaq (2012); Fouzia Ishaq and Amir Khan (2013). A large number of studies covering a wide variety of ecosystems and organisms suggest that species richness tends to vary strongly with ecosystem production and habitat heterogenicity (Rosenzweig, 1995). This is particularly so with freshwater fauna (zooplankton), which plays a key role in preservation and maintenance of ecological balance and its basic study is wanting and is absolutely necessary. The present investigation attempts to analyze the spatial and temporal pattern of zooplankton assemblage in response to limnological factors of Instream ecosystem of River Yamuna.

Material and Methods

Study area

River Yamuna is a perennial Himalayan river which originated from Yamunotri glacier in the banderpoonch peak of lower Himalayas in Uttarkashi district of Uttarakhand India at an elevation of 6,387 m (20,955 ft) located geographically 38°59′N 78°27′E latitude and 38.983°N 78.45°E longitude and travels a total length of 1376 Km. The catchment area of river Yamuna is 366,220 Km^2 out of which 74,208 Km^2 lies in Uttarakhand.

Dehradun is capital of Uttarakhand famous for its beauty, basmati rice, litchi and also a centre of various research institutes as well. It is bounded in the north by the higher range of lesser Himalaya and in the south by the younger Shivalik ranges. Geographically the Doon valley (Dehradun) lies between latitude 29° 55'N

and 38° 30'N, longitude 77° 35'E and 78° 20'E covering an area of about 3,088 sq. km, with a population of 12, 82,143 (as per 2001 census) (Chauhan, 2008).

Study sites

The study was carried out on river Yamuna from April 2012 to March 2013 to assess the spatial and temporal pattern of zooplankton assemblage in response to limnological factors. The sampling sites; Site I Kalsi (S-I), Site II Dakpathar (S-II) and Site III Asan lake (S-III) were selected on 50 Km stretch of river Yamuna.

Site I: Kalsi is a small town approximately 56 kilometers from Dehradun in Uttarakhand and is a dream destination in the Doon valley. The place is just ideal for such recreational activities like kayaking, parasailing and fishing and also witnesses the glory of King Ashoka. Picturesquely it is located at the bank of River Yamuna and surrounded by beautiful hills and greenery all around. The River is beyond doubt the most striking attraction moving by in a frenzy after meeting up with Tons and Asan Rivers. In this stretch of river only anthropogenic activities were seen and domestic waste from residential areas is the main stress on the river.

Site II: Dakpathar is newly developed recreation centre of Garhwal Mandal Vikas Nigam and is about 45 Kms from Dehradun, located at the foothills of Shivaliks. It is a beautiful tourist spot in the western Doon Valley and one of the major attractions of the place is hydel power station which is located on the downstream of the dakpathar barrage. Its sheer size and dimension would awestruck from every angle you see it. Strategically positioned near the banks of mighty River Yamuna, it is naturally exotic surrounded with forests and bejeweled with lush green lawns and gardens. Thus the site being a tourist resort, the wastes from tourist activities is deteriorating the quality of water.

Site III: The Asan Reservoir is a small man-made wetland of ca. 4 sq km area, located 40 km west of Dehradun, in Doon valley on Dehradun-Paonta road. Geographically it is situated between latitude 30º 24'-30º 28' N and longitude 77º 40'-77º 44' E, near the confluence of the two perennial rivers, river Asan and Yamuna. The barrage is 287.5 m long, the river bed being 389.4 m above sea level, with minimum and maximum water levels respectively at 402.4 m and 403.3 m. The asan reservoir attracts 53 species of water birds of which 19 are winter migrants from Eurasia. During winter months 90% of the water bird population comprises the following 11 migratory species, namely Brahminy Duck, Pintail, Red Crested Pochard, Gadwall, Common Pochard, Mallard, Coot, Wigeon, Common Teal, Tufted Duck, Shoveller. The birds listed as globally threatened species and in the IUCN Red Data Book have been observed at Asan. The site is mostly influenced by tourism and people from different far flung areas visit the place leaving behind lot of waste. The activities like skiing, boating, rowing, kayaking, canoeing etc are stressful for the reservoir.

Sampling strategy

Water samples collected for the purpose of estimation of various parameters were brought to the laboratory and subjected to analysis immediately as far as possible. Standards Methods for Estimation of Water and Wastewater 20th edition, 1998 were referred for estimation of parameters viz., temperature, transparency, velocity, turbidity, conductivity, pH, total alkalinity, total hardness, calcium, magnesium,

dissolved oxygen, biological oxygen demand, phosphate and nitrate. The data collected was subjected to standard deviation, and correlation coefficient for the comparison between sites.

For qualitative and quantitative analysis of zooplankton, collections were made by using cone shaped plankton net made up of nylon bolting silk plankton net (No. 25 mesh size 50 μ) attached with collection tube at the base of net. The net was hauled for a distance of 10 m collected samples were transferred to labeled vial bottles containing 4% formalin. The volume of filtered sample was calculated by multiplying the area of mouth of the net by the depth of the river (i.e., the length through which the net was towed). After sedimentation, 100 ml of sample is subjected to centrifugation and used for further investigation. Counting the protozoa, rotifera, copepoda and ostracoda was carried out using a Sedgewick Rafter cell (Needham and Needham, 1962). Zooplankton diversity was calculated using the Shannon Weiner index, which has moderate sensitivity to the sample number (Magurran, 1988).

Shannon Weiner index

$$\mathbf{H' = \sum_{i=1}^{S} Pi \log 2\, pi}$$

Where,

S = Total number of species

$Pi = n_i/N$ = proportion of individuals of the total sample belonging to the *i*th species

n_i = Number of individuals (N) belonging to the ith species

N = Total number of individuals of all the species

Results

Physico-chemical factors

Table-1 presented the values recorded for physico-chemical variables in the samplings stations carried out during the study period. The mean variation in the surface water temperature of the three stations is presented in table 1. The temperature ranged between the lowest values of 17.0±2.59 ºC obtained from station-I and the highest of 18.58±2.90 ºC obtained from station-III and showed no substantial differences between the sampling sites. The temperature in Asan lake (S-III) was higher than that reported for other two sites that may be due to the fact of standing condition of water and activities of tourism and recreation where as at sampling station, lower temperature is on account of the high altitude location as well as less sources of pollution from surrounding areas. Kolo and Yisa, (2000) reported higher temperature in Suka lake and Adam et al. (1990) reported low temperature at high altitude rivers. Secchi's disc transparency was the highest at S-I with a mean value of 42.62±26.82 cm and S-I recorded the least Secchi's disc transparency value with a mean of 20.32±9.24 cm (table 1). The transparency was found positively and significantly correlated with temperature ($P<0.05$). The maximum transparency may be attributed to increasing depth, low pollution load and minimum transparency may be attributed to minimum depth as well as pollution load and increased concentration of suspended solid and other materials that Results in low light penetration and less transparency. Ali et al. (2000) reported similar Results in Ravi and Chenab rivers in Pakistan. The velocity of the river ranged from lowest of 0.405±0.104 m/s at

S-I to the highest of 2.57±0.32 m/s at S-II (table 1). Sampling site III was significantly different from other stations and velocity was significant at ($p < 0.01$). The highest mean water current observed in station II may be due to regulation from the dam site and the release from this station Resultsing in a constant flow of water. A factor of potential limnological significance is water movement within the barrage produced by discharge from the upstream and the release downstream (station II). The actual effects of the current on the chemistry and the biota of the water are not known, but one may speculate that significant water movement through the surface of the river may prevent development of anoxic conditions and thereby preclude release of sediment bound nutrients. Similar observations have been recorded by Dobriyal (1985) and Nautiyal (1990) in the snow fed rivers of Garhwal Himalayas. The velocity was higher in River Yamuna during summer and monsoon months as it is a glacier fed river Resultsing in more and more water due to melting of ice and snow and also heavy rainfall Results in maximum runoff hence increasing its velocity. Values for turbidity marked spatial variation from lowest of 213.79±329.78 JTU at sampling station-I to 264.58±311.51 JTU at sampling station-III. Turbidity showed positive significant relation with temperature and transparency ($p< 0.05$). The high turbidity may be due to the factors contributing which include soil erosion, elevated nutrient inputs and an abundance of bottom feeders that stir up sediments as well as heavy rainfall in monsoon period (Schlesinger, 1991). As water becomes more turbid, less sunlight is able to penetrate its surface, therefore the amount of photosynthesis that can decrease. This Results in a decrease in the amount of oxygen produced by aquatic plants. This also limits the amount of dissolved oxygen water can hold (Merritts, 1998). The quality may not, however, have adverse effect on the aquatic organisms in the river. The mean conductivity value was highest at sampling station-I 0.210±0.030 $\mu mhocm^{-1}$to 0.150±0.010 $\mu mhocm^{-1}$ at sampling site-III. Conductivity showed significant relation with temperature and velocity ($p< 0.05$). Compared with the Results of Taheruzzaman and Kushari, (1995) the conductivity of River Yamuna was low. Low ionic content in natural waters is generally attributed to slow chemical weathering in the catchments (Blakaret al. 1990). Moreover, apart from slightly random spatial and yearly fluctuations; water of this river does not express definite seasonal trends in conductivity value. Occasional fluctuations in conductivity at a particular season during the study period were quite normal as in all natural systems and were due to differences in the rate of local in flow.

The surface water pH fluctuated between lowest of 8.03±0.293 at sampling site-I to highest of 8.28±0.17 at sampling site-III (table 1). No acidic pH was reported during the study and alkaline pH was recorded at all the sampling sites. The variation in pH was significant throughout the study at all the stations ($p< 0.05$). Kaul and Handoo, (1980) found that increased surface pH in water bodies is due to increased metabolic activities of autotrophs, because in general they utilize the CO_2 and liberate O_2thus reducing H^+ ion concentration . The same authors are also of the opinion that in the bottom of water bodies, liberation of acids from decomposing organic matter under lowO_2 concentration Results in low pH. A pH

range of 6 to 8.5 is normal according to the United States Public Health Association (De, 1999). When compared to all these standards, pH observed in river Yamuna in general was within the safe limits. The highest total alkalinity was recorded at station I (187.33±23.97 mg/l) followed by 181.08±20.71 mg/l at station- III. The lowest mean value of 162.0±16.85 mg/l was recorded at sampling station-II, which was not significantly different from stations I and III. The higher alkalinity might be due to increase in atmospheric temperature and the consequent increase in the photosynthetic process in summer. In pure natural waters alkalinity is mostly due to dissolved CO_2 or bicarbonate ions and it represents the main carbon source for assimilation during photosynthesis (Ansa Asare, 1992). Total hardness, calcium was reported lowest at sampling station-II and highest at sampling station –I. whereas magnesium was reported in higher concentration of 11.94±1.74 mg/l at site-II and lowest of 11.18±4.24 at site –I. Total hardness also showed significant relation with turbidity and pH ($p<0.01$) and transparency and total alkalinity ($p<0.05$). Higher total hardness at sampling site I may be due to presence of salts in the catchment area and may be attributed to presence of high calcium and magnesium levels. Khanna et al. (2012) observed the similar trend of hardness in River Yamuna and Tons. The source of Ca and Mg in natural waters is basically leaching from Ca rich mineral rocks such as lime stone or mineralization of organic matter by the bacteria. Therefore, Ca in natural waters differs according to difference in geographic regions or anthropogenic impact (Jain et al. 2002). Dissolved oxygen fluctuated between lowest of 10.70±0.412 mg/l at sampling station-I and the highest mean of 11.41±0.775 mg/L recorded from sampling station- I (Table-1). Dissolved oxygen was found significantly correlated with temperature, velocity and pH ($P<0.05$). The cause of maximum DO may be due to the reduced rate of decomposition by decreased microbial activity at low temperature in winter season (Prasad and Singh, 2003). Depletion of oxygen may occur in summer due to increase in temperature as well. The oxygen cycle in water involves a rapid decrease during summer at a steady increase through autumn till maximum content is reached in winter, following the well known solubility of gases (Kaul and Handoo, 1980). The low temperature and high speed of water flow increase dissolved oxygen in upstream and high temperature with low speed of water flow decrease dissolved oxygen in downstream. So the fast moving water and colder water contain higher dissolved oxygen than slow or stagnant water and warmer water. Chopra et al., (1994) also got the same Results and have revealed that low temperature in winter season increases the oxygen retaining capacity of water and solubility of oxygen in water. There was no significant variation between the values of biochemical oxygen demand (BOD) recorded for all stations. The mean values varied between lowest of 2.87±0.490 mg/l at sampling station-I and highest of 3.35±0.318 mg/l at sampling station-III (table 1). However, there was significant difference ($P < 0.01$) between the values recorded for all stations. BOD increases as the bio-degradable organic content increases in waters. The higher BOD recorded in station III could probably be due to organic matter degradation which utilized oxygen within the river. According to Kolo and Yisa (2000)

organic matter in the form of increased decomposition of domestic sewage can increase the BOD. The highest BOD may be due to addition of high organic content leading to lower oxygen concentration indicating deoxygenation rate due to higher biological decomposition of organic matter. The mean values of phosphate recorded varied between 0.569±0.152 mg/l at sampling site- I to 0.659±0.080mg/l in sampling station- III. Phosphate showed significant correlation ($P < 0.05$) with temperature, turbidity, pH and total hardness. Phosphate is the nutrient considered to be the critical limiting nutrient, causing eutrophication of fresh water systems (Rabalais, 2002). It is a major nutrient that triggers eutrophication and required by algae in small quantities (Bandela, et al. 1999). Each Phosphate ion promotes the incorporation of seven molecules of N and 40 molecules of CO_2 in algae (Wetzel, 2001). Phosphate enters surface water from human-generated wastes and land run off; domestic waste contains approximately 1.6 kg per person, per year of which 64 % is from synthetic detergents (Kataria, et al. 1995). P additions to landscape enter water via waste water effluents and soil erosions, and also from detergents. Therefore, Phosphate in large quantities in water is an indication of pollution through sewage and industrial waste. In instances where phosphate is a growth limiting nutrient, the change in its concentration can cause the stimulation or inhibition in the growth of photosynthetic aquatic micro and macro organisms such as phytoplankton and green bacteria (APHA, 1998).The highest mean value for nitrate was 1.525±0.1 46mg/l at sampling site-III, while the lowest mean value of 0.532±0.082 mg/l was recorded at sampling site- I. Nitrate showed significant correlation of ($P< 0.05$) with temperature, conductivity, pH and dissolved oxygen (table 2). No significant difference occurred between the years of study in nitrate and phosphate concentrations. The most important source of NO_3 in waters is biological oxidation of nitrogenous organic matter of both autochthonous and allochthonous origin, which include domestic sewage, Agricultural run-off and effluents from industries (Saxena, 1998). NO_3 concentration depends on geochemical conditions such as degree of use of agricultural fertilizers and industrial discharges of nitrogenous compounds (Kataria, et al. 1995). The increased concentration of nitrate in river at sampling site- I may be due to domestic activities and agricultural runoff. High Levels of both phosphate and nitrate can lead to eutrophication, which increases algae growth and ultimately reduces dissolved oxygen levels in the water (Murdoch, et al. 2001).

Table-1 Mean values of physico-chemical parameters at three sampling stations (S-I, S-II and S-III) of river Yamuna

Parameters	S-I	S-II	S-III	Avg. ± S.D
Temperature ºC	17.0±2.59	17.83±2.20	18.58±2.90	**17.80±0.790**
Transparency Cm	20.32±9.24	40.90±20.94	42.62±26.82	**34.61±12.408**
Velocity m/s	1.84±0.51	2.57±0.32	0.405±0.104	**1.60±1.101**

Turbidity JTU	213.79±329.78	249.16±351.67	264.58±311.51	**242.51±26.04**
Conductivity µmhocm^{-1}	0.210±0.030	0.208±0.041	0.150±0.010	**0.189±0.034**
pH	8.03±0.293	8.13±0.30	8.28±0.17	**8.14±0.125**
Total alkalinity mg/l	187.33±23.97	162.0±16.85	181.08±20.71	**176.80±13.195**
Total Hardness mg/l	103.16±20.30	96.50±13.71	98.91±18.46	**99.52±3.372**
Calcium mg/l	57.32±8.32	47.54±9.44	52.23±12.49	**52.36±4.891**
Magnesium mg/l	11.18±4.24	11.94±1.74	11.38±2.00	**11.5±0.39**
Dissolved Oxygen mg/l	11.41±0.775	11.35±0.705	10.70±0.412	**11.15±0.393**
B.O.D mg/l	2.87±0.490	3.21±0.497	3.35±0.318	**3.14±0.246**
Phosphates mg/l	0.569±0.152	0.637±0.086	0.659±0.080	**0.621±0.0469**
Nitrates mg/l	0.532±0.082	0.544±0.061	1.525±0.146	**0.867±0.5699**

Table 2: Pearson correlation coefficient between physico-chemical parameters of river Yamuna

	Temperature	**Transparency**	**Velocity**	**Turbidity**	**Conductivity**	**pH**	**Total alkalinity**	**Total Hardness**	**Calcium**	**Magnesium**	**D.O**	**B.O.D**	**Phosphates**	**Nitrates**
Temperature	1													
Transparency	0.91*	1												
Velocity	-0.63	-0.25	1											
Turbidity	0.98*	0.97*	-0.47	1										
Conductivity	-0.87*	-0.58	0.93*	-0.75	1									
pH	0.99*	0.84*	-0.73	0.94*	-0.92*	1								
Total alkalinity	-0.27	-0.63	-0.58	-0.44	-0.25	-0.12	1							
Total Hardness	-0.65	-0.90*	-0.18	-0.78	0.18	-0.53	0.90*	1						
Calcium	-0.55	-0.84*	-0.31	-0.69	0.05	-0.41	0.95*	0.99*	1					
Magnesium	0.28	0.65	0.56	0.46	0.23	0.14	-0.99*	-0.91*	-0.90*	1				

D.O	-0.89*	-0.62	0.91*	-0.78	0.99*	-0.94*	-0.20	0.23	0.10	0.19	1			
B.O.D	0.97*	0.97*	-0.46	0.99*	-0.74	0.93*	-0.45	-0.79	-0.71	0.47	-0.77	1		
Phosphates	0.96*	0.98*	-0.41	0.99*	-0.71	0.92*	-0.50	-0.82*	-0.74	0.52	-0.74	0.99*	1	
Nitrates	0.85*	0.56	-0.94*	0.74	-0.99*	0.92*	0.27	-0.16	-0.03	-0.3	-0.99*	0.73	0.69	1

Significant at p < 0.05 and 0.01*

Zooplankton composition and diversity

Zooplankton is rarely important in rivers because they cannot maintain positive net growth rates in the face of downstream losses. These communities are highly sensitive to environmental variation. As a Results, changes in their abundance, species diversity, or community composition can provide important indications of environmental change or disturbance. Zooplankton species inhabiting freshwater habitats offer an excellent model system to study spatial interactions.

The composition and occurrence of zooplankton recorded at various stations showed significant variations and fluctuations during the study period (Table 3). Twenty nine genera of zooplankton were identified from the river. They belong to Protozoa (10 genera), Rotifera (11 genera), Copepoda (6 genera) and Ostracoda (2 genera). The qualitative analysis of zooplankton in river Yamuna revealed that the Protozoan's were represented by *Actinophrys, Actinosphaerium, Euglena, Paramecium, Peridinium, Campenella, Epistylis, Vorticella, Arcella* and *Diffugia*. The total zooplankton that belonged to protozoa was recorded maximum at sampling station – III (178.25±63.0 Ind. /m^2) and minimum at sampling station-II (141.92±56.28Ind. /m^2). Among these genera *Paramecium* was reported with highest number of individuals ranging from 14.58±7.37 Ind. /m^2 at S- I to 25.58±12.65 Ind. /m^2 at S-III and *Epistyl is* was reported with minimum number of individuals ranging from 8.16±4.28 Ind. /m^2 at S-II to 13.25±5.86 Ind. /m^2 at S-III. However the higher number of individuals that belonged to protozoa was recorded from sampling station-III during the period of investigation. Protozoa showed a negative correlation with velocity, conductivity and dissolved oxygen ($p<0.05$) (table 4) but showed a positive significant relation with all other parameters ($p< 0.01$). The Rotifera was represented by *Keratella, Nolthoca, Rotaria, Testudinella, Ascomorpha, Polyarthra, Philodina, Asplanchna, Pompholix, Brachionus* and *Trichocera.* The total zooplankton that belonged to Rotifera was recorded maximum at sampling station –III (197.5±66.19 Ind. /m^2) and minimum at sampling station-II (163.5±62.55 Ind. /m^2). Among these genera *Testudinella* was reported with highest number of individuals ranging from 12.75±8.28 Ind. /m^2 at S- II to 24.0±9.70 Ind. /m^2 at S-I and *Trichocera* was reported with minimum number of individuals ranging from 9.16±5.11 Ind. /m^2 at S-I to 15.25±6.34 Ind. /m^2 at S-III. However Rotifera was dominating the river Yamuna with maximum diversity and density at all the three sampling stations. The number of genera as well as number of individuals belonging to each genus was highest in case of Rotifera. Rotifera also showed a positive significant relation with temperature,

transparency, pH and nutrients ($p<0.01$) and negative correlation with conductivity and velocity ($p<0.05$) (Table 4). The Copepoda was represented by *Cyclops*, *Diaptomus*, *Daphnia*, *Bosmina*, *Helobdella* and *Nauplius* andthe Ostracoda was represented by *Cypris* and *Stenocypris*. Among Copepoda the maximum number of individuals was reported from sampling station-III (116.25±34.02 Ind. /m^2)and minimum number was recorded from sampling station –I (92.5±36.57 Ind. /m^2). *Daphnia*was reported with highest number of individuals and *Bosmina* with lowest number of individuals during the study period among Copepoda at all the three sampling stations. Ostracoda was found with maximum number at S-III (30.25±10.63 Ind. /m^2) and minimum number at S-I (19.5±5.91 Ind. /m^2). *Cypris* was observed with highest number of individuals and *Stenocypris* with lowest number of individuals throughout the study period. Copepoda and Ostracoda was positively significantly correlated with temperature, transparency, pH and nitrates ($p<0.05$).

Table-3 Mean values of zooplankton at S-I, S-II and S-III of river Yamuna

Zooplankton	S-I	S-II	S-III
Protozoa			
Actinophrys	14.66±5.51	19.16±7.37	16.50±5.55
Actinosphaerium	11.75±4.59	14.41±7.14	14.83±6.20
Euglena	16.91±8.57	14.08±5.69	23.41±8.35
Paramecium	14.58±7.37	15.66±8.51	25.58±12.65
Peridinium	17.83±8.67	11.41±6.11	18.66±5.21
Campenella	12.0±6.84	17.08±8.77	18.0±5.83
Epistylis	11.0±6.98	8.16±4.28	13.25±5.86
Vorticella	15.0±6.0	13.5±6.38	14.75±7.35
Arcella	19.25±9.57	13.91±5.99	17.33±7.03
Diffugia	18.33±8.01	14.5±5.79	15.91±7.77
Total	151.33±61.92	141.92±56.28	178.25±63.0
Rotifera			
Keratella	14.75±6.06	19.0±7.08	17.75±6.98
Nolthoca	15.91±8.44	11.5±5.50	16.0±5.41
Rotatoria	16.08±7.02	15.66±7.79	19.0±6.41
Testudinella	24.0±9.70	12.75±8.28	13.33±5.43
Ascomorpha	13.83±7.96	17.58±6.03	21.58±9.48
Polyarthra	17.66±9.03	14.25±6.64	23.25±9.69
Philodina	17.5±9.84	17.25±7.43	18.16±6.42
Asplanchna	19.58±9.07	12.75±8.09	25.75±8.74
Pompholix	15.66±7.11	16.41±7.56	10.33±4.83
Brachionus	11.41±6.99	15.75±5.49	17.08±7.39
Trichocera	9.16±5.11	10.58±4.99	15.25±6.34
Total	175.58±71.76	163.5±62.55	197.5±66.19
Copepoda			
Cyclops	21.0±10.98	13.16±5.95	19.58±6.25
Diaptomus	17.08±8.08	14.58±6.28	20.5±6.97
Daphnia	16.25±5.77	23.66±8.60	24.33±9.90
Bosmina	12.66±7.22	14.75±6.73	16.0±5.20
Helobdella	11.33±7.48	18.25±5.73	12.25±4.86
Nauplius	14.16±5.28	12.66±5.85	23.58±7.52
Total	92.5±36.57	97.08±32.74	116.25±34.02
Ostracoda			
Cypris	8.25±4.02	11.33±5.66	16.33±4.77
Stenocypris	11.25±3.25	8.66±4.61	13.91±7.02
Total	19.5±5.91	20.0±9.76	30.25±10.63

Table-4 Pearson correlation coefficient between zooplankton andphysico-chemical parameters

	Temperature	Transparency	Velocity	Turbidity	Conductivity	pH	Total alkalinity	Total Hardness	Calcium	Magnesium	D.O	B.O.D	Phosphates	Nitrates
Protozoa	0.69	0.33	-0.99*	0.54	-0.96*	0.79	0.51	0.09	0.22	-0.49	-0.94*	0.53	0.48	0.96*
Rotifera	0.61	0.23	-0.99*	0.45	-0.92*	0.72	0.59	0.19	0.32	-0.58	-0.90*	0.43	0.39	0.93*
Copepoda	0.93*	0.70	-0.86*	0.85*	-0.98*	0.97*	0.10	-0.33	-0.20	-0.08	-0.99*	0.83*	0.80*	0.98*
Ostracoda	0.87*	0.59	-0.92*	0.76	-0.99*	0.93*	0.24	-0.19	-0.06	-0.22	-0.99*	0.75	0.71	0.99*

Significant at p < 0.05 and 0.01*

Zooplankton abundanceand distribution

The abundance and richness of zooplankton is presented in (table 5). The zooplankton was more prevalent during the rains and winter season while their population declined during the dry season. The three stations showed similar genera composition. The only difference was in their abundance and distribution within the sampling stations. Among all the genera *Daphnia* was recorded with maximum abundance (21.41±4.48 Ind. /m^2) followed by *Asplanchna.* Qualitatively the zooplankton fauna of each sampling site was dominated by rotifera followed by protozoa, copepods and ostracoda following the order *rotifer > protozoa > copepod > ostracoda*. The rotifers constitute the largest group of zooplankton recorded at all the sites during the study period. During the study period, Shannon–Wiener diversity was relatively high and ranged from 0.2234 to 1.8551(table 5). Shannon–Wiener diversity index indicated that a more even or equitable distribution among species tends to increase diversity and did not showed significant variation between the sampling stations. Twenty-nine genera of zooplankton belonging to 4 groups were recorded. The percentage occurrences of these genera are presented in table 5. The genera with highest percentage among four groups were *Paramecium* (11.83 %), *Asplanchna* (10.82%), *Daphnia* (21%) and *Cypris* (51.48%). The genera with lowest percentage occurrence among all the four groups was Epistylis (6.87%). The genera belonging to Copepoda was reported with highest percentage occurrence during the study period. Accordingly, Rotifer taxa representing different genera recorded their maximum densities at different sites and as such kept the rotifer diversity relatively high throughout the year.

Table-5 Diversity richness, abundance and distribution of zooplankton

	Avg.± S.D	Shannon-Weiner Index (H')	% of zooplankton in each genera
Protozoa			
Actinophrys	16.77±2.26	0.5386	10.66
Actinosphaerium	13.66±1.67	0.3573	8.69

Euglena	18.13±4.78	0.6295	11.53
Paramecium	18.60±6.06	0.6626	11.83
Peridinium	15.96±3.96	0.4878	10.15
Campenella	15.69±3.23	0.4715	9.98
Epistylis	10.80±2.55	0.2234	6.87
Vorticella	14.41±0.80	0.3977	9.16
Arcella	16.83±2.70	0.5425	10.70
Diffugia	16.24±1.93	0.5051	10.33
Rotifera			
Keratella	17.16±2.18	0.4955	9.59
Nolthoca	14.47±2.57	0.3523	8.09
Rotatoria	16.91±1.81	0.4812	9.45
Testudinella	16.69±6.33	0.4688	9.33
Ascomorpha	17.66±3.87	0.5249	9.87
Polyarthra	18.38±4.54	0.5685	10.27
Philodina	17.63±0.47	0.5231	9.85
Asplanchna	19.36±6.50	0.6308	10.82
Pompholix	14.13±3.31	0.3360	7.90
Brachionus	14.74±2.96	0.3556	8.24
Trichocera	11.66±3.18	0.2288	6.51
Copepoda			
Cyclops	17.91±4.17	0.9472	17.56
Diaptomus	17.38±2.97	0.8919	17.04
Daphnia	21.41±4.48	1.3536	21.00
Bosmina	14.47±1.68	0.6183	14.19
Helobdella	13.94±3.75	0.5738	13.67
Nauplius	16.80±5.91	0.8334	16.48
Ostracoda			

Cypris	11.97±4.07	1.8551	51.48
Stenocypris	11.27±2.62	1.6445	48.47

Discussion

In the present study temperature fluctuations in water were influenced considerably by air temperature, humidity and solar radiation. The higher concentration of pH was observed during summer season could be attributed to enhanced rate of evaporation coupled with human interference are partly to enhance photosynthetic activity. The higher values of transparency were recorded during summer season, which may be due to increased solar radiation and thus, considerably good standing crop of zooplankton productivity. The Yamuna river water was in moderate hard water conditions, which in turn useful for the higher productivity. The zooplankton of Yamuna river consists of Protozoa, Rotifers, Copepoda and Ostracoda; a total 29 genera were recorded from the river Yamuna during the present study. There was a distinct seasonal fluctuations and composition of the zooplankton in the river Yamuna with

productive (October to March), retardation (June to August) and recovery (September onwards) periods. The total zooplankton population was dominated by rotifera followed by Protozoa, copepoda and ostracoda respectively. Among rotifera*Asplanchna, Polyarthra* and *Ascomorpha* were dominated in the present in the present investigation of rotifers. The composition of rotifera population showed higher population during monsoon and summer, while lower population observed in the month of August. This perhaps may be due to influence of copious quantity of rainwater and turbidity, which gets drained into the river. Ostracoda occupied fourth position of zooplankton and represented very low population diversity compared to other groups. The densities of various zooplankton were in the order of rotifera> protozoa > copepod > ostracoda. The Results indicated that the maximum number of genera occurred during winter season than summer and monsoon season which also reported by Abdus et al (1995), Kumar (2001). The less number of genera might be attributed to the less nutrients in the river which consequently Results in less productivity or might be due to the depletion of important limnological. The reduction in the number of genera may be due to predation, variation in the pH of water is always associated with the genera (species) composition of zooplankton inhibiting among them (Jhingran 1982). In winter, it is biotic interaction operating through feeding pressure rather than water quality it seems to affect the zooplankton diversity and density particularly the stocked fish species play an important role in harvesting species of copepoda and ostracoda, thereby reducing their predatory pressure on other groups. The rotifers and protozoa were higher in winter can be linked to favorable temperature and availability of abundant food in the form of bacteria, nano plankton and suspended detritus (Edmondson, 1965). Wetzel (2001) reported that maximum diversity of freshwater ecosystems occurs where the terrestrial wetland-littoral interface regions are strongly coupled to open waters of lakes and river channels. The waters of the river Yamuna represent such a habitat and hence support diverse biotic communities in general and rotifers in particular, although the total number of species is less than half that expected in eutrophic lakes (Dumont and Segers, 1996). In addition, seasonal flushing

of these low-lying areas by the river plays a crucial role in maintaining high zooplankton diversity, as these lentic ecosystems provide refuge to the resting eggs carried by the river from its headwaters.

In addition, the rotifer taxocoenos is had a tropical character, with an abundance of 'tropic centered' genera such as *Lecane* and *Brachionus*. This is supported by the presence of fewer species of 'temperate-centered' brachionid genera such as *Keratella* and a restricted occurrence of the cold-water genera *Notholca.* These observations are in concurrence with reports by Sharma and Michael (1980) and Sharma (1998) with regard to Indian rotifer fauna. During the one-year cycle, the rotifer density reached maxima during spring–early summer and minima during winter. In earlier studies on river Yamuna, the zooplankton density was found to be maximum during summer (Rai, 1974b: Kaur, 1996). This difference could reflect the different ecological conditions prevailing in the backwaters. A number of investigators (Pontin and Shiel, 1995; Duggan et al. 1998) also observed that macrophytes in aquatic ecosystems provide a rich variety of microhabitats for a diverse zooplankton fauna. In general, high densities of zooplankton reflect the availability of a wide range of natural sestonic food particles, which zooplankton may consume (Dumont, 1977; Gulati, 1990). Various investigators reported that the rapid increase in zooplankton numbers may be attributed to their intrinsic high fecundity supported by favorable food and environmental conditions (Dumont, 1977; Lynch, 1979; Gulati, 1999). Thus, the presence of favourable physical–chemical characteristics (e.g., temperature, nutrients and pH) as also relative abundance of diatoms were possibly responsible for promoting the growth of zooplankton densities during this study (Michaloudi et al. 1997). Hofmann (1977) suggested that temperature (a conditional factor) and oxygen (a material factor) are the main but not the only determinative factors which influence the occurrence and diversity of zooplankton. De Zwart (1991) and Kaur (1996) observed dominance of zooplankton with low species diversity in the segments of the Yamuna having high levels of BOD and COD. In comparison, the present study sites recorded highly diverse zooplankton fauna with relatively low BOD and COD values.

The zooplankton genera found in the river Yamuna agrees with the observations of Rocha et al. (1999) about zooplankton assemblages in rivers. The rotifer was noted to be main and first dominating group of total zooplankton during the entire study. The present study has also revealed that rotifer densities depend on the quantitative changes of organic decaying materials and temperature the same view was given by Alfred, et al. (1973). The zooplankton assemblage in river Yamuna was attributed to several biotic and abiotic factors interacting together. These include temperature, transparency, nutrients, food availability and river morphometry. Monthly and seasonal variation in zooplankton was significant in river Yamuna. The seasonal, monthly and altitudinal variation of zooplankton in the river Yamuna was related with temperature and dissolved oxygen which favors the survival of zooplankton in water. In summer and winter zooplankton showed a rise in their population but the zooplankton volume and population was low in monsoon due to high temperature, high turbidity, high solids, high conductivity and low DO. Similar Results were obtained by Nath and Srivastava, (2001) in river Narmada and Pathani and Mahar, (2006) in some lotic systems of Himalayas in Uttarakhand. The velocity of water, and transparency of water in different spots were studied and it was found that the

number and quality of zooplankton were higher in low altitude than the high altitude. On the Other hand the velocity of water was very high in monsoon due to which plankton population was found low in river Yamuna. Zooplankton advective losses are especially great in rivers having high velocities (Pace et al. 1990), Most of the zooplankton encountered in the study area appears to be normal inhabitants of river Yamuna and increase in primary production (phytoplankton) tends to be the reason for increased zooplankton number and biomass. The species composition of zooplankton with dominance of rotifers was observed in river Yamuna. Arora and Mehra, (2003) also observed richness and dominance of rotifers among zooplankton in Yamuna river. Rotifers were numerically dominant in the lower zone and most of genera had a high frequency of occurrence in samples. In the present investigation, the observation clearly revealed that zooplankton represents a sensitive indicator of pollution and the maximum zooplankton density in winter and minimum in monsoon season may be due to water temperature, water velocity, and turbidity been lower in winter months and these provide favorable environment for the growth of zooplankton. This has been confirmed by Sinha et al. (2000) and Valecha and Bhatnagar, (1998).

Our study suggests that physical and chemical factors largely influence the spatial and temporal dynamics of the zooplankton populations in the waters of the Yamuna. However, an additional investigation of some top-down factors and on the influence of competitive interactions on the seasonal succession of zooplankton communities is required to reach a definite conclusion.

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Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Unveiling the potent antioxidant capabilities of essential oil derived from *Artemisia roxburghiana* leaves: A comprehensive exploration

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Abstract-Plants have potential due to their capability to synthesize an array of phytochemicals as secondary metabolites to defend themselves and respond on spot to a variety of environmental biotic or a biotic stresses. Such plants metabolites are used as drugs, medicines and also for many other industrial purposes including most attractive fragrances and nutraceuticals. Fragrance or flavour, the aroma behind is visualized by olfactory responses to the odorant molecules of different structures and forms. These molecules even with similar looking structures often express variation in quality of aroma because of electricity and stereocity Resultsing range of olfactive odors. De-stressing the world of its hectic lifestyles coupled with competitive ambience in all spheres is an area that aromaceuticals has occupied and thus has opened up a new horizon of aromatics research as well as commercial and industrial applications in near future taking lead in preventive healthcare scenario attracting global attention. Keeping in view of the above, the proposed study is aimed to carry out antioxidant potential form of essential oil from wild plant species namely *Artemisia roxburghiana* Wall. Ex Besser. Oil was extracted and studied for its physico-chemical characteristics and antioxidant potential.

Introduction

Essential oils, known for their strong aroma, are complex and volatile compounds produced naturally by aromatic plants as secondary metabolites. These oils are recognized as rich sources of biologically active compounds. Recent attention has focused on exploring the antimicrobial properties of extracts from essential oils hold significant importance as antioxidants, contributing to their widespread utilization in various fields[1-4] their antioxidant properties stem from a rich array of bioactive compounds, such as to copherols, phenols, and other volatile components. These compounds play a pivotal role in

neutralizing free radicals, unstable molecules that can cause cellular damage and contribute to various health issues, including aging and chronic diseases. As potent antioxidants, essential oils exhibit the ability to inhibit oxidative stress, a process associated with the imbalance between free radicals and the body's antioxidant defenses. By scavenging free radicals, essential oils help protect cells from damage, thereby potentially reducing the risk of oxidative stress-related conditions. Moreover, the antioxidant nature of essential oils extends their shelf life by preventing the oxidation of fats and oils in food products, thus serving as natural preservatives. This preservation quality makes them valuable in the food industry, offering a natural and health-conscious alternative to synthetic preservatives. The plant Artemisia roxburghiana wall ex: Besser, also known as Roxburgh's Wormwood is one of the least explored folk medicinal plants of the genus Artemisia[6-9]. The plant grows in the lower or mid-altitude of lower Himalayas from 500 to 4000 m. It is used as a folk medicine in various Asian countries for the treatment of malarial fever, skin disorders and intestinal wormsThe primary objective of this study is to evaluate the antioxidant potential of *A. roxburghiana* wall ex:Besser.

Material and Methods

Preparation of substrate

The aerial part of *Artemisia roxburghiana*was collected from Suwakholi (near Dhanaulti) at an altitude, latitude and longitude ranging from 2060 m, 30.45004º N, 78.15853º E - 2385 m, 30.44582º N , 78.19557º authenticated by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. The leaves and the rhizomes were cut into small slices followed by oven drying at 60 °C - 80 °C for 48 hrs. The dried parts were then stored at room temperature and then used for the experiment.

Extraction of oil from leaves *Artemisia roxburghiana*

Fresh material was cut into small pieces and then subjected to hydro-distillation directly for 4 hours. The isolation of essential oil was carried out by using Clevenger assembly, according to standard method (Guenther, 1948). The extracted essential oils were dried using anhydrous sodium sulfate and stored in sealed vials at low temperature (4^0C) for further analysis. 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.

Studies of characteristics of extracted oil

(a) Physicochemical characteristics provide a base line for aptness of oil. These characteristics studied were colour, Specific gravity, Specific Viscosity, Refractive index, Optical Rotation **,** Acid number, Saponification number, Ester value, percentage of phenol and hydroxyl value

(b) **Specific gravity**

1. A cleaned and dried empty pycnometer was taken and weighed.
2. The pycnometer was filled upto the mark with double distilled water and weighed again.
3. The weight of water was recorded. After removing the water the pycnometer was dried in the oven and filled upto the mark with the essential oil under experimentation.

4. The weight of pycnometer with oil was calculated using the following formula.

$$\textbf{Specific gravity} = \frac{wt.\ of\ the\ oil}{wt.\ of\ an\ equal\ volume\ of\ water}$$

(c)**Specific Viscosity**

1. An Ostwald viscometer was cleaned and dried. Ten ml of 1% solution (in acetone) of the oil under experimentation was filled in the bulb of the viscometer.
2. The solution was sucked upto upper mark and the viscometer left as much.
3. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.
4. The process was repeated by filling the viscometer with pure acetone. The relative viscosity of the oil was calculated as per following formula:

$$\eta Rel = \frac{\text{Flow time of 1\%solution of essential oil in solvent}}{\text{Flow time of pure solvent}}$$

The value of relative viscosity thus obtained, was converted to specific viscosity by the formula as under:

$$\eta sp = \eta\ rel^{-1}$$

where,

ηsp = Specific viscosity

ηrel^{-1} = relative viscosity

(d)**Refractive index**

1. Abbe type of refractometer was used to determine the refractive indices of the oils.
2. The double prisms of the apparatus were cleaned with alcohol and one drop of the oil was placed between them.
3. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minutes to equate the temperature of the oils and the apparatus.
4. The alidade of the refractometer was moved backward of forward to get a broader line which was a band of colour.
5. A sharp colourless line was obtained by rotating the screw heads of the compensator. Finally, the line was adjusted in such a way that it fell on the point of intersection of the cross hairs.
6. The refractive index was head directly on the scales of the sector.

(e) **Acid number**

1. One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask.
2. Three drops of 1% phenolphthalein were added to the contents of the flask and it was titrated against 0.1 N sodium hydroxide solution.
3. The first appearance of pink colouration that did not fade within 10 second was considered as the end point.
4. Another set without oil was also run parallel to treatment and the difference in the amount of alkali used while titrating the treatment and the set without oil gave the amount of alkali consumed for determination of the acid number of the oil.
5. The acid number was calculated by the following formula :

$$\textbf{Acid number} = \frac{\text{Volume of 0.1 N Alkali consumed}}{\text{Weight of 1ml essential oil}} \textbf{ X 5.61}$$

(f) **Saponification number**

1. One ml of the essential oil was taken into a 100 ml saponification flask. Ten ml of 0.5 N alcoholic sodium hydroxide solution was added to the flask and an air cooled glass condenser (1 meter in length and 1 cm in diameter) was attached to it. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature.
2. The contents were the titrated against 0.5 N aquous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator.
3. Another set, without oil was also run parallel to the treatment set and the

difference in the amount of acid consumed for the determination of saponification number of the oil, which was calculated by the following formula:

$$\textbf{Saponification number} = \frac{\textbf{Volume of 0.5 N Acid consumed}}{\textbf{Weight of 1ml essential oil}} \textbf{ X 28.05}$$

(g)Optical Rotation

1. Placed the 100mm polarimeter tube containing the oil or liquid under the examination in the trough of the instrument between the polarizer and analyzer.
2. Slowly turned the analyzer until both halves of the field, viewed through the telescope, show equal intensities of illumination.
3. At the proper setting, a small rotation to the right or to the left will immediately caused a pronounced inequality in the intensities of illumination of the two halves of the field.
4. Determined the direction of rotation. If the analyzer was turned counter-clockwise from the zero position to obtain the final reading, the rotation is leave and if clockwise, it was dextro.
5. After the direction of rotation had been established, carefully readjusted the analyzer until equal illumination of two halves of the field was obtained. Adjusted the eyepiece of the telescope to give clear, sharp lines between the two halves of the field.
6. Determined the rotation by means of protectors; read the degrees directly , and the minutes with the aid of either of two fixed Vernier's, the movable magnifying glasses will aid in obtaining greater accuracy.
7. A second reading should be taken, it should not differ by more than 5' from the previous reading.

Antioxidant Activity

DPPH Radical Scavenging Assay

Essential oil was tested spectrophotometrically for their ability to scavenge free radicals against stable DPPH (2,2-diphenyl-2-picrylhydrazyl). DPPH is reduced when it interacts with an antioxidant substance that can donate hydrogen. Using a UV visible light spectrophotometer, the transition from purple to yellow was measured at 517 nm. Fresh solution of DPPH in methanol was prepared each day. Before measuring absorption, the oil solutions and DPPH solution were combined in the ratio of 3:1 and kept in the dark for 30 minutes at room temperature. The experiment was carried out a total of three times. The following formula was used to determine the amount of radical scavenging activity:

(A_B-A_A/A_B)% Inhibition×100

Where, A_B = Absorption of the Blank Sample in this case

A_A is the tested sample's absorption.

Ferric Chloride Reducing Power Assay

A quick and easy screening technique for determining antioxidant potential is the assay for lowering power. According to Banerjee procedure (10), the reducing power of oil was assessed. Tannic acid and gallic acid were employed as a standard. The reagents used were 0.2 M Sodium phosphate buffer, pH 6.6, 1%w/v Ferricyanide, 10% w/v Trichloroacetic Acid, 0.1%w/v Ferric Chloride. Oil was combined with 5.0 ml of potassium ferricyanide and 5.0 ml of sodium phosphate buffer at concentrations ranging from 1.0 to 5.0 mg/ml of water. 5.0 ml of trichloroacetic acid were added after the mixture had been incubated at 50°C for 30 min. The mixture was then centrifuged at 980Xg for 10 min. at 50°C in a refrigerator-based centrifuge (VHM-17L Kokusan Denki, Tokyo, Japan). One ml of ferric chloride was added after the 5.0ml of the upper layer of the solutions were diluted with 5.0 ml of distilled water. Using a spectrophotometer (U-2001, Hitachi

Instruments Inc.), absorbance was measured at 700 nm.

Increase in reducing power (%) = (A_T-A_B/A_B) ×100

Where, A_B =Absorbance of the Blank Sample

A_T= Absorbance of Tested Sample.

Results

Results of the above studies are summarized in the **Table (1-3)** and discussed at appropriate place.

Table-1 Yield of extracted oil

Part of the plant used	Initially amount taken (in gm)	Oil obtained(in gm)
Leaves	900	3.4690

Table-2 Characteristics of Essential oil of*Artemisia roxburghiana*

Parameter studied	Value
Colour	Dark green
Specific gravity	0.924
Specific viscosity	0.110
Refractive index	1.553
Acid number	13.253
Saponification number	37.24

Table3:Antioxidant Potential of *Artemisia roxburghiana* Essential Oil

S.NO	Concentration(µg/ml) IC_{50} (µg/ml) in DPPH assay	Absorbance	% Antioxidant activity
1.	control	1.623	-
2.	Ascorbic acid IC_{50} (µg/ml) in DPPH assay	0.055	96.61
3.	Artemisia *roxburghiana* IC_{50} (µg/ml) in DPPH assay	0.273	78.03%
4	Artemisia *roxburghiana* µM (AAE)/100g dry mass in FRAP assay	0.787	60.98%

Discussion

The study focused on the preparation of essential oil from the aerial parts of Artemisia roxburghiana and subsequent characterization of its physicochemical properties. The plant material was collected from Suwakholi near Dhanaulti, and after thorough authentication, the leaves and rhizomes were sliced, oven-dried, and stored for further use.

The essential oil extraction process involved hydro-distillation using a Clevenger assembly. The obtained essential oils were then subjected to various analyses, including quantitative determination and physicochemical characterization. Parameters such as color, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, and percentage of phenol were assessed using standard methods.

The specific gravity was determined by comparing the weight of the oil to that of an equal volume of water. Specific viscosity was evaluated using an Ostwald viscometer, and refractive index was measured using an Abbe refractometer. Acid number and saponification number were determined through titration methods. Optical rotation was assessed using a polarimeter. The Results indicated that the essential oil of Artemisia roxburghiana exhibited specific characteristics, including a dark green color, specific gravity of 0.924, specific viscosity of 0.110, refractive index of 1.553, acid number of 13.253, and saponification number of 37.24.

Additionally, the antioxidant activity of the essential oil was assessed through DPPH radical scavenging and ferric chloride reducing power assays. The oil demonstrated substantial antioxidant potential, as evidenced by its ability to scavenge DPPH radicals and exhibit reducing power comparable to the standard antioxidants, ascorbic acid.[9-13]

The potential mechanisms of action underlying the relationship between antioxidant potential and essential oils are complex and multifaceted. Essential oils, derived from aromatic plants, have demonstrated significant antioxidant properties, and their effects can be attributed to various bioactive compounds present in these oils. Essential oils are rich in compounds like phenols, terpenes, and flavonoids, which have been shown to effectively scavenge free radicals. Free radicals are highly reactive molecules that can cause oxidative damage to cellular components. Antioxidant compounds in essential oils donate electrons to neutralize free radicals, thereby preventing cellular damage. Certain constituents of essential oils, such as phenolic compounds, are known for their ability to donate hydrogen atoms. This process inhibits the chain reaction of free radical formation by stabilizing and neutralizing radicals. It is crucial to note that the specific mechanisms can vary among different essential oils due to variations in their chemical composition. The synergistic effects of multiple compounds within the oils likely contribute to their overall antioxidant potential. Further research is needed to elucidate the specific pathways and interactions involved in the antioxidant activity of individual essential oils.

Conclusion

The study provided valuable insights into the physicochemical characteristics and antioxidant activity of the essential oil extracted from Artemisia roxburghiana, suggesting its potential applications in various industries, including pharmaceuticals and cosmetics. Further research could explore its specific bioactive components and therapeutic properties.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Phytochemically tailored zinc based ayurvedic nano-medicine: Therapeutic Importance and Prospects

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Abstract- Ayurveda, a time honoured traditional system of medicines, has thrived on its profound understanding of the correlation between human body constitution and the elements of the universe. Yashad Bhasma (Zinc Oxide), a unique Ayurvedic metallic preparation, has been employed for centuries in treating various ailments, offering a holistic approach as an immune booster and a healing agent. Phytochemically tailored Zinc based herbomineral novel preparations/formulations have been explored to be an important wide spectrum therapeutic, based on blended pharmacy methods.

The article introduces the concept of zinc-based Ayurvedic nano-medicine, emphasizing the therapeutic potential of zinc oxide nano-particles, an evidence based herbo-mineral formulation which has embraced reverse pharm. principles. There lies resemblance between Ayurvedic Bhasmas and chemically metal oxides, Physicochemically, Biochemically and also in-turn physiologically. The trans-formation of metallic zinc into assimilablezinc oxide as per modified Ayurvedic methodology may be of paramount significance. This conversion not only eliminates the toxicity of the metal but also enhances biocompatibility, paving the way for targeted drug delivery and medical imaging on the lines of ADMET.

Furthermore, the study calls for comprehensive standardization measures, addressing qualitative and quantitative aspects. The article highlights the necessity of scientific strategies to navigate challenges in Yashad Bhasma research, ensuring its safety, quality, and medicinal efficacy by phytochemical blending. The research advocates for a harmonious blend of traditional wisdom and modern scientific rigor to propel Ayurvedic medicinal system, specifically Yashad Bhasma, into mainstream healthcare. Standardization and redesigning may emerge as the cornerstone for bridging the gap between ancient healing practices and contemporary healthcare to foster global acceptance and utilization of Ayurvedic treatments, accepted as have been realised, practiced and proved during pandemic Corona as Repurposed therapeutic strategy.

Key words: Yashad Bhasma, Biochemically, Biocompatibility, Nano-medicine, Thermo gravimetric.

Introduction

Ayurveda is one of the most renowned traditional systems of medicine that has survived and flourished from ages till date. With the enormous knowledge of nature based medicine, the relationship of human body constitution and function to nature and the elements of the universe that act in coordination and affect the living beings, this system will continue to flourish in ages still to come. There are many avenues still to be explored by the researchers, practitioners and experts in the field who carry the responsibility of keeping the traditional systems of medicine (TSMs) alive and contributing to their growth in the future. These futuristic goals can be accomplished when one gains insights about the systems, the principles and histories and works upon the strengthening aspects common between the various TSMs. With a rich knowledge of plants, minerals and animal based products, and the above based principles of doctrine, Ayurveda has achieved its widespread acceptance globally.

Metals play an important role in the human body, the deficiency of which leads to various disorders. In Ayurveda, metals such as gold, silver, copper iron, tin, zinc are described as medicinally important for the body. Perfect health is attributed to the state of equilibrium of these metals in body tissues. Any imbalance, whether excess or deficiency, disturbs the body metabolism. It has been described that metal-based formulations, called Bhasma, are highly effective in the prevention and cure of various diseases related to the organs where they are naturally found. Bhasmas are unique Ayurvedic metallic preparations with herbal juices/fruits, used in the Indian subcontinent since the seventh century BC and widely recommended for the treatment of a variety of chronic ailments. The Bhasmas are in fact products of classical alchemy inorganic compounds of certain metals and gems in a very fine powdered form, mostly oxides, made in an elaborate calcinations process known as bhasmikarana. It is believed that bhasmikarana process converts the metal into its specially desired chemical compound which eliminates the toxicity of the metal and has the necessary medicinal benefits[1,2]. Various minerals like iron pyrite, copper pyrite and bitumen; salts such as common salt, alkaline salt, black salt, and fossil salt; certain compounds like realgar, iron sulphate, copper sulphate and antimony sulphide were used in the preparation of Bhasmas due to their medicinal value. Some of the commonly used Bhasmas are Kajjali, Abhrak Bhasma, Naag Bhasma, Vang Bhasma, Jasad Bhasma, Tamra Bhasma, Mandoor Bhasma, Swarnamakshik Bhasma, Rasa Sindoor, Makardhwaj and Lauha Bhasma. They will be available as nano-particles and are taken along with milk, butter, honey or ghee; thus making the metals easily assimilable, eliminating their harmful effects and enhancing their biocompatibility[3]. The methods of bhasma preparation vary so much for each metal such that bhasma with different colours are produced. The Resultsants are considered to be same medicinal substances with the ascribed indications even though these may differ in the composition between them and should

ideally be addressing different ailments. In short, there is no standard bhasma of a metal as such. Ayurveda provides a list of tests for the efficacy of the bhasmikarana process. The tests are essentially qualitative and ensure that the Resultsing drug is very fine (small grains), has no metallic shine and does not alloy with silver even at higher temperature to which it was subjected[4-7]. However, these qualitative tests do not provide any quantitative information about the composition and the structure of the final drug. For any drug containing heavy metals (for example lead, mercury), such structural information is an absolute necessity. In view of such ambiguity and the risk due to their inconsiderate use, there is an urgent need to bring about a standardization of the preparation-process and the end product, as also to resolve the prospective indications and strengthen the regime to monitor the manufacturing, and admin.

Zinc is known to play a crucial role in biological processes, and adding it to nano-medicine can have a number of therapeutic advantages. Utilizing zinc oxide nano-particles is one such strategy, as zinc oxide has been researched for its biocompatibility and possible medicinal benefits. Zinc's special qualities combined with the adaptability of nano-materials make zinc-based Nano-medicine a promising therapeutic approach. The creation and characterisation of zinc oxide nano-particles for targeted medication delivery and medical imaging are the main objectives of this work. Zinc is a desirable option for use in Nano medicine since it is a vital micronutrient that is involved in many different cellular functions. Because of its adjustable qualities and biocompatibility, zinc oxide nano-particles provide a platform for developing novel therapeutic approaches.

Correspondence between Bhasma and metal oxide

In Ayurveda, bhasma is a special preparation that uses herbs and metals. Metal oxide forms are created from the initial forms of the metals employed in Bhasma1. The conversion is accomplished by heating the metals in a closed crucible using cakes of cow dung, a procedure called calcination.

The majority of the metals found in Ayurveda come from deliberate additions in the form of Bhasma. These metals are different from ambient metal forms in that they go through rigorous processing to change their molecular forms. For example, minerals utilized in Ayurvedic and Tibetan medicine, such as HgS, As_4S_4, PbS, PbO, etc., are treated to create Bhasma or Zuotai, which are distinct from ambient metal forms like $HgCl_2$, MeHg, $NaAsO_2$, NaH_2AsO_4, $Pb(CH3COO)_2$, etc.

The organically formed nano-particles known as Bhasmas are consumed with ghee3, milk, butter, or honey. This improves these elements' biocompatibility and facilitates their easy assimilation, hence removing any negative effects3. Significantly smaller particle sizes (1-2 μm) may aid in the drug's absorption and assimilation into the human system.

The primary correspondence lies in the conversion of metallic zinc into zinc oxide during the calcination process. This transformation is significant because zinc oxide is known for its unique properties,

including antimicrobial, anti-inflammatory, and wound-healing effects. The repeated incineration cycles not only transform the zinc into its oxide form but also contribute to the reduction in particle size. This nano-sized structure may enhance the bioavailability and therapeutic efficacy of Yashad Bhasma. Zinc oxide has been studied for its medicinal properties, and the correspondence between Yashad Bhasma and zinc oxide suggests that the Ayurvedic preparation may inherit some of these therapeutic benefits. These properties may include anti-inflammatory, antioxidant, and immunomodulatory effects.The addition of herbal extracts during the preparation of Yashad Bhasma introduces a holistic approach to healing. The combination of zinc oxide and herbal components may create a synergistic effect, providing a more comprehensive therapeutic impact[(11)].

Yashad Bhasma: an ancient Zinc based nano-medicine

Biological nanocrystals are called bhasmas. Nano crystalline materials are solids made up of crystallites smaller than 100 nm in at least one dimension, according to the field of nanotechnology. The obtainable grain size is influenced by milling parameters such as product type and milling temperature. Particle size is decreased by applying the Ayurvedic principles of marana (trituration) and bhavana (levigation). Scanning electron microscopy, transmission electron microscopy, fast freeze fracture, fluorescent microscopy, X-ray photo-electron spectroscopy, atomic absorption spectroscopy copy, gel electrophoresis, and enzyme expression are some of the techniques used to find nano-particles in bhasma. Three steps were included in the testing of nano-particles in Bhasma:

1. To determine whether a test sample contains nano-particles .

2. To determine the homogeneity of a chemical substance.

3. The crystalline or amorphous nature of the nano-particles .

Standardizationof Yashad Bhasma

Standardization is a measurement for ensuring quality. It refers to all actions taken during the production process and quality control that produce a reproducible quality. Plant life cycles, from seed to finished product, are greatly influenced by herbal compositions. It also refers to the process of incorporating excipients or mixing herbal remedies or drug preparations in order to achieve a desired level of a constituent or group of compounds with established therapeutic activity, respectively, in the herbal drug preparation. Standardization is a challenging task since numerous factors influence the repeatable therapeutic impact and bio-efficacy. To produce high-quality herbal products, attention must be paid from the outset of the preparation process.

There are very few scientific analytical investigations on standard bhasma preparations, and the ones that do exist are often incomplete. As a Results, a scientific strategy that takes the following actions is absolutely necessary.

i.Standardization of raw materials and completed products in terms of form and function.

ii. Determining the metals' level of oxidation and connecting them to acidic radicals in the final product.
iii. Metal extraction from tissues or the use of tracer techniques to study the pharmacokinetics of the main metallic component of bhasma.
iv. Research on metal buildup in various organs and tissues. e. Both acute and long-term toxicity.
v. Heat shock protein expression.
vi. How bhasmas affect typical antioxidant and physiological markers.
vii. Bhasmas's therapeutic reaction at the cellular and molecular levels on the suggested disease model (based on claims made in writings related to ayurveda).
viii. Bhasmas' function as drug transporters, and
ix. The function of bhasmas in digestive tract physiology and immuno modulation of the organism.

Standardization techniques

Organoleptic test

Organoleptic tests are an essential part of the quality control process for Ayurvedic bhasma. The organoleptic test of Ayurvedic bhasma is performed to evaluate the sensory properties of the substance, such as its color, odor, taste, and texture. The test is used to ensure that the bhasma is of high quality and free from impurities. The test is typically performed by trained professionals who have experience in evaluating the sensory properties of substances. The Results of the organoleptic test are used to determine whether the bhasma meets the required quality standards for use in Ayurvedic medicine. This information is important for researchers who are studying the efficacy of Ayurvedic bhasma and its potential use in treating various health conditions.

Physicochemical evaluation

Anherbo-mineral concoction called Ayurvedic Bhasma is utilized in Ayurvedic medicine. Ayurvedic Bhasma's physico-chemical analysis is a crucial component of quality assurance. Several physical and chemical characteristics of the Bhasma, such as its surface area, shape, particle size, and chemical composition, are analyzed as part of the evaluation process. Organoleptic testing are another step in the evaluation procedure that evaluate the Bhasma's sensory attributes. The Ayurvedic Bhasma is checked for impurities and high quality using the Results of the physicochemical examination. Researchers looking into the effectiveness of Ayurvedic Bhasma and its possible applications in treating a range of illnesses should find this information to be very useful[6].

Microbiology evaluation

- One of the most important aspects of guaranteeing the quality and safety of different products is microbiological examination. The evaluation of the total fungal, total Enterobacteriaceae, and total viable aerobic counts is part of it. An overview of the microbial load in a sample is given by these tests[3].
- Certain pathogens, such as Salmonella spp., E. Coli, S. aureus, and Pseudomonas aeruginosa, are also screened. These bacteria' presence may be a sign of pollution and possible health hazards. Regulatory agencies like the Food and Drug Administration (FDA) in

the United States and the World Health Organization (WHO) determine the acceptable limits for certain infections and microbial loads. For example, the FDA requires that an ingredient's live microbial content, measured by aerobic plate count, be less than 10,000 organisms/gram. The FDA advises against using any analytical unit with an MPN of 9.2 or more coliform organisms per 100 milliliters for E. Coli.

- When it comes to Salmonella species, a method that finds three MPN species per 4 grams (or milliliter, if a liquid sample is being taken) of total solids should not be used to detect them. The Bacterio-logical Analytical Manual published by the FDA offers instructions for identifying and counting S. aureus. Regarding Pseudomonas aeruginosa, the FDA offers guidance for its identification even though precise allowed levels are not easily accessible. It's crucial to remember that these restrictions may change, based on the particular product and how it will be used. Consequently, in order to guarantee the security and calibre of their goods; manufacturers need to abide by these rules[(4)].

Analytical evaluation

Analytical evaluation of Bhasma is essential to ensure the quality and safety of the product. Several modern analytical techniques are available for this purpose. Among them X-ray Diffraction (XRD) is a significant technique that can detect the compounds of the material and free metals in it, if they are within detectable limits. High Power Thin Layer Chromatography (HPTLC) is another method used to identify the active ingredients in Bhasma[(7)]. SEM (Scanning Electron Microscopy), TEM (Transmission Electron Microscopy), and AFM (Atomic Force Microscopy) are advanced microscopy techniques employed for the characterization of materials at the nanoscale. In the context of Yashad Bhasma, these techniques are utilized to investigate the morphological and structural features of the nano-particles formed during its preparation [(14)].

In addition to SEM, TEM, and AFM, several other scientific techniques can be employed for the comprehensive characterization of Yashad Bhasma. These techniques provide valuable information about its chemical composition, structure, and physical properties. Here are some additional techniques:X-ray Diffraction (XRD): Fourier Transform Infrared Spectroscopy (FTIR),Energy-Dispersive X-ray Spectroscopy (EDS or EDX), Nuclear Magnetic Resonance (NMR), Thermogravimetric Analysis (TGA), Raman Spectroscopy etc.

Integrating these techniques allows for a thorough understanding of Yashad Bhasma's chemical and physical characteristics, aiding in its standardization and quality control. Each method contributes unique insights that collectively contribute to a comprehensive characterization of this Ayurvedic nano-medicine.

Additionally, an Atomic Absorption Spectrometer (AAS) can be used to analyse bhasma elementally by determining the concentration of metallic elements in Bhasma. This is especially crucial because Bhasma should include levels of harmful metal components that are safe for humans to consume.

To sum up, Bhasma's analytical assessment is essential to guaranteeing both its safety and medicinal efficacy. It enhances the manufacturing of high-quality Ayurvedic products and reduces product irregularities.Further more, to enhance the ease of bioabsorption, it should further be tailored as per chelation therapy practices; this facilitates the therapeutic concentration limits *in-vitro* and *in-vivo* ; this regulates MIC also[10].

Navigating Challenges in Yashad Bhasma Research: Considerations and Concerns

Ayurveda has a rich history; however, there were certain drawbacks in approaches towards it, which inhibited its growth like the Western system of medicine. The active components of the herbal drugs prescribed were not known, and even today many drugs still need further exploration for their active constituent characterization and elucidation of the mechanism of action. Even after decades of applying advanced analytical techniques for drug analysis, herbal drugs still face some drawbacks. The administration of combinations of several drugs adds to the complexity of the study of the activity of these medications. A merit of traditional medicine systems as discussed earlier is that they consider every individual as the prime focus of treatment rather than the disease. But this factor also possesses a hurdle to the applicability of medications on a general population basis. Several issues like, the variation in the potency due to difference in species, absence of an integrated coding for every species used commonly in TSMs, varying geographical location of growth, and incorrect identification and adulteration of drugs, non-uniform quality control standards, differences in processing methods, direct an alarming need towards comparative study of drugs used in both these systems of medicine[12].

While Yashad Bhasma has been traditionally used in Ayurvedic medicine and is believed to have therapeutic benefits, it's essential to acknowledge potential drawbacks and limitations in the study and analysis of this preparation. Some of the drawbacks include:lack of standardization, heavy metal contamination, limited scientific research, bioavailability issues, interaction with herbal additives, individual variability, adulteration and quality control etc.The use of animal-based ingredients or unsustainable sourcing practices in the preparation of Yashad Bhasma may raise ethical concerns, especially for individuals who prioritize cruelty-free or environmentally friendly products[13,14].

In conclusion, while Yashad Bhasma has a rich history in Ayurvedic medicine, addressing these drawbacks through rigorous scientific research, standard-ization, and quality control measures is crucial to ensure its safety and efficacy in contemporary healthcare practices. Individuals considering the use of Yashad Bhasma should consult with qualified healthcare professionals to make informed, decisions based on their specific health needs and conditions.

Conclusion

The discussion of the research article revolves around the significance of Yashad Bhasmas in Ayurveda and the need for

their standardization and uses. Bhasmas, being unique Ayurvedic metallic preparations, have been used for centuries to treat various ailments; and thus Reverse-pharmacology principle validates it for therapeutic practices. Therapeutic dozing based on tolerable concentration regulation, is essential as per pharmacological guidelines. This variability presents a challenge in ensuring the consistency and efficacy of these medicines.

Furthermore, it would ensure that the Resultsing drug is very effective, thereby increasing the global acceptance and utilization of Ayurvedic treatments. The research underscores the importance and prospect of standardizing zinc-based Ayurvedic nano-medicine, paving the way for future studies and advancements in this field.The research establishes a connection between Bhasma and metal oxide, highlighting the transformation of metallic zinc into metallodrug.

The study introduces the concept of zinc-based nanomedicine, recognizing zinc's vital role in biological processes. The utilization of zinc oxide nano-particles is explored, offering a promising strategy for targeted drug delivery and medical imaging. The creation and characterization of these nano-particles emerge as primary objectives, aligning with modern therapeutic approaches on the lines of Chelation-therapy.

The research article calls for a comprehensive standardization and formulation of zinc-metallodrug as Ayurvedic nano-medicine. Addressing drawbacks, such as lack of standardization and heavy metal contamination, through rigorous scientific research and quality control measures is crucial. The study opens avenues for future research, tailoring and monitoring to encourage advancements in Ayurvedic medicinal system to pave the way for the integration of traditional wisdom with contemporary modern healthcare practices. This opens the Gates to Review, Revise, Reform and Revive the Ayurvedic and Traditional Indian Medicinal System.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Qualitative phytochemical analysis of ethanolic and aqueous extracts of *Origanum majorana*

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Abstract- Preliminary phytochemical analysis has been performed in aqueous and ethanolic extracts of *Origanum Majorana* (leaves and flower), the various phytochemical constituents like, alkaloids, triterpenoids, anthraquinone, emodin, terpenoids. steroids, flavonoids, glycolsides, tannin, phenol and saponin are present in all the extracts. All the phytochemicals are abundantly found in leaves extract followed by in flower extract.

Key words: *Origanum majorana*, Phytochemical analysis, Extracts

Introduction

Plant parts such as, leaves, seeds, vegetables, fruit, spices etc with value have been used to cure many diseases since ancient time. Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. There is a wide diversity of compounds, especially secondary metabolites (like, alkaloids, triterpenoids, anthraquinone, emodin, terpenoids. steroids, flavonoids, glycosides, tannin, phenol and saponin) found and isolated from plants and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent **(Cai et al., 2004; Miliauskas et al., 2004).**

Origanum majorana is a medicinal plant of the Lamiaceae family, known as Maruwa in traditional Indian medicine. This plant is distributed around the Mediterranean regions, Asia, and North Africa, in particular, Morocco, Algeria, Egypt, Spain, and Portugal (**Vasudeva P and Neeru 2015).** *Origanum majorana* showed various biological activities such as allergies, fever, hypertension, respiratory infections, anti-diabetic, painful menstruation, Kidney Yang deficiency, stomach ache, cough, rheumatism, headache, insomnia, also in intestinal antispasmodic. Moreover, *Origanum majorana* exhibits a wide effect spectrum with antioxidant, antibacterial, antifungal,

nephroprotective, anti-proliferative, anti-cancer activities **(Hajlaoui H et al, 2016; Kozáowska M et al, 2010, Rao S et al, 2014; Abdel-Massih RM et al, 2018].** These effects are mediated by the presence of bioactive compounds such as thymol, carvacrol, tannins, hydroquinone, sito-sterol, cis-sabinene hydrate, limonene, terpinene, camphene, and flavonoids. The objective of this study was to carry out preliminary phytochemical screening and to determine the contents of *Origanum majorana*.

Plant material and sample preparation

Flowers and leaves of *Origanum majorana* were collected from botanical garden of D.A.V. College, Muzaffarnagar. They were rinsed with tap water followed by distilled water to remove the dirt on the surface. They were then air dried for 2 days and then freeze dried until a constant mass was obtained. Dried samples were ground into fine powder and kept in desiccators until extracted. The extraction was carried out in a soxhlet apparatus for 12 hours using 50% of aqueous and ethanolic solvent at room temperature. The extracts were filtered using Whattman filter paper and filtrates were used as an extract. Extracts were kept at 4 °C until the bioassay analyses.

Phytochemical screening

The aqueous and ethanolic solvent extracts of flowers and leaves were tested for the presence of alkaloids, steroids, tannins, flavonoids, terpenoids, saponins and glycosides etc. The qualitative Results are expressed as (+) for the presence and (−) for the absence of phytochemicals using standard procedures to identify the constituents as described by (**Sofowara, 1993; Trease and Evans, 1989; Harborne, 1973 and 1984).**

Test for Tannins: 1ml of every sample is boiled in 20 ml of distilled water in a test tube and then filtered separately. A couple of drops of 0.1% ferrous chloride are added Resultsing into brownish green or a blue-black colouration.

Test for Saponins: 2 ml of every sample is boiled in 20 ml of distilled water in a water bath and filtered separately. 10ml of the filtrate is mixed with 5 ml of distilled water and jolted smartly for a stable persistent froth. The frothing is mixed with three drops of olive oil and jolted smartly, Resultsing into the formation of emulsion.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a little of the liquid filtrate of every plant extract followed by addition of targeted H_2S0_4. A yellow colouration in every extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Steroids: 2 ml of acetic anhydride were added to 1ml of extract of every sample with 2 ml H_2SO_4. The colour modified from violet to blue or inexperienced in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski test): 5 ml of every extract was mixed in 2 ml of chloroform, and targeted H_2SO_4 (3 ml) is rigorously added to create a layer. A venetian red colouration is pointecd out positive Results for the presence of terpenoids.

Test for Triterpenoids: One ml of extract is added to 1 ml of chloroform; 1 ml of acetic anhydride is additional following the addition of 2 ml of diluted H_2SO_4.

Formation of blood-red violet colour indicates the presence of triterpenoids.

Test for Alkaloids: Mayer's test: To a couple of (one) ml of every extract, a drop of Mayer's chemical agent was added by the side of the test tube. A creamy or white precipitate confirms presence of alkaloids.

Test for Anthraquinones: 5ml of every extract solution is hydrolyzed with diluted H_2SO4 extracted with benzene. 1 ml of dilute ammonia is added to this solution. Pink coloration steered the positive response for anthraquinones.

Test for Polyphenols: Plant product (4 ml) is added to every extract (1ml) and the ensuing resolution is transferred and warmed in a water bath (15 minutes). 3 drops of freshly made ferrous cyanide resolution were added to the extract resolution. Formation of a blue color indicated the presence of polyphenols.

Test for Glycosides (Keller-Killani test): Five ml of every extract is treated with 2 ml of glacial acetic acid containing one drop of ferrous chloride resolution. This is often under layed with 1 ml of targeted H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring could seem below the brown ring, whereas within the carboxylic acid layer, a green ring could be seen simply step by step throughout skinny layer.

Test for Emodins: 2ml of NH_40H and 3ml of benzene were added to the extract. Appearance of red colour indicated the presence of emodins in the test solution.

Results and discussion

The phytochemical screening of 50% ethanolic and aqueous extracts of leaves and flowers samples of *Origanum majorana* revealed the presence of some biologically active metabolites such as, alkaloids, triterpenoids, anthraquinone, emodin, terpenoids. steroids, flavonoids, glycosides, tannin, phenol and saponin etc as shown in **Table-1.**

Table-1 Qualitative analysis of phytochemical in different parts of *Origanum majorana* in Aqueous and ethanolic extract

S.No.	Name of bioactive compound	Flower		Leaf	
		Aq.	Et.	Aq.	Et.
1	Alkaloids	++	++	++	+++
2	Triterpenoids	+	++	+++	+++
3	Anthraquinones	++	++	++	+++
4	Emodins	++	+	++	+++
5	Glycosides	++	++	++	+++
6	Tannin	+	+	+++	+++
7	Terpenoids	++	+++	-	++
8	Steroids	-	-	-	-
9	Phenols	++	+++	+	++
10	Saponin	++	++	+++	+++
11	Flavonoids	-	++	+++	+++

aq=
aqueous extract, et=ethanolic extract, + = presence, - = absence

Tannins were detected in the leaf extract but not in the flowers extract of *Origanum majorana*. Terpenoids are absent in aqueous leaf extract of *Origanum majorana*. Steroids are found to be absent in all studied parts of *the Origanum majorana*. All the phytochemicals are abundantly found in leaves extract (aqueous and ethanolic) followed by in flower extract. This phytochemical screening is more prominent in ethanolic (organic) extract as compared to aqueous extract, as bioactive compound are organic in nature and soluble in organic solvent.

The phytochemical compounds detected are known to have medicinal importance. The flavonoids and phenols are major compounds that act as antioxidants or free radical scavengers **(Bhandary et al., 2012).** The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported **(Kubmarawa et al. 2007.)**For example, many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory antimalarial ,antimicrobial and pharmacological effects. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties They are very often used in medicines due to their well-known biological activities. Tannins, according to research, are known to have antibacterial **(**Augusto et al., 2011**;**Benbott et al., 2012**;** Hisanori et al., 2001**).** Phytochemicals such as cardiac glycosides have been used to treat congestive heart failure **(Vladimir and Ludmila, 2001)**.

Conclusion

From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. These phytochemical compounds identified in the leaf and flower extracts may be responsible for the biological activities shown by *Origanum majorana* and the reason for their use as a traditional medicine. This phytochemical screening test may be helpful to provide a therapeutic platform to detect and develop new drugs.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Green synthesis of silver nano-particles (AgNPs) from flowers of *Rhododendron campanulatum* and its potential applications as photo-catalyst and antioxidant

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Abstract-The present work demonstrates the Green synthesis of silver nano-particles (AgNPs) using the *Rhododendron campanulatum* flower extract and their potential applications as photo catalyst and antioxidant. Several spectral approaches viz., UV-Vis, XRD, SEM-EDAX, BET isotherm and TEM were used to characterise the biosynthesized AgNPs in detail.The UV-Vis spectroscopy showed that the biosynthesized AgNPs have surface plasmon resonance (SPR) peak at 472 nm. The X-ray diffraction (XRD) studies confirmed the crystalline nature of biosynthesized AgNPs with mean crystalline size ~22.97 nm. According to the TEM examinations, the synthesized AgNPs were poly dispersed, smooth in morphology, irregularly shaped, and had an average grain size of 43.75 nm. The adsorption studies showed that the biosynthesized AgNPs have the single point surface area (at P/Po) was 0.1398 m^2/gm, BET surface area was 0.1057 m^2/gm, Langmuir surface area was 0.1465 m^2/gm, and the micro pore area was 0.7834 m^2/gm. The antioxidant efficacy of as synthesized AgNPs was determined using DPPH scavenging method, and the IC_{50} value was observed 38.68 µg/mL. Photocatalytic activity of synthesized AgNPs was determined by studying the adsorption/desorption equilibrium between the AgNPs and the solutions of MB, and RB dyes. The synthesized AgNPs showed the dye degradation efficacy of 73.88% for methylene blue (MB) dye, and 76.62% for Rose Bengal (RB) dye; however, in the absence of AgNPs, the degradation efficacy was observed as17.55% and 48.80% in 4 hours under photo-irradiation for MB and RB dye, respectively.

Keywords: *Rhododendron campanulatum* , Green Synthesis, Agnps, Antioxidant, Photo-Catalytic Degradation, Methylene Blue, Rose Bengal Dye.

Introduction

Nano-technology is one of the most fascinating and emerging area of research across all discipline of sciences. The controlled fabrication of materials at

different nano size scale is the major advantage of nanotechnology, which offers liberty to researchers to design the material of their need. These nano sized particles exhibit some unique characteristics compared to those of macro sized particles of bulk materials. If the nano-material synthesis is achieved via Greener synthetic approach it makes the process more acceptable due to strict environmental protocols associated with the uses of chemicals in synthesis. Therefore, in the synthesis of nano-material Greener approach in synthesizing the nano-material is presently an area of greater interest among the researchers due to its biocompatibility, less toxicity, easy adaptability and cost effectiveness. These nano-structured materials synthesized via green approach are applicable as a purifying agent, sensors, nano-medicines, optoelectronic devices, detoxifying agents, and many more[1-5]. Green mediated nano-material from plants, microorganisms, algae[6], and employing biological materials with enhanced properties, such as greater cost-effectiveness, low hazard, more eco friendly nature and well-defined shape and size[7]. Nanotechnology is a revolutionary technology for the modern society due to its enhanced applications in agricultural and food industries[8].The formation of toxic and non-degradable chemicals is grim point of discussion in present time, which pollute the major resources of our environment *viz.* air, water, plant growth, ecology and many more[9-10]. Therefore, the researchers are focusing on detoxification and recycling of waste-water by applying nano-material in the treatment of waste water[11].

The development of such nano-materials with outstanding physical, chemical, and biological properties, such as biosensing, optical, antioxidant, catalytic capabilities comprising antibacterial, antiviral, drug transport, and so forth, has been the main emphasis of the current effort[12-16].The novel aspect of this work is the creation of silver nano material using an extract of flowers from a *Rhododendron campanu-latum* tree in the Garhwal Himalaya region of Uttarakhand, India. This was done for the first time using this plant material and is in accordance with the principles of green chemistry. The reason behind the selection of this plant was the richness of phenolic compounds in its extract, which is responsible for anti-oxidant activity[17]. Selected plant *R. campanulatum* has of medicinal properties like it is quite useful in cold, chronic fever, sciatica and hemicrania, while its bark is useful for digestive and respiratory disorders, and roots are useful to recover from many diseases like boils, headache, fever and so on[18]. Antioxidants play an important role in scavenging radicals and thus providing protection against infections and degenerative diseases[19-20]. By eliminating the free radical intermediates, antioxidants stop these chain reactions and stop additional oxidation processes. A potential source of bioactive substances with antioxidant effects is plants. Several medicinal plants include antioxidants, such as vitamin C (ascorbic acid), vitamin E, lycopene, etc., which aid in scavenging free radicals[21-23].For the breakdown of harmful dyes like methylene blue, methyl orange, alizarin red, acridine orange, malachite green, and many others, the majority of silver nano particles (AgNPs) have excellent photo-catalytic activity[24].

Material and methods

Chemicals- $AgNO_3$, methylene blue

(MB) and rose bengal (RB) dyes were purchased from Sigma Aldrich and plant material flowers of *Rhododendron campanulatum* voucher specimens GUH 0743[18] deposited at HNB Garhwal University were collected from Tungnath region of Garhwal Himalaya, Uttarakhand (India) and were utilized in this study.

Methodology

In the preparation of flowers extract of *Rhododendron campanulatum* (RCF), fresh flowers of *R.campanulatum* **(see** Figure**.1)** were collected and dried in the absence of sunlight at room temperature for 10-15 days for the preparation of non-volatile as well as stable compounds of nano particles, then the dried flowers were crushed in powder form. In a clean round bottom flask, 5 gm of crushed flowers was boiled in 100 mL of distilled water at 75−80°C for 30 min. The extract was cooled and filtered twice with Whattman filter paper no. 1. The freshly prepared extract was collected for analysis of targeted object[18-19].

Synthesis of silver nano particles (AgNPs) takes place via green route chemistry, in which flowers extract of *R. campanulatum* added into 5 milli molar aqueous solution of $AgNO_3$ in the ratio of 1:11, respectively. After three to four days the colourless solution of plant extract and silver nitrate was turned into dark brown red colour due to surface plasmon resonance (SPR) as well as presence of some phytochemical compounds present in the plant extract, which stabilize the formation of nano particles. After the reduction of solution, it was centrifuged at 5000 rpm at room temperature for 30 minutes, then residue as AgNPs collected and washed thrice by deionised water and kept at cool and dry place for further analysis. Different instrumental methods, such as the UV double beam spectrophotometer, XRD, SEM-EDAX, BET isotherm, and TEM, were used to characterise the synthesised nano materials[12,18-19].

Evaluation of Antioxidant activity

Antioxidant activity of synthesized silver nano particles (AgNPs) had been done by DPPH scavenging method. For this stock solution of sample was prepared by dissolving 100 mg of AgNPs in 100 mL of DMSO (Dimethyl sulphoxide), which was separated in several dilutions*i.e.*10µg/mL to 80 µg/mL for the test of antioxidant activity. Antioxidant activity was done against the ascorbic acid as a standard at similar dilution (10µg/mL to 80µg/mL) of stock solution. After 15 minutes, the DPPH sample's final absorbance at various concentrations was measured at 517 nm. Protocol for antioxidant activity has been already described in our previous work[20-24]. Percentage inhibitions of DPPH radical given by green synthesized AgNPs were determined by the following formula:

$$\%\ \textbf{inhibition} = \frac{(\textbf{Ac} - \textbf{As})}{\textbf{Ac}} \textbf{x100}$$

A_C and A_S represents the absorbance of control and sample respectively.

Determination of Catalytic activity

To evaluate the photo catalytic activity, 10 mg of biosynthesized AgNPs, were separately added to 100 mL aqueous solution containing 5 mg of methylene blue (MB) and rose bengal (RB) dyes separately. To establish adsorption / desorption equilibrium between the AgNPs and the solutions of MB and RB dyes, the mixture was constantly agitated in the dark for 1 hour. The suspension was then exposed to photo-radiation. The

suspensions were removed from the reactor, centrifuged at 10min intervals, and their distinctive absorption spectra were recorded using de-ionized water as a reference on a UV-vis spectro-photometer[25-30,33]. The concentrations of the dyes were calculated using calibration curves. Methodology also discussed in previous paper Sati *et.al.*, 2021.

The following formula was used to determine how well synthesized nanomaterial (AgNPs) degraded dyes:

$$\textbf{Degradation}\ (\%) = \frac{(\mathbf{Co} - \mathbf{Ct})}{\mathbf{Co}} \mathbf{x100}$$

Where;

C_o and C_t are the concentration of dye sat t=0 and at time *t* after solar irradiation respectively.

Results and discussion

Characterization of silver nano-particles

Green synthesis of metal nano particles (AgNPs) was characterized by several instrumental techniques. These Results are as follows:

First of all, the formation of AgNPs was confirmed by bio-reduction of Ag^+ metal ion into AgNPs, which shows the UV absorption maxima peak (UV model 3375 Electronics India) for AgNPs at 472 nm for $5RCF_{1:11}$.2θ values of XRD (PAN analytical, X'PERT PRO) peaks were 37°, 43° and 63° correspond to the (111), (200), and (220) planes, respectively, for *fcc* crystals of synthesized AgNPs and the crystal size was calculated 22.97 nm (approx.). SEM images indicated the presence of agglomerated nano-material of AgNPs. To confirmation of AgNPs formation, EDAX was performed, in which a strong peak of elemental Ag (69.14 weight %) and other peaks of O (22.24 weight %) and C (8.62 weight %) elements were present. The synthesized powdered sample of nano material additionally used for TEM analysis in which it was observed that the green AgNPs were polydispersed, smooth morphology and irregular shaped grains having average size of 43.75nm[12,18-19,31-36]. BET isotherm plot **(see** Figure**.2)** of synthesized sample revealed that the synthesized AgNPs have single point surface area (SPSA at P/Po) was 0.1398 m^2/gm, BET surface area was 0.1057 m^2/gm, Langmuir surface area (LSA) was 0.1465m^2/gm, and the micro pore area was 0.7834 m^2/gm[28], on the basis of observed data due to which it may become a corrosion free material and may use as a good anti-corrosion material as well as protecting material.

Figure**ure1-** Flowers of *R.campanulatum* (RCF)**;**

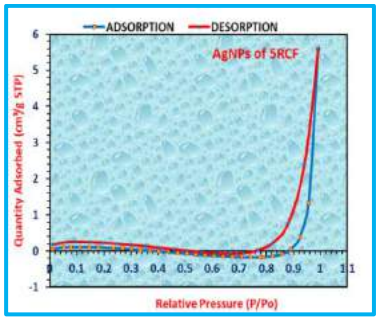


Figure **ure 2-** BET isotherm plot of AgNPs.

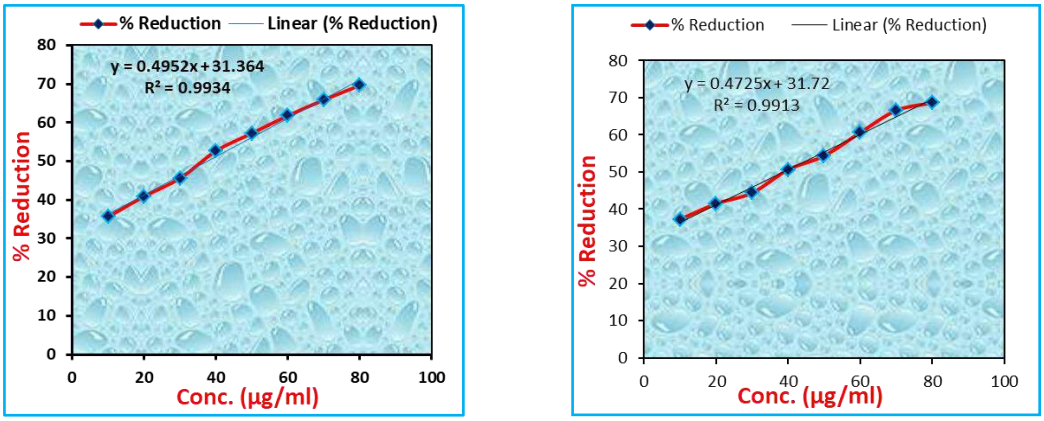
Evaluation of Antioxidant activity

Antioxidant activity of synthesized nano materials had been performed by DPPH method with respect to ascorbic acid as

standard and AgNPs as sample. In the determination of antioxidant activity, the following (Concentration v/s Percentage Reduction) graph and data shows that synthesized nano-material (AgNPs) were a good antioxidant material, for this IC_{50} value is 38.68µg/mL (**see Table-1;** Figure**ure-3**), which is just near to the IC_{50} value of the standard ascorbic acid (*i.e.* 37.63µg/mL)[20-24,32,34].

Table-1 Antioxidant activity data of Standard and sample (AgNPs)

S. No.	Conc. (µg/mL)	Absorbance		% Reduction		IC_{50} Value (µg/mL)
		Standard	AgNPs	Standard	AgNPs	
1.	10	0.300	0.310	35.60	37.28	
2.	20	0.290	0.280	40.89	41.47	**Standard 37.63**
3.	30	0.275	0.265	45.59	44.39	
4.	40	0.250	0.250	52.65	50.64	
5.	50	0.220	0.243	57.23	54.35	
6.	60	0.187	0.230	61.78	60.58	**AgNPs 38.68**
7.	70	0.164	0.218	65.86	66.54	
8.	80	0.147	0.210	69.60	68.60	



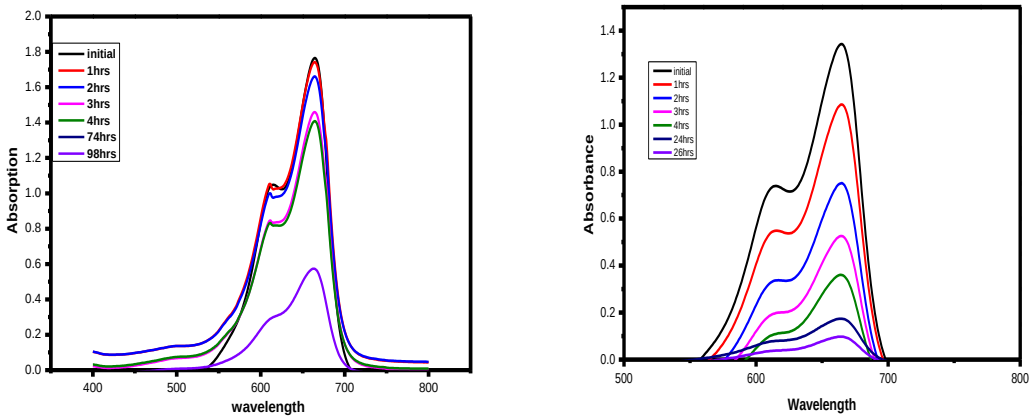
(a) **(b)**

Figure**ure 3-** Graph of Antioxidant activity for **(a)** Standard (Ascorbic acid); and **(b)** Sample (AgNPs).

Determination of Catalytic activity

Catalytic degradation of methylene blue and rose bengaldye was analyzed by UV spectrometer, in which the absorption maxima peak of MB and RB dye was shifted towards lower absorption maxima value *i.e.* hypochromic shiftin characteristic peak at the time intervals of 1 hour. The hypochromic shift of characteristic peak of MB and RB dye was the indication of dye degradation process. The amount of dye degradation was

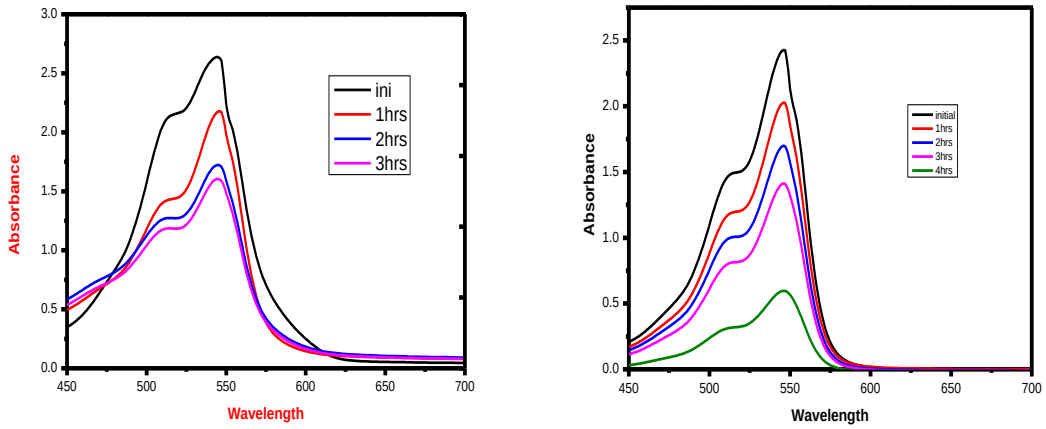
calculated 73.88% in 4 hours **[**Figure**ure 4 (b)]** in the presence of AgNPs as a nano catalyst, while in the absence of nano catalyst (AgNPs) it shows 17.55% degradation of methylene blue dye **[**Figure**ure-4(a)]**. These findings show unequivocally that the MB dye decayed very quickly in the presence of produced AgNPs, but this degradation proceeded very slowly in the absence of AgNPs[23-27,29-30, 33].



(a) (b)

Figure**ure 4-** Degradation of MB dye (a)without AgNPs, and (b) with AgNPs as catalyst.

Similar Results were obtained in the photo-catalytic degradation of the dye rose bengal (RB), which was likewise facilitated by artificial AgNPs. The UV graph showed a reduction in the RB absorption peak within four hours. Initially (at t=0 hr), the value of absorption maxima peak of MB dye was 2.541 (at 543 nm) which was decreased very sharply upto 0.594 value of absorption maxima peak due to 76.62% degradation of dye on exposing to sunlight in the presence of synthesized NPs[see Figure.5(b)], It shows that the photo-catalytic degradation of the RB dye was completed to a 76.62% level within 4 hours, however in the absence of AgNPs, it was discovered that the nano catalyst only degraded the RB to a 48.80% level of its starting amount within 4 hours as seen by a UV-Visible spectrophoto-meter. These findings show unequivocally that green nano-catalyst caused MB and RB dyes to degrade quickly[23-28]. Therefore, AgNPs are good catalyst for toxic dye degradation.



(a) **(b)**

Figure**ure 5-** Degradation of RB dye (a) without AgNPs, and (b) with AgNPs as catalyst.

Conclusion

In the present study, AgNPs were synthesized by using flowers extract of *R. campanulatum* via green chemistry synthesis. Due to richness of various phyto-chemical compounds in flowers extract which played an important role as a reducing as well as capping agent in the formation of AgNPs from $AgNO_3$ solution of 5mM.In this environment friendly

procedure, average size of synthesized NPs was 43.75 nm, and the BET isotherm plot revealed that the surface area of synthesized AgNPs was 0.1057 m^2/gm, which shows the significant presence of silver metal in synthesized sample. In the application part of nano material, synthesized silver nano material showed significant antioxidant property for which the IC_{50} value was 38.68µg/mL with respect to ascorbic acid as standard (IC_{50} value was 37.63µg/mL).The photo-catalytic degradation of the poisonous dyes methylene blue (MB) and rose bengal (RB) dyes demonstrated the remarkable catalytic properties of green produced nano materials. Within 4 hours of solar exposure, the MB dye was degraded by 73.88% in the presence of AgNPs, compared to 17.55% in the absence of the nano catalyst (AgNPs). Similar to this, after 4 hours of solar irradiation, the RB dye decomposed 76.62% in the presence of AgNPs and 48.80% in the absence of AgNPs. As increasing the time period of solar irradiation of the MB dye degraded 93.22% with AgNPs and without AgNPs degraded only 23% within 26 hours.These facts demonstrate the superior antioxidant and catalytic properties of the environment friendly nano-material (AgNPs) for the photo degradation of harmful colours. So, we can conclude that synthesized nano material may be applicable in the development of strong oxidizing agent, anti-corrosion material, water purifying agents as a removal of toxic contaminants as well as dye degrading agent in pure water conservation and in industries e.g. pharmaceutical, cosmetics, electronics and many more.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Chemical characterization and antimicrobial activity of *Sesamum indicum* (Sesame oil)

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Abstract- It has been noted from the past few decades, researchers are striving for the development of natural alternate to antibiotic due to their resistance problems. Bacterial infection and its resistance is a major health issue that affects millions of people throughout the world. *Sesamum indicum* L. is one of the important seed crops worldwide to get edible seed and oil. In the present study we evaluate the chemical characteristics including refractive index, wt/ml, pH, viscosity, acid value, iodine value and peroxide value and found that the obtained Results are under the specification. The antimicrobial activity of oil shows that the minimum inhibitory concentration (MIC) is in the range of 17 µl to 460 µl. the best MIC is 17 µl against *Salmonella species.* The zone of inhibition of sesame oil against the tested microorganism is in the range of 18 mm to 25 mm. the best zone of inhibition is 25 mm against *Salmonella species.* So we can say that the antimicrobial activity of sesame oil is best against *Salmonella species*out of the microorganism which we have taken for activity.

Introduction[1-2]

Sesamum indicum L. is one of the important seed crops worldwide to get edible seed and oil. This crop is cultivated since ancient times. Various species of sesame were found in Africa and generally believed that it is originated in Africa while a smaller number in India. Sesame seed is used wholly in cooking because of its rich nutty flavor and oil. Commonly the paler varieties of sesame seem to be more valued in the West and Middle East, while the black varieties are highly valued in the East. It is used as a cooking oil, as a soap fat, in pharmaceuticals and as a synergist for insecticides. In many countries sesame oil is very popular as cooking oil and is more expensive than other vegetable oils. Sesame oil apply for darker hair and also for loss of hair. Refined sesame oil is used in herbal drugs manufacturing company. Sesame oil is a source of anti-oxidant as it contains vitamin E and its anti-oxidant activity has been reported. Sesame oil is also used in lowering cholesterol levels. Sesame oil contains magnesium, copper, calcium, iron, zinc and vitamin B6. Copper present in sesame oil can provide relief in rheumatoid arthritis. Magnesium of sesame oil supports vascular and respiratory health systems while Calcium helps to prevent colon cancer, osteoporosis, and migraine. The aim of the present study is to chemically characterize

as well as evaluate the antimicrobial activity of sesame oil.

Material and Methods

Sesame oil is purchased from local supplier of Delhi (Shiv Sales Corporation). All chemical and reagent are HPLC or Analytical grade.

Chemical Characterization[3]

Refractive index

The refractive index (η) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. The Abbe's refractometer is used for measurements of refractive index. To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25^0.

Apparatus is used per the manufacturer instruction or as per the SOP.

Weight per ml

The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25 ^{0}C. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25 ^{0}C and weighing the contents. Assuming that the weight of 1 ml of water at 25 ^{0}C when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the Results of a determination significantly). Adjust the temperature of the substance to be examined, to about 20 ^{0}C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25 ^{0}C, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

pH

The pH value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and a pH meter. Operate the instrument as per the manufacturer instruction or as per the SOP.

Viscosity

Viscosity is a property of a liquid, which is closely related to the resistance to flow. Viscosity is measured by the help of Brookfield Viscometer using spindle no. 3 and speed of 50 RPM.

Acid Value

The acid value is the number of mg of potassium hydroxide required to neutralize the free acids in Weigh accurately about 10 g of the substance into a 250 ml flask and add 50 ml of a mixture of equal volumes of alcohol and solvent ether, which has been neutralized after the addition of 1 ml of solution of phenolphthalein. Heat gently on a water-bath, if necessary until the substance has completely melted, titrate with 0.1 N potassium hydroxide, shaking constantly until a pink color which persists for fifteen seconds is obtained. Note the number of ml required.

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

where 'a' is the number of ml of 0.1 N potassium hydroxide required and 'W' is

the weight in g of the substance taken.

Iodine Value

The Iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of the substance, when determined by one of the following methods:-

Place the substance accurately weighed, in dry iodine flask, add 10 ml of carbon tetrachloride, and dissolve. Add 20 ml of iodine monochloride solution insert the stopper, previously moistened with solution of potassium iodine and allow to stand in a dark place at a temperature of about 25 ^{0}C for thirty minutes. Add 15 ml of solution of potassium iodine and 100 ml water; shake, and titrate with 0.1 N sodium thiosulphate, using solution of starch as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of ml of 0.1 N sodium thiosulphate required (b).

Calculate the iodine value from the formula:-

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{W}$$

Where 'W' is the weight in g of the substance taken. The approximate weight, in g, of the substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

Peroxide Value

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance. Method Unless otherwise specified in the individual monograph, weigh 5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow color almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue color just disappears (ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression

Peroxide value = 10 (a – b)/W

Where W = weight, in g, of the substance.

Anti-Microbial Activity[4-12]

The following microorganisms were used for anti-microbial activity. Staphylococcus aureus, Escherichia coli, Salmonella species and Pseudomonas aeruginosa. All the microorganisms were maintained at 4°C on nutrient agar slants.

Determination of minimum inhibitory concentration (MIC) using micro dilution method:

The Minimum Inhibitory Concentrations (MICs) of sesame oil found to be active by the diffusion test were determined based on the macrodilution method (Berghe and Vlietinck, 1991) with some modifications as follows. The sesame oil was serially diluted (two-fold) in a

series of test tubes using nutrient broth supplemented with 10% glucose and 0.05% phenol red (colour indicator). These were later inoculated with 0.2ml suspension of the test organisms. The final concentrations were in the range 1000 to 10 µl/mL in the medium. Microbial growth was determined by observing for color change in the tube (red to yellow when there is growth). The lowest concentration that showed no change of color was considered as the MIC.

Anti-microbial activity

Cup plate method using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of sesame oil against different microbial strains. The agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. The cups each of 9mm diameter were made by scooping out medium with a sterilized corkborer in a petri dish which was streaked with the organisms. The sesame oil (50 µL) was added separatelyin thecups andpetri dishes were subsequently incubated. Kenamycin (30 µg) were used as standard reference drugs. Zone of inhibition produced by sesame oil was measured in mm.

Results and discussion

The chemical characterization of sesame oil shows that it has the quality required to use in the purpose of herbal product manufacturing.

The Results shows in the below table:

Sr. No	Parameters	Value ± SD (n=3)
01	Refractive index	1.471 ± 0.003
02	Weight/ml (g/ml)	0.914 ± 0.002
03	pH	4.40 ± 0.020
04	Viscosity (cp)	50 ± 1.000
05	Acid Value (%)	0.982 ± 0.007
06	Iodine Value	112.333 ± 0.007
07	Peroxide Value	3.354 ± 0.061

Antimicrobial activity

Minimum inhibitory concentration (MIC) of sesame oil

The lowest minimum inhibitory concentration of sesame oil is 17 µl against salmonella species. The MIC for other microorganism was found to be 330 µl for Pseudomonas aeruginosa, 380 µl for *Staphylococcus aureus*and 460 µl *Escherichia coli* **as shown in table below**:

Sr. No	Micro-organism	MIC Value of sesame oil (µl)
1	*Escherichia coli*	460
2	*Salmonella species*	17
3	*Pseudomonas aeruginosa*	330
4	*Staphylococcus aureus*	380

The sesame oil show antimicrobial activity against the tested microorganism with the inhibition zone ranging from 18 mm to 25 mm as shown in below table. Sesame oil show comparable Results with the kenamycin used as standard antibacterial drug.

Sr. No	Micro-organism	Zone of inhibition (mm)	
		Kenamycin	**Sesame oil**
1	*Escherichia coli*	20	18
2	*Salmonella species*	25	25
3	*Pseudomonas aeruginosa*	21	21
4	*Staphylococcus aureus*	20	23

Conclusion

The chemical characterization of oil justify that the quality is good and in the range of specification. The Results of the study shows that the MIC of oil is 17 µl against *Salmonella species* is the lowest against microorganism used in the test.Zone of inhibition for most of the microorganism is comparable with the standard drug kenamycin used. Now we can conclude that sesame oil have good chemical qualities and antimicrobial activity against the microorganism used in the test.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Spermatogenic arrest in guinea pig (*Cavia porcellus*) after administration of *Abrus precatourius* seeds

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Abstract- *Abrus precatorius* seeds are said to possess fertility regulating activity. Hence, the50% alcoholic extract of seeds at doses of 50, 100 and 200 mg/kg b.w. administered orally for 60 days to 03 groups (does wise) each includes 05 male Guniea pigs. First group served as control (vehicle treated). After 60 days of feeding of doses the body and genital organ weight were noted histopathological changes were observed in genital organs such as testes, epididymes and vasa deferentia and compared with control animals. The body and genital organ weight were not much affected. A significant weight reduction of genital organ was noted. At higher doses, an arrest of spermatogenesis was observed at various stages in sominiferous tubules. No mature spermatozoa could be seen in somniferous tubular lumen. The epididymes and vasa deferentia were also devoid of spermatozoa. It is concluded that at higher doses, the treated male G. pigs became a zoospermic. It is clearly indicated that the seeds of this plant has potentiality to arrest spermatogenesis in male and thus regulate the fertility of male animals.

Key words: Spermatogenesis, Anti-Spermatogenic activity, Male reproduction, Reproductive Biology, contraception, Herbal Drug, *Abrus precatorius* seeds.

Introduction

Worldwide search is going on fertility regulating agents to curb the problem of 'Population Explosion'. Hormonal drugs and other surgical methods are available for the purpose but they are not free from side effects. Hence, the search for suitable product from herbal plants is proposed which could be effectively used in place of the 'Pill'.

Herbal plants associated with fertility regulating activity are found abundantly in India. They have been listed by Chaudhury (1966). Saxena (1973). Farnsworth *et.al.* (1975) Kamboj & Dhawan (1982) and Chaudhary et.al (1990) as antifertility plants. It is interesting to note that these plants belong to different genera & species. Thus, they exhibit diversity in nature and activity. Some plants have shown antifertility activity in male while

others have shown activity in female and even some have shown their effect on both male and female animals.

Abrus precatorius Linn. (family – Leguminoceae) is one of them. The plant, a climber, is known as Gumchi or Ratti. It is also known as "Blacksmith's weight". The seeds of this plant are considered as a local contraceptive by tribals of India. Desai & Rupawala (1967). Agarwal et.al (1970) and Jain & Khan (1996) reported its antifertility activity in female albino rats. Baijal et.al 1981) and Sinha & Mathur (1990) carried out fertility regulating activity in male albino rats. Authors had conducted the experimental work with alcoholic seed extract of this plant on male Guinea pigs (Cavia porcellus) to explore the fertility regulating activity through spermatogenic arrest.

Material and Methods

The seeds of A. precatorius were purchased from local medicinal plant stores, Dehradun and powdered after removing the hard seed coat. The 50% alcoholic extract of powdered seeds was obtained using "Soxhlet apparatus". It was dried under reduced pressure and low temperature.

The three doses (50, 100 and 200 mg) of dried extract powder were prepared with 0.5mg./dose of gum acacia powder as vehicle. All the doses were dissolved in distilled water in such a way that each dose comprises 01ml of solution.

Adult & healthy, male Guinea pigs weighing between 400-450 gms. were purchased from I.V.R.I. Izzatnagar, Bareilly (UP) and acclimatized in the laboratory for one week prior to experimentation. The 05 male G. pigs were used in each group, control as well as a treated and were housed in large animal cages. Standard animal feed (Hindustan Lever Ltd.), Leafy vegetables and water was given them twice daily.

The three doses 50, 100 and 200 mg/kg b.w./day were administered orally separately into 01ml solution through knobbed needle fitted into a syringe for 60 days to different groups (2^{nd}, 3^{rd} and 4^{th}) of male Guinea pigs. The first group was served as control in which the vehicle (01ml (0.5mg) G.pig administered orally for 60 days. After 60 days of feeding of doses, the G. pigs were killed under either anaesthesia. Before killing, the body weight of each G.pig was noted. These G. Pigs were quickly dissected and their genital organs (testes, epididymies & vasa deferentia) were taken out. These organs were processed for histopathological examination. The weight of organs were also noted, and presented in tabular form Guinea pigs were maintained as per the protocol outlined in publication of the committee for the purpose of control and supervision of experiments on animals. Standard guidelines and approval obtained from college animal ethical committee appointed by the then Principal for laboratory animals.

The data were statistically analysed by fisher's test (1950). $P<0.05$ was considered as significant in comparison of control.

Results and Discussions

The oral administration of doses did not reduce the body weight at any dose, but at higher doses, the significant reduction in genital organ weight was noted (**Table-I**).

The dose 50mg/kg/day 60 days did not cause any histopathological change in

genital organs. These organs resembled genital organs of control G.pigs.

The higher doses (100 and 200 mg/kg/day) for 60 days of administration caused arrest of spermatogenesis. The *sominiferous* tubules became disfigure and reduced in size with distorted germinal epithelium. The leakage of germ cells, Karyolysis, Karyorrhexis, vacuolization and atrophy of Leydig's cells in the interstitium of the testes were noted. (Figure 1 & 2) The lumen of epididymal tubules were devoid of spermatozoa. Reduction of tubuler size and lack of stereocilia were other changes (Figure 3 & 4). The vasa deferentia were also devoid of spermatozoa. Stereocilia were deteriorated in reduced lumen. (Figure 5 & 6). In control male G.pigs, histopathological changes in genital organs were not noted in this study.

The observations / Results of the present study clearly indicate that *Abrus percatorius* seed's 50% alcoholic extract at doses of 100 and 200 mg/kg/day for 60 days of administration to male G.pigs caused histopathological changes in genital organs (Testes, epididymes and vasa deferentia) which made them azoosermic. No effect on body weight but significant reduction in genital organ weight was noted at higher doses (Table-I) The arrest of spermatogenesis in testes at higher doses within 60 days (one spermatogenic cycle) and reduction of genital organ weight are androgen dependent (Jackson, 1966).

Conclusion

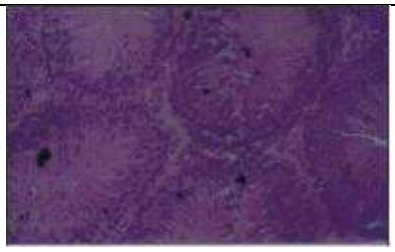
It appears that the infertility was due to absence of spermatozoa in the genital organs (epididymes and vasa deferentia) which is evident from histological preparation. The study on A. precatorius seeds also support the studies made by Baijal et.al. (1981) and Sinha and Mathur (1990) on arrest of spermatogenesis in testes of male albino rats and effect on epididymies and vasa deferentia had shown by Sinha (1991). Similar studies were also carried out by Singh (1985) and Das (1986) on semicarpus anacardium (seed kernel) and carica papaya seeds respectively in male albino rats with suppression of spermatogenesis. No toxic effects were noted at any given doses in this study. The Results of the present study suggest that A. precatorius seed's 50% alcoholic extract may be a good fertility regulating agent from plant origin for male animals.

Table-1 Effect of A precatorius alcoholic seed extract on body (gm) and genital organ weight (mg) of male Guniea pigs administered at different doses for 60 days. Five animals used in each group.

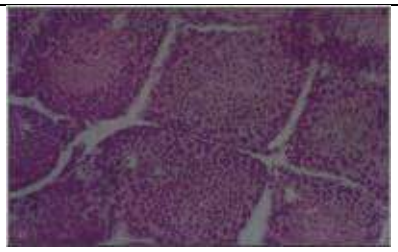
Group	Doses (mg/kg)	Body weight (gm)		Genital organ weight (mg)	
		Initial	Final	Tests	Epididymes (pig)
1	Central	502.10 ± 01.17	540.20±07.95	02.70±01.90	800.20±05.10
2	50 mg	490.20±04.70	520.35±05.15	02.50±01.75	798.30±02.15
3	100mg	498.05±02.10	518.15±02.35	02.60±02.20	789.10±02.75
4	200mg	491.75±01.20	480.10±02.78	01.15±01.20*	610.10±06.15*

Values are mean ±_S.E.

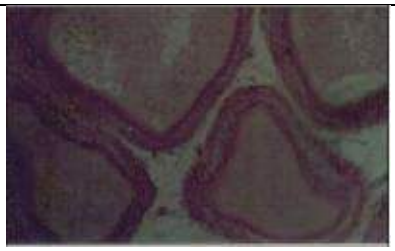
*p values < 0.05



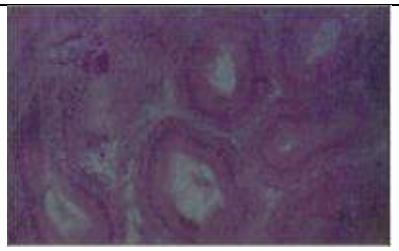
(Figure.1)
T.S. of testis of G.pig of control group. Note the normal supermatogenesis with all all spermatogenic elements in *sominiferous* tubules including spermatozoa in lumen and Leydig's cells in the interstituim. X400.



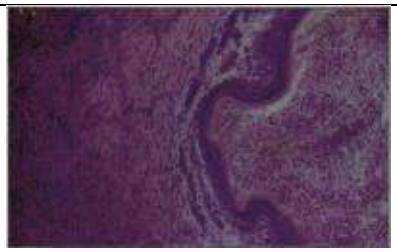
(Figure.2)
T.S. of testis of G.pig of treated group with 100 and 200 mg/kg. doses of *Abrus Precatorius* for 60 days. Note the arrest of spermatogenesis, disFigureured *sominiferous* tubules, distorted germinal epithelium, vacuolization, leakage of germ cells and atrophied Leydig's cells in interstitum. X400.



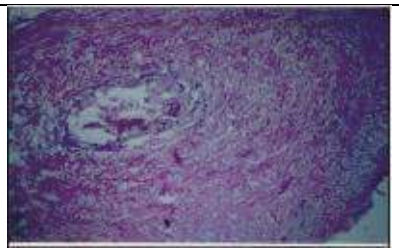
(Figure.3)
T.S. of caput epididymis of G.pig of control group. Note the normal histological structure of cells of epididym al tubules, stereocilia and lumen packed with spermatozoa. X400.



(Figure.4)
T.S. of caput, epididymis of G.pig of treated group with100 and 200 mg/kg doses A. precatorius for 60 days. Note the reduced epithelial cell height and lumen of tubules, deteriorated stereocilia and lack of spermatozoa. X 400.



(Figure.5)
T.S. of vas deferens of G.pig of control group. Note the normal musculature, mucosal lining, folds, stereocilia and lamina propria. Lumen packed with spermatozoa. X400.



(Figure.6)
T.S. of vas deferens of G.pig of treated group with 100 and 200 mg/kg doses of A. precatorius for 60 days. Note the distorted mucosal lining, epithelial cells and stereocilia, Empty lumen without spermatozoa. X400.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Comparative Quantitative estimation of total tannin content of leaves of *Ocimum sanctum* (tulsi), *Mentha* (pudina) and *Camellia sinensis* (tea leaves)

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Abstract- Ocimum sanctum or Tulasi is a perennial plant belonging to the family of Lamiaceae, native to the Indian subcontinent and widespread as a cultivated plant all over the Southeast Asian tropics. *Mentha* or mint, is a genus of plants in the family Lamiaceae, distribution across Europe, Africa (Southern Africa), Asia, Australia Oceania, North America and South America. *Camellia sinensis* (tea) belonging to the Theaceae family is a plant that generally cultivates in tropical and subtropical climates. Present study was to estimate total tannin content of leaves of Tulsi, mint and tea. Tannic acid was used as a standard and the total tannin content were expressed as tannic acid equivalents (TAE). Absorbance was measured using a spectrophotometer at 720nm. The study shows that the tannin contents of pudina and tulsi leaves is comparable whereas the tannin content of tea leaves is much more as compare to above pudina and tulsi leaves. The research will be continue to determine the other phytochemical constituent like flavonoid, Bitter, Alkaloids and Saponin etc.

Key words: *Ocimum sanctum* or Tulasi, Mint, *Camellia sinensis* (tea), Tannin

Introduction

The plant kingdom is an excellent and natural source of medicine. Nowadays there has been an increasing awareness about the importance of medicinal plants. Plants are rich source of therapeutic medicines and produce various bioactive molecules. Herbal plant extracts are very useful in controlling various types of pathogens and as growth promoters[1]. These are the economical source for therapeutics and viable solution for number of pathogens. The medicinal plants are rich in a wide variety of secondary metabolites such as tannins, phenolics, alkaloids and flavonoids etc. which enhances growth, innate immune response and disease resistance against pathogenic bacteria in human[2]. A large numbers of people uses various medicinal plants as anticancer drugs antimicrobial drugs, antifungal etc[3]. A large number of phytochemicals are widely uses in human therapy, agriculture, veterinary, various scientific researches along with inhibitory effects on most of microorganisms[4-5]. Ocimum sanctum also known as Tulsi or Holy basil is an aromatic plant and it belongs to the family Lamiaceae. Tea, also

known as *Camellia sinensis* (Theaceae family), is a lesser-known variety of the world-famous Camellia sinensis plant and beverage, it possess health advantages, cheap cost, and energizing effects. *Mentha Arvensis* L. belongs to the family Lamiaceae and is typically known as Pudina, menthol mint, corn mint. In this study we are evaluate and compare the tannin contents of these three herbs.[6-8]

Material and Methods

The plant specimens (leaves) for the proposed study were collected from the store of Himalaya wellness company Faridabad Haryana. The Collected plants were carefully examined and authenticated by Dr. Mayaram Uniyal, Ex. Advisor Medicinal plant UP govt.

Estimation of Total Tannin Content[9]

The tannins were determined by A.M. Diaz, Analytical method. Absorbance for test and standard solutions were measured against the blank at 720 nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Sample preparation

The collected leaves were washed with running tap water to remove adhering materials and cut into small pieces. Then, the leaves were dried at a temperature not exceeding 50 °C. These dried materials were cut into small pieces and then pulverized mechanically into coarse powder. The fine powder was separated by passing through sieve No: 60.weight accurately about 0.1 g finely powered sample in 100 ml of purified water at 100^0C using water bath for 1 hr, cool and decant the dissolved extract in to 500 ml volumetric flask. Washed the residue with purified water and make the volume up mark with same solvent. Filter the extract through whatman filter paper no.1. Discard first 50 ml of filtrate use next filtrate for analysis.

Standard preparation

Weight accurately about 100 mg of standard tannic acid in 100 ml standard flask and make up to volume with water (standard stock solution). Pipette out 1 ml from the above solution and makeup to 100 ml with water (standard solution).

Reagent preparation

1. Prepare 1 % potassium ferri cyanide in water
2. Prepare 1 % ferric chloride in water

Procedure

Take 1 ml of standard solution in 10 ml volumetric flask. Add 1 ml potassium ferri cynide and 1 ml of ferric chloride. Mix well and make the volume up to 10 ml with purified water. Exactly 30 min after addition of the reagent read the optical density at 720 nm against reagent blank. Reagent blank is prepared by a diluting 1 ml potassium ferri cynide and 1 ml of ferric chloride to 10ml with purified water.

Test solution: Take 0.2 ml of test solution and follow the same procedure as thatas standardand measure the test absorbance (T) against reagent blank.

Test blank: Take 0.2 ml of test solution and make up to 10 ml with purified water and measure the absorbance (TB) against water.

Note-All the optical density readings should be taken exactly 30 min after additions of the reagents.

Calculation

Subtract the reading of test solution from test blank and calculate the content of tannic acid from the standard curve express as % w/w of tannins.

$$\frac{\text{Absorbance of sample (T- TB)}}{\text{Absorbance of standard}} \times \frac{\text{weight of standard mg}}{100} \times \frac{1}{100} \times \frac{\text{Volume of stand. taken for reaction(ml)}}{\text{Volume of sample}} \times \frac{\text{Total volume of Sample ml}}{\text{Weight of sample}} \quad \text{\% Purity of Standard} = \text{Total tannins \% w/w}$$

Results and Discussion

We observe at the tannin content of all three leaves and found that the tea leaf contained more tannin than the other two leaves, Tannic acid was used as a standard and the total tannin contents of pudina, tea leaf and tulsi leaves is shown in(**Table-1**)

Table-1

Sample name	Sample absorbance	Sample blank absorbance	Standard absorbance	Standard weight	Sample weight	Purity of standard	Results Percent%
Pudina	0.3674	0.0026	0.4822	101.4	104.0	95.6	3.53
Tea	1.9505	0.0063	0.4677	100.1	101.4	95.6	19.62
Tulsi	0.3591	0.0018	0.4622	100	106.8	95.6	3.46

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavanoids, glycosides, tannins, steroids etc. some of these plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and srtoke by scavenging free radicals which are implicated in the pathogenesis of many diseases. The present study indicated that the aqueous extract of these plant leaves show good amount of total tannins. The study shows that the tannin contents of pudina and tulsi leaves is comparable whereas the tannin content of tea leaves is much more as compare to above pudina and tulsi leaves. The research will be continue to determine the other phytochemical constituent like flavonoid, Bitter, Alkaloids and Saponin etc.

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Indian Herbs a potential source of antimicrobial drugs as Non-antibiotics

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Abstract- New sources of antimicrobial drugs need to be identified to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating anti-microbial action needs to be exploited in modern phytomedicine as Non-antibiotics and in combinational therapy.

The present investigation reveals the scientific evaluation of the root of *Hemidesmus indicus (*anantamool*)* against pathogenic bacteria and it is suggested that this plant could be exploited in the management of infectious diseases caused by the test pathogenic bacteria and *Candida albicans* in the human system.

The *in vitro* antibacterial activity of 3 different extracts (Hexane, Methanol and Aqueous) was performed. The most active extract was found to be the hexane extract showing the maximum zone of inhibition of 22 mm against *Staphylococcus Aureus* followed by *E.coli* (18mm) and *Candida Albicans* (18mm).

Key words: *Hemidesmus Indicus,* antibacterial/ anticandidal activity, bioactive compound

Introduction

Infectious disease are the world's leading cause of premature deaths, killing almost 50 000 people every day. Infections due to variety of bacterial etiologic agents such as pathogenic *Escherichia coli, Salmonella* spp., *and Staphylococcus aureus* are most common. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh et al 1992 and Mulligen et al; 1993). With the continuous use of antibiotics micro-organism have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions (Lopez et al.2001 and Idsoe et al.1968).This has created immense clinical problems in the treatment of infectious diseases (Davis 1994). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important recourse to combat serious diseases in the world. According to WHO (1993), 80% of the world's population is

dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field.

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity (Shelef 1983; Zaika 1988; Beuchat and Golden 1989 and Juven et al.1994). In recent times, the search for potent antibacterial agents has been shifted to plants. Most plants are medicinally useful in treating disease in the body and in most of cases the antimicrobial efficacy value attributed to some plants is beyond belief. Claims of effective therapy for the treatment of dysentery ,diarrhea, respiratory disorders, skin diseases, syphilis, fever, leprosy, eye diseases and kidney and urinary disorders by traditional herbalist in India have prompted our interest in the scientific investigation of such herbal medications (Mukherjee, 1953; Chopra et al; 1956; Kritikar and Basu,1980; Anonymous,1986 and Nadkarni,1989).Conservative estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists. There are about 120 plant-based drugs prescribed worldwide and they come from just 95 plant species. Approximately 250,000 species of flowering plants and only 5000 have had their pharmaceutical potential assessed. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics (Kunin 1993). Due to increased resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable (Anonymous 1986). There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, basic health needs in the developing countries.

One possible approach is to screen/unexplored Indian medicinal bioactive plants extracts for their potential to be used against multi resistant bacteria. India has one of the world's richest flora with about 120 families of plants comprising 1,30,000 species and about 119 secondary plant metabolites are used globally as drugs. The WHO reported that 80% of world population rely chiefly on traditional medicines/herbs for primary healthcare have steadily increased worldwide in the recent years. Keeping in view this study is designed to evaluate the antimicrobial activity of *Hemidesmus Indicus.*

Material and Methods

Collection of plant materials

Hemidesmus Indicus roots were collected from the Himalaya Wellness Company Dehradun India .The collected plant material was identified by the department of Pharmacognosy, Himalaya Wellness Company Dehradun. Roots were washed under the running tap water 2-3 times and once with sterile distilled water and dried under shade and then homogenized to fine powder and stored in air tight container till further use.

Preparation of solvent root extraction

The method of Alade and Irobi, (1993) was adopted for preparation of plant extracts with little modifications.

The dried 25 g powdered root soaked separately in 100 ml Hexane, methanol, and aqueous .Each solvents were kept in separate flasks with powdered sample were kept in a rotating shaker for 3 days. The extracts were filtered through whatman Filter paper No.1 and the extracts were reduced to half of its original volume. The organic solvents were concentrated in vacuum using rotary evaporator, while aqueous extract was dried using water bath.

Culture media

The media used for antibacterial test was Soyabean Casein Digest Agar/broth of Hi Media Pvt. Ltd. Bombay, India.

Inoculum

The bacteria were inoculated into soyabean casein digest agar /broth and inoculated and incubated at 37 ℃ for 4 h and the suspension was checked to provide approximately 10^5CFU/ml.

Microorganisms

The antibacterial activity of the extract and the essential (bioactive compound) were tested individually on G+ve and G-ve bacterial strains .All bacterial strains were obtained from IMTECH, Chandigarh India. The G+ve strain used was *Staphylococcus aureus MTCC 737 and* G-ve bacterial strains were *E.coli MTCC 1687; Psuedomonas aeruginosa MTCC 1688* and *Salmonella enteric MTCC 3858.and Candida albicans MTCC 3017.*

Determination of antibacterial / anticandidal activity

The agar well diffusion method (Perez et al; 1990) was modified. Soyabean casein digest agar (SCDA) was used for bacterial cultures. The culture medium is inoculated with the microorganisms suspended in soyabean casein digest broth. A total of 8mm diameter wells were punched into agar and filled with plant extracts and solvent blank s(distilled water, hexane and methanol as the case may be).Standard antibiotic was simultaneously used as positive control. The plates were then incubated at 37°C for 18 h. The antibacterial / anticandidal activity was evaluated by measuring the inhibition zone diameter observed. Wells were filled with 0.1 ml of 20 mg/ml concentration of each sample (2 mg/well).Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm.

Results and Discussion

Among all the tested extracts hexane extract was found to have maximum zone of 22mm against *Staphylococcus aureus* (Table-1; Figure-1 and 2, Plate-1) followed by *E.coli* (18mm), *Candida albicans* (18mm), *Pseudomonas aeruginosa* (16mm) and *Salmonella enteric* (15mm).

The significant antimicrobial effect of *Hemidesmus Indicus* against all the pathogen confirmed that the compound present in the crude extract are responsible for the effective antimicrobial activity.

Table- 1 Antimicrobial activity of *Hemidesmus indicus* root extract

S.No.	Test microorganisms	Diameter of zone of inhibition(mm)			
		Hexane extract	Methanol extract	Aqueous extract	**+VE Control Ciprofloxacin 30µg/ml**
1.0.	*Staphylococcus aureus MTCC 737*	22	16	NAD	25
2.0.	*E.coli MTCC 1687*	18	14	NAD	21
3.0.	*Pseudomonas aeruginosa MTCC 1688*	16	12	NAD	22
4.0.	*Salmonella enterica MTCC 3858*	15	13	NAD	21
5.0.	*Candida albicans MTCC 3017*	18	16	NAD	----

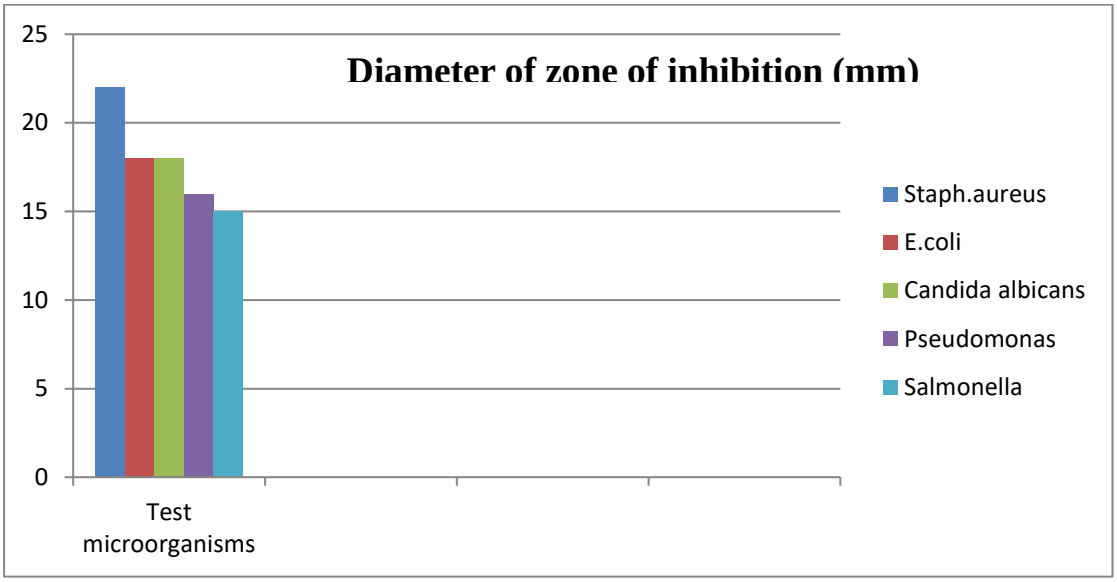

Figure-1 Susceptibility of test microorganisms against Hexane extract of *Hemidesmu sindicus* in **the form of diameter of zone of inhibition (mm).**

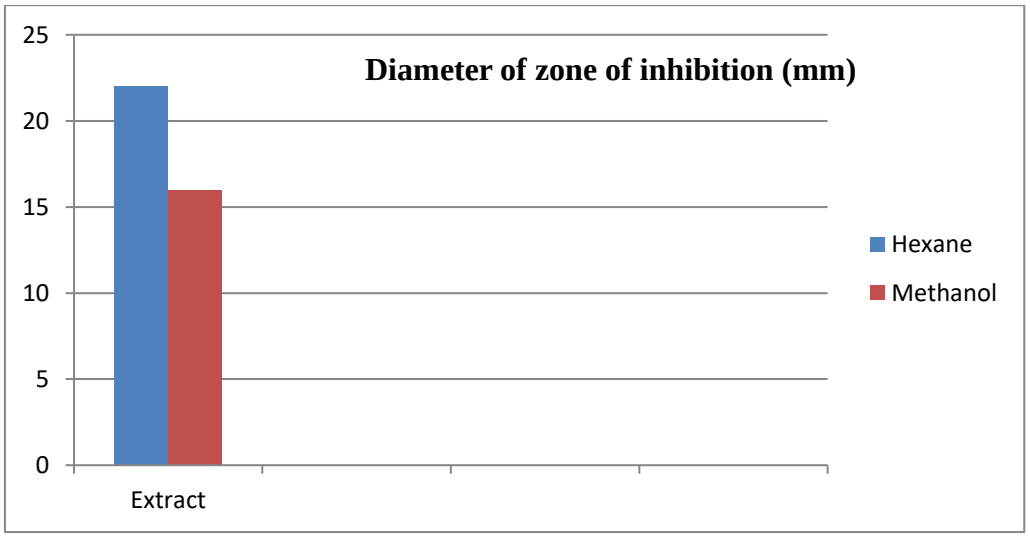

Figure-2 Antimicrobial efficacy of Hexane and Methanol extract of *Hemidesmus indicus* **in the form of Diameter of zone of inhibition**

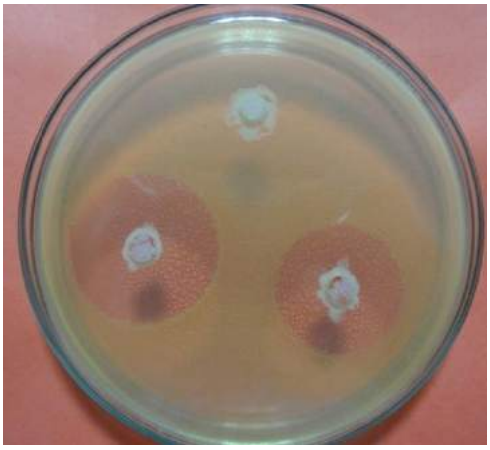

Plate-1 Antibacterial activity of Hexane extract against *Staph. aureus* **(MTCC 737)**

The traditional therapeutic indications of *Hemidesmus Indicus* studied appear to have a fairly good degree of correlation with their antimicrobial activity. The herb *Hemidesmus Indicus* appear to have broad spectrum of action, it could be useful in antiseptic, disinfectant formulations and in chemotherapy .The antibacterial activities of the herb is particularly noteworthy, considering the importance of these organisms in infections.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Evaluation of secondary metabolites and antibacterial effect of *Boerhavia diffusa* ***roots*** with chromatographic studies

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Abstract- India has a vast diversity of medicinal herbs. Around 3,000 years ago, these herbs were acknowledged and used as medicinal plants helpful for treating people. New scientific research has established that some plants and herbs have presence of many active compounds and possess specific pharmacological properties.*Boerhavia diffusa* is one of the renowned medicinal plants used to treat large number of human ailments as mentioned in Ayurveda. The plant in whole or its peculiar parts (aerial parts and roots) have a numerous medicinal properties. It shows antibacterial, hepato-protective, antistress and also in treatment of stress, abdominal pain, inflammation and jaundice. Various phytochemical, Pharmacological, experimental and clinical investigations were done on *Boerhavia diffusa* by many scientists to clearly understand the ancient ayurvedic usage of punarnava. The evaluation of secondary metabolites and antibacterial activities of punarnava root extract were investigated on pathogens using the well diffusion method. HPTLC analysis of *Boerhavia diffusa* extracts were also carried out in different solvent.

Key word: *Boerhavia diffusa*, Antibacterial activity, Phytochemical analysis and HPTLC.

Introduction

Boerhavia diffusa commonly known as punarnava in Sanskrit is an herbaceous plant of the family nyctaginaceae. The whole plant or its specific parts (roots, leaves and stem) are known to have a long history of use by indigenous people in India; it has many ethanobotanical uses and is medicinally used in traditional, ayurvedic system. Besides, the *Boerhavia diffusa* plant reported to possess many pharmacological and clinical properties (Awasthi and Verma, 2003). The leaves are used in ophthalmic diseases (Shah and Gopa, 1987), as analgesic, in dropsy and jaundice (Raj and Patel, 1978), in rheumatism (Rao, 1981). Dried leaves are used in dhoomapana (smoking) in treatment of bronchial asthama (Satheesh and Pari, 2004, Chude et al., 2001).

Boerhavia diffusa is used to revitalize and clean the liver (Rawat et.al.1997). According to Ayurveda, when the liver is unable to perform well it also leads to an

imbalance of Vata-Pitta-Kaphadoshes. This might lead to liver diseases like jaundice. Consumption of *Boerhavia diffusa* helps to correct the function of the liver by removing toxins from the liver cells. *Boerhavia diffusa* also helps to improve digestive fire due to its appetizer property. It helps to digest the food easily and reduce the burden of the liver. The hepatoprotective activity of roots showed marked protection of serum parameters in thioacetamide toxicity in rats. Furthermore, the aqueous extract of thin roots collected in summer has more activity suggesting the proper time and type of root collection for the most desirable Results. The investigation also validates the use of *Boerhavia diffusa* L. roots in hepatic ailments by the several tribes in India (Rawat et.al, 1997)

Presently, secondary plant metabolites (phytochemicals), previously with unknown biological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju et.al. 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficiency would be used for the treatment of bacterial infections (Balandrin et.al. 1985). *Boerhavia diffusa i*s one of the most widely used plants and secondary metabolites found in medicinal plants are one of the main sources of drugs and health products. Improving of the content and yield of secondary metabolites in medicinal plants has become increasingly important.

Antibacterial activity of *Boerhavia diffusa* were studied against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) compared to Amoxicillin. The present study provides a scientific rationale for the traditional use of *Boerhavia diffusa* in the management of liver and jaundice.

Material and Methods

Collection and Processing of plant

Matured roots of *Boerhavia diffusa* were collected from the Himalaya Wellness company. There plant roots were washed with tap water to remove soil and unwanted dust particles. Then the roots were shaded, dried, and then powdered by using mechanical blender and stored in air tight bottles.

Extract Preparation

The powdered plant roots were soaked (10g/100ml) in different solvents aqueous, methanol and chloroform, for over night in rotator shaker.

HPTLC Profile

Mobile Phase

Chloroform: Methanol: Glacial acetic acid (35:6:1)

Application

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

Preparation of development tank

Used Camag twin trough development tank (10×10cm). Chamber was covered from one side with required size of whatman no-1 filter paper, measured 20ml of mobile phase and transferred into the chamber along the side of the filter paper and allowed for the saturation process.

Visualization and Documentation

Visualized the dried plate under UV 254 nm and 366 nm using Camag Reprostar 3.

Identification Test

The individual sample was subjected to the qualitative phytochemical screening. Phyto-chemical tests were carried out adopting standard procedures (Trease et.al 1983, Kokate et.al 2003 and Harbone, 1998).

All of the reagents were made by adopting standard procedures (Indian Pharmacopoeia 2014).

Test for Alkaloids

To 1 mL of extract was added 1 mL of Mayers reagent and few drop of Iodine solution. Formation of yellow colour precipitate indicates the presence of Alkaliods.

Test for Terpenoids

To 1 mL of crude extract was added 1 mL of concentrated H2SO4 and heated for 2 minutes. A grayish colour indicates the presence of terpenoids.

Test for Tannins

To 1 mL of crude extract added 1 mL of $FeCl_3$. A blue green indicated presence of tannins.

Test for Saponins

To 1 mL of extract added 2 mL of distilled water and was shaken well and formation of 1 cm layer of foamindicates presence of saponins.

Test for Flavonoids

To 1 mL of extract added few fragments of magnesium ribbon and added few drops of concentrated HCl drop wise. Appearance of pink scarlet colour confirmed the presence of flavonoids.

Test for Steroids

1 mL of extract mixed with 1 mL of chloroform and concentrated H_2SO_4 sidewise. A red colour presence at the lower chloroform layer indicated presence of steroids.

Antibacterial activity

Antibacterial activity of *Boerhavia diffusa* was studied against Gram-positive, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli and Pseudomonas aeruginosa* compared to Ciprofloxacin.

Method Used

The zone of inhibition was measured by the Agar well diffusion method (Stoke, 1975) used for antibacterial activity .The sterilized nutrient agar was melted and allowed to set in a sterile petri dish and then inoculated with the nutrient broth containing the test inoculation. Holes were punched in agar of 6 mm diameter to a depth of about 2mm. Each of the holes in a Petri-dish was filled with the extract and incubated for 24 hrs at 37^0C. The active extracts had zone of inhibition which were measured to indicate the degree of sensitivity.

The antibacterial activity of the dried leaves extract of *Boerhavia diffusa* on the test bacteria are shown in **table-1**.

Results and Discussion

The bioactive substances in plant are produced as secondary metabolites. Secondary metabolites are substances manufactured by plants that make them competitive in their own environment.

These small molecules exert a wide range of effect on the plant itself and on other living organisms. The search for new secondary metabolites in plant with the hope of discovery of new products, new approaches for the treatment of disease is an on-going process. Aqueous, methanol and chloroform extracts are rich with secondary metabolites. **Table-1** shows the detection of secondary metabolites in *Boerhavia diffusa* root extract.

Table-1 Detection of secondary metabolites in *Boerhavia diffusa* root extract.

Class of compound	Aqueous	Methanol	Chloroform
Tannin	+	++	++
Flavanoid	++	+++	++
Saponin	+	++	+
Alkaloids	-	+++	++
Steroids	-	+	+
Terpenoids	-	++	+

Where +++ shows strong presence, ++ shows partially strong, + shows week and – shows absence of phytochemical activities.

The antibacterial activity of the roots extracts of *Boerhavia diffusa* on the test bacteria are shown in table-2. The antibacterial activity of *Boerhavia diffusa* roots were carried out. Most of the extract shows an antibacterial activity against the pathogens such as *S.aureus, E.coli* and *Pseudomonas aeruginosa.* The results of this work on chloroform, aqueous and methanol extracts of *Boerhavia diffusa roots had activity on E.coli, S.aureus and Pseudomonas aeruginosa.* The activity of plant extract showed the good antibacterial activity. The plant extracts contained active principle with broad antibacterial spectrum *(Bankole, 1992*). Methanol and chloroform had highest susceptibility. E.coli exhibited the least susceptibility.

HPTLC fingerprinting is proved to be a linear, precise, and accurate method for herbal identification (Cortes et.al 2014). Such finger printing is useful in quality control of herbal products and checking for the adulterants (Teo.P, et.al, 2013). The significant antibacterial effect of *Boerhavia diffusa* against all the three pathogens confirmed that the compounds present in the crude extract are responsible for the effective antibacterial activity. Thin layer chromatography studies of different extracts of roots (Methanol, Chloroform and Aqueous) indicated the presence of more than ten different compounds (Fig. 1), further confirming the synergistic action.

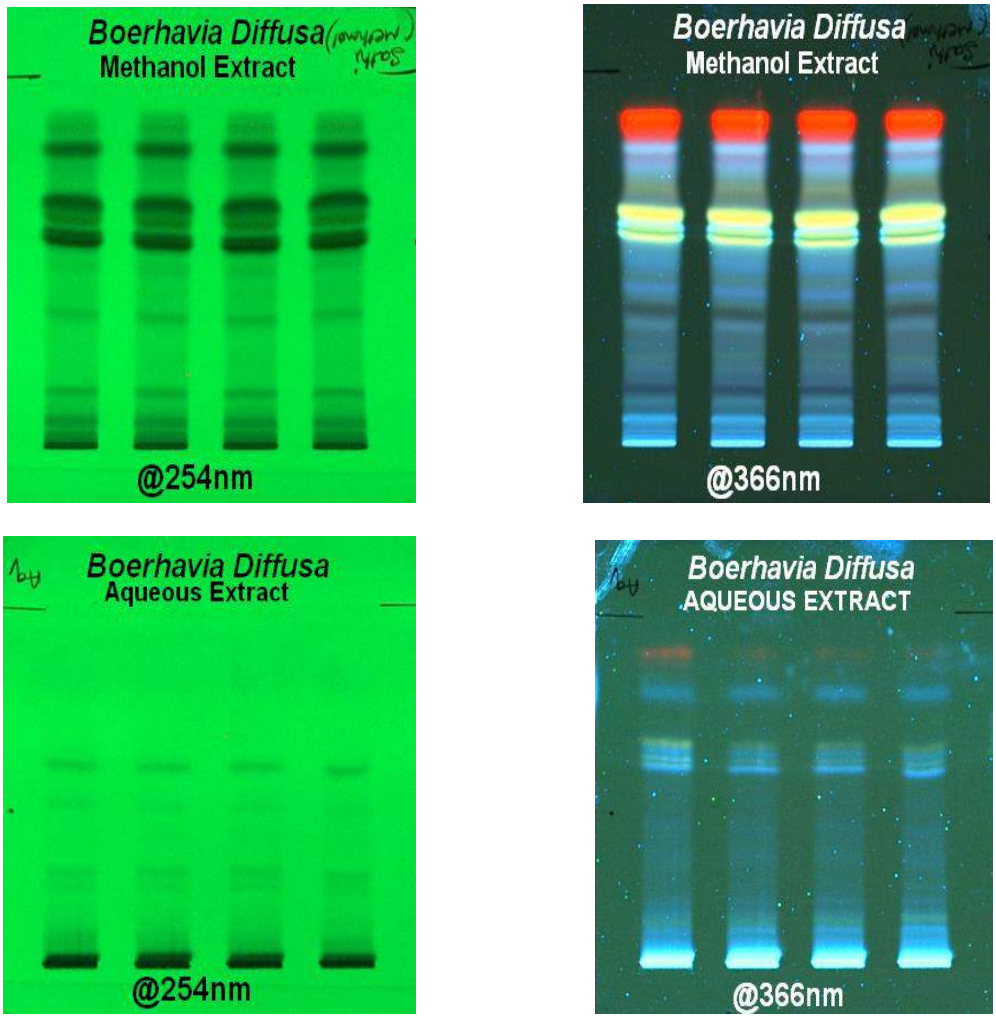

Figure-1 HPTLC profile of root extract of *Boerhavia diffusa in different solvent*

Table-2 Antibacterial activity of *Boerhavia diffusa* root and standard (Ciprofloxacin)

Tested Bacteria	Diameter of ZI (in mm)	Diameter of ZI (in mm)	Diameter of ZI (in mm)	Diameter of Zone of Inhibition (in mm)
	Methanolic Extract	Chloroform Extract	Aqueous Extract	Standard (Ciprofloxacin-50 mcg/ml)
Escherichia coli	20mm	19mm	14 mm	25 mm
Staphylococcus aureus	18mm	19mm	16 mm	28 mm
Pseudomonas aeruginosa	18mm	17mm	15 mm	22 mm

ZI→ Zone of Inhibition

Conclusion

From the above research, it is seen that the results of this investigation support the ethanomedicinal use of this plant by local practitioners. India has a rich flora used in traditional medical treatment. These plants have medicinal properties because of their phytochemical components. These phytochemicals show therapeutic effect on

mankind. From this current study, we concluded that the root of *B.diffusa* showed high therapeutic activity because the presence of potential phytochemicals. Extracts of this plant is abundant in flavonoids, diterpenoids, alkaloids, tannins, saponins. In this study, we concluded that biologically active phytochemicals present in methanol and chloroform extracts of *Boerhavia diffusa* roots. *B.diffusa* root extracts showed good antibacterial activity on *E.coli, S.aureus and Pseudomonas aeruginosa.* However more research has to be carried out so as to characterize the bioactive ingredients of the plants.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Antimicrobial activity of Hawan samagri against pathogens

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Abstract- In India majority of people practise's an ancient ritual almost on every occasion in their homes, which is commonly known as 'Havan'. Hawan is a Sanskrit word which refers to any ritual that involves making offerings into a consecrated fire. During the ritual special herbal/plant medicinal preparations (*Hawan samagri*) are offered in the fire of medicinal woods ignited in a specially designed inverted pyramid shaped fire pit or container (called agni-kunda). Fire lead to sublimation, chemical conversion and/or transformation into vapour phase of the herbal/plant medicinal preparation leading to release of medicinal phytochemicals. This ritual is supposed to clean the environment as well as to cleanse the body from the toxins. Hawan fumes are not only used for the disinfection of air but also it can be environmentally oppressed for the physical, mental, intellectual and spiritual development. It is the easiest way, least taxing,least risky and most effective way for administering a medicine so as to reach every single cell of the body.Havansamagri is the key content to perform the havan,which is a mixture of herbs of superior quality like black till,Kapoorkachri, rose petals,long, kulinjan along with dhoop powders, sandalwood powder, lobaan,ghee, jaggeryetc. This work aims to evaluate its antimicrobial potential against*S.aureus, E.coli,* and *C.albicans* via well diffusion methodand simultaneously evaluates the effect onair quality of the chamber before and after hawan.

Key words: Hawan, Hawan samagri, Ritual, Environment, Ayurvedic,Solvent extraction, Antimicrobial,Well diffusion method etc.

Introduction

Hawan is also known as yagya, yagna, or Agnihotra. It's being used from the Vedic era for preventing and treating various diseases and ailments, and also for refining and maintaining immunity for individuals.Yagya has numerous applications described in Vedic and Ayurvedic literature.

Yajurveda advocates performing of Hawan every day, morning and evening to attain spiritual enlightenment, mental peace, purification of the mind and environment[6]. From time immemorial, human beings have used smoke of medicinal plants for curing disorders. Smoke produced from natural substances has been used extensively in many cultures and famous ancient physicians have described and recommended such use. Under the Saraswati-Indus civilization

7500 BC[7], the great Rishis (saints) used to perform agnihotra-yagnas to purify the environment as described in Rigveda-the most ancient compilation of knowledge on earth by sublimating the *Hawan samagri* (mixture of wood with odoriferous and medicinal herbs) in the fire accompanied by the chanting of Vedic mantras described in Rigveda [8 & 9]. Smoke produced at high temperatures is considered as a simple way of administering a drug, which exhibitsrapid pharmacological activity when inhaled. The sublimated vital elements and herbal medicines inhaled in Yagya first reach the brain, followed by lungs and other subtle components of the body[10 & 11]. Thus, it has a direct healing effect on brain borne diseases and complexities. Ayurveda also recommended nasal route as a preferred mode of administration of drugs[1].

Hawan is a scientific experiment in which special herbs (*Hawan samagri*) are offered in the fire of medicinal woods ignited in a specially designed fire pit called agnikunda. Hawan seems to be designed by the ancient scholars to fight with the diseases of the brain[2-4]. The components of Hawan are having a number of volatile oils that are specifically useful for curing different diseases through one or the other mechanism of action. Due to high temperature of fire the vapours of these oils enter into the central nervous system through nasal route[5]. A consecrated fire is the central element of every Hawan ritual however the procedure and items offered to the fire vary by occasions/ceremony or by the benefit expected from the ritual. The decomposition and transformation of specific substances in the yagya-fire enter the human body in a gaseous form through the nose, lungs and the pores of the skin.

Nosocomial infections

Camphor purifies the air in the diffusive atmosphere and achieves various medicinal benefits when we inhale. It acts as a germ killer, mosquito and fly repellent and bounds spreading of the virus in that place. The chief ingredient in Hawan is mango wood (Mangiferaindica) which when burnt releases formaldehyde a gas which slays harmful bacteria thus purifying the atmosphere. The jaggery burnt in the Hawan also releases the formaldehyde gas[18].

Other health benefits

Medicinal-smoke intake can deliver benefits in curing ailments such as-headache, disease related to sensory organs, migraine, hiccups, asthma, goiter, fungal infection, jaundice, cold, sneezing, insomnia, hair fall etc. The inhalation of medicinal-smoke of a specific herbal powder combination of neem leaves, bach, kooth, harad, saraso and googal ends high fever in patients. Inhalation of the medicinal-smoke of a specific herbal-powder combination made using neem leaves, bach, hing, sendhanamak, saraso was seen to destroy worms and pus[19].

Hawan Samagri

Cow's Ghee

Benefits of burning Cow Ghee in Yagna are

i) Purification of atmosphere,
ii) Production of oxygen in environment,
iii) Disinfects air and cleans environment,

iv) Heal the respiratory system and clear any blood clots and bacterium affecting the nasal, lungs and veins and

v) Helps in combustion process[12 and 13]. The cow‟s ghee the very important ingredient of havan has been referred as an antidote to the poison in VEDAS.

vi) Its fragrance purifies the physical atmosphere. Ghee when burnt in fire goes up in the atmosphere and the fat particles get laden on the dust particles in the atmosphere (somewhat similar to the stickiness on the objects in the kitchen) and comes back to the earth in form of rain thus nourishes the vegetation on the mother earth[1].

Mango wood

The main ingredient in havan is mango wood which when burnt releases **formic aldehyde** a gas which kills harmful bacteria thus purifies the atmosphere. Formaldehyde is sprayed to disinfect walls and ceilings and is also used to preserve the fruits as formic acid which is produced by burning mango wood[14].

Jaggery

The jaggery burnt in the havan also releases the formic aldehyde gas. Since, the formaldehyde is effective only in the presence of water that is why there is a ritual of sprinkling the water around the Havan–Kund and in the air. The water is also available in form of water vapours found in the atmosphere[1 and 14].

Black sesame seeds

Black sesame is very rich in minerals and vitamins, often packed more densely than many other foods. Also, the crop is remarkably stable and hardy. Since it is purported to drive away negative energy, during the funeral rites of ancestors, sesame is used. During the ancestral month (Before Durga Puja), it is used as daily food offering to Pitras[15 & 16].

Camphor

Camphor , or cinnamomum *camphor* a, is extracted from the *Camphor* tree, a close relative of cinnamon. Long considered a very sacred plant with healing powers. Spiritually, *camphor* is very healing. It can give you a sense of liberation from your obligations. *camphor* can also uplift the mood, especially to a higher, more spiritual vibration. As alternative medicine, *camphor* is especially useful for fighting colds and flu. The cool, pungent aroma of *camphor* can instantly clear the sinuses. *Camphor* is also useful for treating aches and pains in the muscles and joints[1 &17].

Barley seeds (Jau)

It may not be as popular as wheat or oats, but barley is called the “King of the cereals”. Barley contains all nutrients that are vital for maintaining optimum health. It protects the heart against various cardiovascular diseases. It also protects the kidney, urinary tract, liver, bones, joints and ensures their normal functioning [15 &17].

Guggal

Antimicrobial herbs like guggal when burnt cause rain and purify the atmosphere. Havan ritual is like giving back to the atmosphere what we have taken from the atmosphere. The aromatic herbs when burnt remove the foul odour in the atmosphere by their fragrance[9 & 17].

Table-1 Effects of various active vaporized constituents of *Hawan samagri* on human body.

Botanical name	Active component	Effects on human body
Coconut (*Cocosnucifera*)	Monounsaturated fatty acids, Saponins	Inhibit convulsions, increase GABA and, serotonin level
Sesame seeds (*Sesamumindicum*)	1-(5-methyl-2-furanyl)-1-propanone	Antioxidant, antimicrobial, anti-inflammatory, anticancer
Clove (*Eugenia caryophyllus*)	Eugenol, β-caryophyllene	Analgesic, antioxidant, anti-inflammatory, anxiolytic, anticonvulsant.
Nutmeg (*Myristicafragrans)*	Myristicin and macelignan	Reduce severity of seizures.
Mango wood (*Mangiferaindica*)	PGG, polyphenolics, flavonoids	Increase GABA levels, anticonvulsant action.
Kulinjan (*Alpiniagalanga*)	1,8-cineole ,α-fenchyl acetate.	Antioxidant,anti-bacterial ,expectorant ,anti-fungal
Ashwagandha (*Withaniasomnifera)*	Withaferin, withanone(withanolides) ,Saponins,sitoinosides, acylsteryl-glucosides	Anti-stress agents, Neuroprotective agent
Cardamom (*Elettariacardamomum*)	1,8-cineole, flavonoids, esters,terpenes	Antiseptic, anti-stress agent

Material and Methods

Collection of the material

Herbs used in making the *hawan samagri* were provided by Himalaya wellness company.

Mixing of the *hawan samagri*

List of the ingredients used are mixed in the ratio as mentioned **(Table-2)**.

Table-2 Ingredients used

Content	Per 500g
Kala til	250g
Jau	75g
Coconut powder	45g
Camphor	5g
Long	15g
Green cardamom	10g
Guggal gum	35g

Nutmeg (*Myristicafragranus* +taizpatta+ misri	15g
Kulinjan + Ashwagandha	50g

Other additives used- Mango wood, cow's ghee

Methodology

i) Hawan experiments was performed using experimental chamber setup to determine its impact of medicinal fumes on air quality
ii) Anti-bacterial activity of the herbal mixusing well-diffusion method.

Experimental chamber to perform the experiment.

1. Hawan chamber of space 6.5ft x 5ft x 7ft, the whole setup was designed within a brick and cement walled room.
2. Nutrient agar plates were prepared and openly exposed plates placed at the four corners of the chamber for 4 hours before hawan.
3. Hawan was performed inside the chamber, the fumes were retained in the chamber for 1 hour.
4. After completion of 1 hour of hawan nutrient plates were placed exposed in same four corners positions for 4 hours.
5. Plates were incubated for 12-24 hours at 30-35°c in incubator.

Determination of anti-microbial activity-

The sample mix was dried and coarsely powdered in the grinder. After that solvent extraction method was used for preparing the extract. 50g mixture was added in 250 ml of solvent and conical was kept on shaker for 6-7 hours. After completion, the extract was filtered using filter paper. The collected extract was then concentrated on water bath for making up the final volume. Three extracts was prepared using methanol, hexane and aqueous

Well-Diffusion method

Pre-inoculated nutrient agar media was poured in petridishes, the plates were allowed to cool and settle inside the laminar air flow. After the plates get solidified well was made using a well cutter. And 100µl sample was loaded in the well. The inoculated plates were incubated in incubator at 30-35°c for 12-24 hrs.

Results and discussion

Laboratory tests were directed by exposing petriplates having agar media in a closed room before and after fumigating 'Hawansamagree'. Results displayed substantial decrease in concentration of microbial load. This method can be successfully applied at actual work places like small flour mills, ginneries, cobbler shops etc.

The fumes lead to purification of air by oxidizing the carbonic compounds and along with that they have antimicrobial properties which benefit the people around.

1- Air monitoring results

Table-3 Plate count data

Sno	Before hawan	After hawan
1	80	42
2	110	60
3	90	45

4	115	80
5	109	75
6	100	50

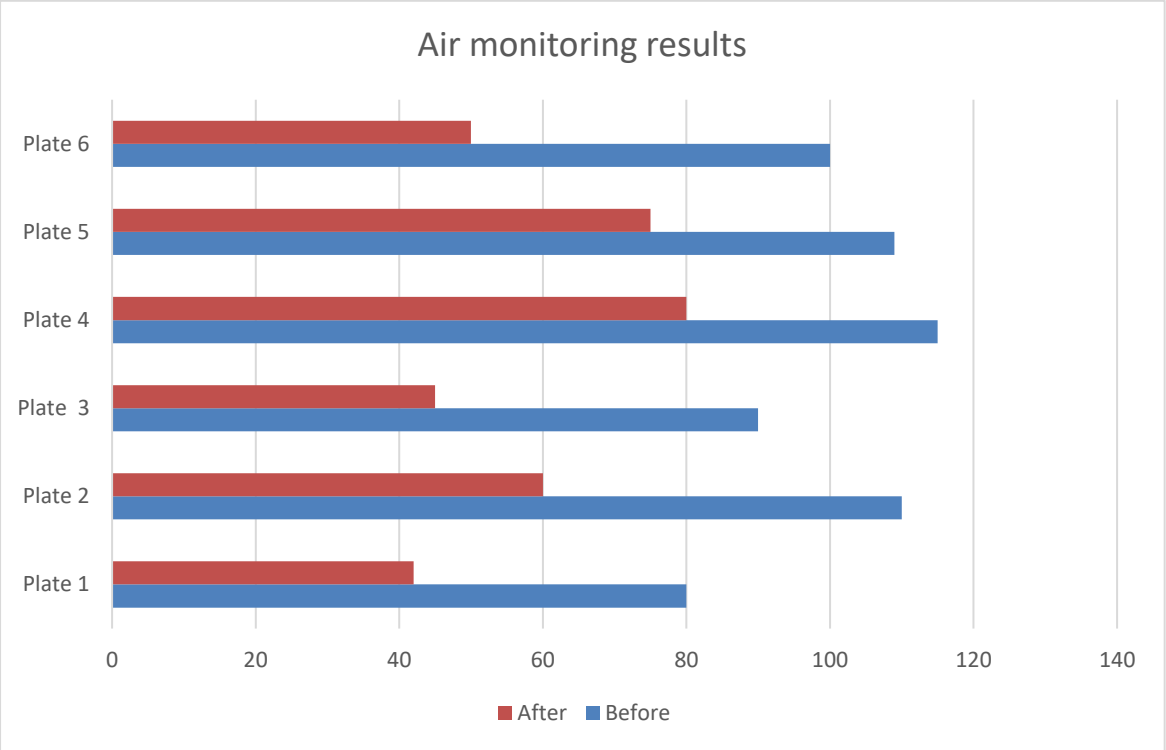

Figure-1 As the this can be seen from the above data (fig-1), decrement of at atlest 50-60% was observed in the microbial load of the air in the chamber after hawan

.

Table-4 Antimicrobial activity results.

Solvent	*S.aureus*	*E.coli*	*C.albicans*
Methanol	26mm	22mm	16mm
Hexane	NA	18mm	11mm
Aqueous	28mm	23mm	10mm
Ash methanol	10mm	20mm	17mm
Ash aqueous	16mm	10mm	NA
Positive Control	31mm	32mm	30mm

Conclusion

From our study and the results, we can conclude, that this mixture have so much potential including health benefits. The mix was found active against tested pathogen, as the extracts showed zone of inhibition against, *S.aureus, E.coli,* and *C.albicans*.Even after burning completely the havan samagri ash do possess anti-microbial activity against the pathogens. This herbal mixture can be used as a

replacement of our chemically prepared antibacterial room freshener sprays if it is used on a regular basis. Therefore, the development of therapeutic agents from indigenous resources will be of great help and further exploration in this direction is needed.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Phytochemical investigations of Pomegranate (*Punica granatum*) rind extracts and their antibacterial activity

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Abstract-Pomegranate (*Punica granatum* L.) is an ancient fruit rich in phytochemical bioactive compounds. *Punica granatum* L belonging to the family Punicaceae is described as an ingredient in remedies and is a widely used plant having medicinal properties. Having served as a symbolic fruit since ancient times, pomegranate (*Punica granatum* L) gained considerable recognition as a functional food in the modern era. Present study reports the phytochemical analysis which has been carried out on the peel (rind) extract. Rind of pomegranate (*Punica granatum*) was extracted using Methanol as solvents. Phytochemical investigations included qualitative detection of phytochemicals .This phytochemical investigation results shows the presence of phenols , tannins, flavonoids, coumarins, quinones, steroids, triterpenoids, and alkaloids in Methanol extracts.The antibacterial activity of extracts were also evaluated by agar diffusion method against *Staph aureus*, *E.coli* and *Pseudomonas aeruginosa*.Methanolic extracts of pomegranate rind was most effective in inhibiting the growth of *Staph.aureus* showing 22 mm of diameter of zone of inhibition followed by Ethanolic extract against *Staph.aureus* with 19 mm diameter of zone of inhibition followed by *E.coli* with 21 mm diameter of zone of inhibition by methanol extract and 17 mm of diameter of zone of inhibition by ethanol extract. Work is under progress on new emerging AI technologies for detailed analysis of phytochemicals in *Punica granatum* rind. AI provides new approaches for screening the main components and pathways of single herb or prescriptions, and predicting the mechanism of action.

Key words: Pomegranate peel/Rind; Phenols; Flavonoids; Phytochemical; Antibacterial

Introduction

Pomegranate is an ancient fruit native to Persia which has been cultivated in the Mediterranean region through years[1 and 2]. Pomegranate, which belongs to the family of Punicaceae, is botanically named *Punica granatum*[3]. Pomegranate trees are considered as shrubs or small trees which grow 5 to 10 meters high[2]. Its flowers are red and can occur either as single blossoms

or clusters of several blossoms[4] and [5].Pomegranate is rich in bioactive molecules, it has shown myriad medicinal properties due to its high phenolic content [1,6]. Pomegranate, as a fruit rich in antioxidants, can intensively and positively contribute in humans' health. Pomegranates strong historical, cultural and religious significances, besides to the researches determining the phytochemicals present in pomegranate triggered analysing and evaluating pomegranate rind.

Resistance to antimicrobial drugs in pathogenic bacteria is a global concern.[7-9]*Pseudomonas aeruginosa* and *Staphylococcus aureus* are important nosocomial pathogens, which both frequently cause multidrug resistance.[7,10] Infections caused by commercial antibiotic resistance isolates have increased greatly during the last decades in hospitals.[8] The spread of these organisms in healthcare settings are often difficult to control, due to the presence of multiple intrinsic and acquired mechanisms of antimicrobial resistance.[8,11] In recent years, study of antibacterial properties of plant extracts is of interest.[12] Pomegranate (*Punica-granatum* L.) is a native fruit in Iran it has a rich history of traditional use in medicine.[13] Pharmacological effects of pomegranate have been mentioned anciently and research on pomegranate is increasing due to its great nutritional values and medicinal uses.[14] In several studies it had been found that pomegranate extracts have many potential effects including antibacterial[15], antifungal[16], antiviral[17]and some other activities.As an emerging discipline, artificial intelligence (AI) technologies have been enthusia-stically explored by Traditional Medicine(TM) researchers in recent years. AI-powered methods, such as machine learning, deep learning, network pharmacology, bioinformatics, systems biology, chemical informatics, and computer vision, can link chemical composition, herbal medicine, drugs, targets, symptoms, and diseases. In other words, AI provides new approaches to exploring ancient literature on TM, enabling the screening of major components of herb or formula, revealing the mechanism of action, and guiding the precise use of TM. The present Research Topic aims to discover novel approaches and strategies for developing and evaluating medicines *via* AI technologies[20.]

The aim of this study was to assess the potential antibacterial activity of *Punica granatum* L. peel in alcoholic extracts against *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *E.coli.*

Material and Methods

Collection and Sample preparation

Fresh pomegranate fruit was bought from the local market and washed thoroughly using water. The rind of the pomegranates was separated, washed with distilled water and dried. The dried rind was powdered using commercial grinder. Samples were kept in sealed plastic bags and stored at low temperature in dark, until use.

Preparation of extracts

Dried and powdered pomegranate peels were kept in air tight plastic containers and stored at freezing temperature until used for extraction. Methanol (100 mL) was added to 0.5 g of dried sample in conical flasks and was stirred for 3h at room temperature (20°C). Methanol extract was capable of recovering many compounds thus Methanol was chosen for the extraction of functional components from

pomegranate peels[16]. The extractions were performed for 48 h and concentrated by slow evaporation process[18]. The obtained extract was kept in moisture free container and used for phytochemcial analysis. Aqueous extract was also prepared using the same method.

Phytochemical screening

Primary phytochemical screening of pomegranate rind

Preparation of extracts- Aqueous extracts of pomegranate rind was prepared by soaking 3.0 g of dried rind in 80 mL distilled waterfor 24 hours, followed by filtration. Alcoholic extracts of pomegranate rind was prepared by soaking 3.0 g of dried rind in 80 mL methanol, separately, for 24 hours, followed by filtration. Chloroform extracts of pomegranate rind was prepared by soaking 0.5 g of dried rind in 5 mL chloroform, for 24 hours, followed by filtration.

Test for phenols and tannins- To 1 mL of aqueous extract, 1 mL of 10% aqueous ferric chloride was added. The presence of phenols is indicated by formation of blue or green colour. The presence of hydrolysable tannins is indicated by formation of dark blue colour, while the presence of condensed tannins is indicated by formation of green colour[8,9].

Test for flavonoids

Test (a)-To 1 mL of aqueous extract, few magnesium turnings were added followed by few drops of concentrated hydrochloric acid. He presence of flavones is indicated by the formation of red colour[8].

Test (b)-To 1 mL of aqueous extract, 1 mL of 10% sodium hydroxide was added. He presence of flavonoids is indicated by the formation of yellow to orange colour[8,9].

Test (c)-To 1 mL of alcoholic extract, 1 mL of concentrated sulphuric acid was added. He presence of flavanones is confirmed by the formation of orange to crimson red[8].

Test for anthocyanins: To 1 mL of alcoholic extract, 1 mL of 10% sodium hydroxide was added and heated for 5 minutes in 100°C water bath. He presence of anthocyanins is indicated by the formation of blue colour[8,9].

Test for coumarins: To 1 mL of alcoholic extract, 1 mL of 10% sodium hydroxide was added. He presence of coumarins is indicated by the formation of yellow colour which fluorescence under ultraviolet light[8,9].

Test for quinones: To 1 mL of alcoholic extract, 1 mL of concentrated sulphuric acid was added. He presence of quinones is indicated by the formation of red colour[8].

Test for saponins: To 1 mL of aqueous extract, 5 mL of distilled water was added, the tube was vortexed for 2 minutes. He presence of saponins is indicated by lather formation[8,9].

Test for steroids: To 2 mL of chloroform extract, 2 mL of concentrated sulphuric acid was added slowly on the tube's wall. The presence of steroids is indicated by formation of two layers, an upper red layer and a lower yellowish-green layer[10].

Test for triterpenoids: To 2 mL of chloroform extract, 1 mL of acetic anhydride was added, followed by the slow addition of 1 mL of concentrated

sulphuric acid. The presence of triterpenoids is indicated by formation of **reddish white colour**[10].

Test for alkaloids: Test (a): Dragendroff Test-To 2 mL of chloroform extract, few drops of Dragendroff reagent was added. The presence of alkaloids is indicated by formation of orange colour[8]

Test (b): Mayer's Test-To 2 mL of chloroform extract, 2 mL of Mayer's reagent was added. The presence of alkaloids is indicated by formation of white precipitate[8].

Antibacterial assay

Agar well diffusion method was used for the antibacterial assay[19]. The bacteria chosen for the antibacterial assay were obtained from American Type Culture Collection (ATCC) *Staphylococcus aureus* (ATCC 6538) [gram positive], and *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027) [gram negative]. The cultures of the chosen bacteria were grown on 25 mL nutrient agar (Hi Media) plates . Overnight nutrient broth cultures of bacteria (10^5 per 0.5 mL) were aseptically mixed with 20 mL of nutrient agar cooled down to 50°C in Petri dishes following that, wells of 7 mm diameter were made in the solidified agar medium using a sterilised steel cork borer. A volume of 100 µL of each extract was slowly loaded into the wells using micropipettes of sterilised tips, the plates were then incubated for 24 hours at 37°C. The diameter of inhibition zone surrounding the agar well for each plate was measured. Triplicate tests were done for each extract[19].

Results and Discussion

Due to the presence of a wide range of phytochemicals in pomegranate rind, it can be predicted that they have significant medicinal values. Phytochemical screening of pomegranate rind revealed that pomegranate rind contained phenols, tannins, flavonoids, quinones, coumarins, steroids, triterpenoids and alkaloids. (**Table-1**).

Table-1 Primary phytochemical screening of Pomegranate Rind.

S.No.	Phytochemicals tested	Pomegranate Rind
1.0.	Phenols	+
2.0.	Tannins	+
3.0.	Flavonoids	+
4.0.	Anthocyanins	-
5.0.	Coumarins	+
6.0.	Quinones	+
7.0.	Anthroquinones	-
8.0.	Saponins	-
9.0.	Steroids	+
10.0.	Triterpenoids	+
11.0.	Alkaloids	+
Key:(+)=Present,(-)= Absent		

Antibacterial activity

Table-2 Antibacterial activity of solvents extract of *Punica granatum* rind

S.No.	Solvent extracts	Diameter of zone of inhibition(mm)		
		Staph.aureus-ATCC 6538	***E.coli ATCC 8739***	***Psuodomonasaeruginosa ATCC 9027***
1.0.	Methanol extracts	22	21	17
2.0.	Ethanolic extract	19	17	16
3.0.	Aqueous Extracts	15	12	NAD
4.0.	Ciprofloxacin (Positive control)	38	36	37
5.0.	Solvents(Negative control)	NAD	NAD	NAD
Key : NAD= No Activity Detected				

Antibacterial activity of pomegranate rind extracts was determined Well agar Diffusion method. The methanolic extract of pomegranate rind was the most effective in inhibiting the growth of *Staph.aureus* ,*E. coli* and *Pseudomonas aeruginosa* by 22 mm ,21mm & 17 mm respectively (Table-2) followed by Ethanolic extract. Nevertheless, the pomegranate rind aqueous extract was also significantly effective in inhibiting the growth of *Staph aureus* and *E.coli* by 15 mm& 12 mm. The growth of *P. aeruginosa* was again only inhibited by the methanolic and Ethanolic extracts of pomegranate rind. Overall, the most effective extract in inhibiting the growth of the chosen bacteria was the methanolic extract of pomegranate rind.

When a new drug to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. The present study showed interesting preliminary phytochemical constituents in solvent peel extracts of *Punica granatum*. Further characterization and quantitative assay may be carried out to test the peel extracts for various therapeutic and pharma-cological activity. In any case, if any significant bioactive natural product is present, it is necessary to separate that compound from the mixture of compounds by using suitable chromatographic technique[21].

Conclusion

The present study provides more evidence on the importance and value of pomegranate fruit, especially pomegranate's rind which is usually considered as a waste product. According to the phytochemical screening done pomegranate rind contain phenols , tannins, flavonoids, quionones, coumarins, steroids, triterpenoids and alkaloids. The methanolic and s extract of pomegranate rind was the most effective in inhibiting the growth of a number of bacteria according to the well agar diffusion method. Further studies could be conducted on the antibacterial capacity of pomegranate rind components, which may led to the discovery of new antibacterial agents.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Deciphering the role of *Andrographis paniculata* in pharmaceutical field and chromatographic evaluation of *Andrographis paniculata* in the solution of methanol and hexane through HPTLC

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Abstract- This article explores the medicinal potential of *Andrographis paniculata* (Kalmegh) in Ayurveda, focusing on its hepato protective and anti febrile properties. The paper emphasizes the importance of herbal medicines in primary health care due to their availability, compatibility, and affordability. Additionally, it discusses the rich herbal medicinal wealth in India and the historical significance of Ayurveda.

The study delves into the pharmacological actions of *Andrographis paniculata* including anti-inflammatory, anti-diabetes, anti-viral, and immune stimulating properties. The main objective is chromategraphic evaluation using High-Performance Thin Layer Chromatography (HPTLC) to analyze the chemical composition of *Andrographis paniculata*. The HPTLC method is detailed, which is used in qualitative analysis of *Andrographis paniculata.*

The article concludes with the qualitative analysis of *Andrographis paniculata*, comparing chromatograms in different solvents and wavelengths. Methanol is identified as the superior solvent providing a clearer chromatogram. Chemical constitution analysis reveals the number of visible chemicals at different wavelengths, offering insights into the herb's composition. The study provides valuable information for pharmaceutical quality control and the analysis of medicinal plant.

Key words: *Andrographics paniculata,* HPTLC, Pharmacological, Ayurvedic

Inroduction

Ayurveda is a medical science thatgives us a profuse knowledge of Dravyas or drugs which we get from plants, animals and minerals. The medicinal plants are the potential source of Ayurvedic medicines and are a core component at primary health care level due to their availability, compatibility and affordability. India is perhaps the richest nation with regard to herbal medicinal wealth (about 15000-20000 plants have been found to have good medicinal value and it also has the oldest medicinal system in the form of Ayurveda. Ayurvedic classics have mentioned many efficacious herbs to treat a variety of ailments. One such herb is being Kalmegh i.e. *Andrographis paniculata* (Burm.f.) Wall. ex Nees is a potent hepatoprotective and antifebrile herb, It can be easily grown in major types

of soil and uptakes minimal amount of water in the several areas and well recognized medicinal plant widely utilized in Thailand, China, India, and several other Asian countries.

Andrographis paniculate Burm. Nees (AP), commonly known as 'King of bitters', is an indigenous South Asian herb belonging to the Acanthaceae family. It is widely used traditionally in managing diabetes, hypertension, cancer, rheumatoid arthritis and other inflammatory conditions. In Indian and Chinese traditional medicine, AP has been used to address coughs, colds and influenza, indicating its potential effectiveness in managing respiratory tract diseases.

According to phytochemical research, *A. paniculata* encompasses a wide range of bioactive components of pharmacological significance including high quantity of flavonoids, quinoids, xanthones, tannins, alkaloids and other compounds (Hossain et al., 2014)

Pharmacological properties and the rapeuticactions of Kalmegh

In Ayurveda, the therapeutic actions of any drug is based on it's properties such as Rasa, Guna, Virya, and Vipaka**.** According to Ayurvedic Pharmacopeia of India the properties and actions of Kalmegh are listed below.

Rasa (Taste) – Tikta (Bitter)
Guna (Qualities) – Laghu (Light for digestion),
Ruksha (Dryinnature) Veerya (Potency)- Sheet (Cold)
Vipaka (Metabolic Property)- Katu (Transforms into Pungent / Spicy taste after digestion)
Karma (Actions)- Kaphapitta shamaka (reduces vitiated kaphaand pitta dosha), Dipana (appetizer), Pachana (digestive), Yakrut uttejaka (stimulates liver), Jwaraghna (antipyretic), Krimighna (wormicidal), Raktashodhak (purifies blood), Shothahar (reduces oedema), Svedajanana (stimulates sweating).

Chemical Constituents

Andrographolide, neo andrographolide, and rograpanin, 14-Deoxy–11, 12-didehydro andrographolide, are the principal chemical constituents found in the plant.

These chemical constitutents play a important role in pharamaceutical field.

a) **Andrographolide-** Widely used as a bitter tonic, for snake bite and for the treatment of hepatitis.
b) **Neoandrographolide-** Mainly used for treating acute bacterial dysentery, acute gastroenterit is upper respiratory tract infection, acute tonsillitis, pharyngitis.
c) **Andrograpanin-**Andrograpanin, a bio-active compound from *Andrographis paniculata*, exhibits anti-inflammatory and anti-infectious properties.
d) **14-Deoxy-11, 12-didehydo and rographolide-** a diterpenoid in *Andrographis paniculata* (Burm.f.) Nees, acts as a bioactive phyto nutrient that can treat many diseases.

Analysis of pharmaceutical compounds and drugs is commonly used in all the stages of drug discovery and development process. These analytical techniques provide more accurate and precised data, not only supporting drug discovery and development but also postmarket surveillance. Pharma-ceutical analysts work regularly to improve the reliability of existing techniques to cope up the demands for better chemical measurements. Modern pharmaceutical analysis is mainly dominated by costlier

instrumental analysis. Hence, many analysts focus is on developing newer applications, discoveries and new methods of analysis to increase the specificity and sensitivity of a method.

Analytical methods used in drug analysis are diversified and are still being improved to find better solutions to satisfy manufacturers and institutions that test drug quality. Official documents dealing with the problem of QC of pharmaceutical products recommend diversified analytical techniques, with chromatographic methods playing a significant role in pharmaceutical analysis.

The usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step a head when compared with other tools of chromatography.

HPTLC (High performance thin layer chromatography)

HPTLC (high-performance thin layer chromatography) is sophisticated form of TLC, which provides superior separation efficiency. The HPTLC concept includes validated methods for qualitative and quantitative analysis, and fulfills all quality requirements for use in fully regulated environments.

HPTLC Principle- The HPTLC works on the same principles as TLC such as the principle of separation is adsorption. The mobile phase or solvent flows through the capillary action. The analytes move according to their affinities towards the stationary phase (adsorbent). The higher affinity component travels lower towards the stationary phase. A low-affinity component travels rapidly toward the stationary phase. On a chromatographic plate, then, the components are separated.

HPTLC Application Fields- HPTLC plates offer superior separation performance for quantitative evaluation of highly complex samples in

1. Pharmaceutical quality control
2. Analysis of medicinal plants and herbs
3. Analysis of pesticide mixtures

Instrumentation

a) Linomats
b) Chromatographic chamber
c) Visulizer
d) Scanner

The Advantages of HPTL Care as follows -

1.More than one analyst works on the system simultaneously.
2.HPTLC can be sharable, as it is not devoted to any sample.
3.The pre-coated plates of HPTLC are available at low prices.
4.There is less maintenance cost as compared toothe requipment.
5.HPTLC has no risk of contamination, since the use of the freshly prepared mobile phase and stationary phase.
6.Mobile phases are not required for filtration and degassing such as HPLC.
7.It is highly sensitive, reproducible and precise as compared with a thin layer chromatography

Material and Methodology

Material required – Dried kalmegh, oven, mixer grinder, conical flask, measuring cylinder, spatula, methanol, hexane, whatman filter paper, Aluminium foil, chloroform : methanol – 90 :10 (For mobile phase), silica gel plate, dryer, HPTLC

Procedure for chromatographic evaluation of *Andrograph paniculata*

We followed following steps for chromatographic evaluation of *Andrographis paniculata.*

1. Sample drying – Firstly, dried the given sample of the *Andrographis paniculata* in the oven for 25 – 30 minutes and after that put it out from the oven.

2. Powdering of the sample– Made a powder of the given sample in mixer grinder.

3. Sample prepration -For the prepration of sample, took 2 gm powder of the kalmegh in the conical flask with the help of spatula and then mixed it with a 20ml hexane, using as solvent. Same process repeats for the preparation of the other sample, mixed the 2 gm powder of the kalmegh in the methanol (using as solvent), separately in the other conical flask. Closed the conical flask with the help of aluminium foil. Kept the above mixture for overnight. Filtered the above mixture by using whatman filter paper.

4. Sample application– Used the linomat, filled the mixture in syringe and then pressed the run button, then sample started to apply in silica gel plate.

5. Put the plate in Mobile phase of HPTLC – Put the above silica gel plate in mobile phase (chloroform: methanol – 90: 10) untill solution was reached at the marked point.

6.Plate drying –Plate was dried with the help of dryer.

7.Putting the plate in visualizer–Put the Plate invisualizer and then saw the chromatograms in win CATS Software in computer (which was attaches to a HPTLC Instrument) in a 254nm and 366nm wavelength**.**

8.Report reading– Lastly, read the report, in computer.

After reading report carefully, we did qualitative analysis of *Andrographis paniculata* and also compared the chromatogram of the both samples in n-hexane solution and methanol solution in 254nm and 366nm wavelength**.**

Result

Conclusion

After seeing above result, we could do qualitative analysis of *Andrographis paniculata* and concluded the following things:

Best solution – Methanol is the better solution than hexane because methanol gave good result, more visible and clear chromatogram than hexane.

With hexane

Wavelength	No. of the chemical
At 254 nm	Not visible clearly
At 366 nm	Only 4 are visible

Methanol gaves best chromatogram at 366 nm wavelength.

Chemical constitution – After seeing above result, we can count number of the chemicals which are visible in chromatogram of kalmegh at different wavelengths with hexane and methanol as given here under.

With methanol

Wavelength	No. of the chemical
At 254 nm	Only around 4-5 are visible
At 366 nm	Around 15 are visible

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Volatile constituents of *Nardostachys jatamansi* DC. rhizomes: from Uttarakhand Himalaya (India)

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Abstract- The rhizomes of *Nardostachys jatamansi* DC. were collected from two alpine Himalayan locations of Uttarakhand (India). The essential oils were obtained by hydro-distillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) in order to determine the variation of concentration in their constituents. A total of 22 compounds were identified in both the oils, accounting 96.41–98.22%. The major constituents of *N. jatamansi* oils were characterised as patchoulol (46.0–54.11%) and calarene (9.10–15.6%). Due to the higher relative area quantum of patchoulol in *N. jatamansi* populations growing in Uttarakhand, there is need to develop propagation protocol for mass multiplication and in-situ and ex-situ conservation.

Key words: *Nardostachys jatamansi* DC.; Valerianaceae; essential oil, patchoulol

Introduction

Nardostachys jatamansi DC. (family-Valerianaceae), commonly known as Indian Nard, spikenard or balchar, is a 10-60 cm high perennial herb found in alpine Himalayas [1] (Anonymous, 1997). The species has very long history of use as medicine in Ayurveda, Homeopathy, Ethno-medicine and Indian system of medicine (ISM) to modern medicine industry which is distributed in the Himalayas from Pakistan, India (Jammu and Kashmir, Himanchal Pradesh, Uttarakhand and Sikkim) to Nepal, Tibet and China between 3300 to 5000 m asl. It has been reported the species has become critically endangered depending on habitats [2-4] (Nayer and Sastry, 1988; Airi et al., 2000; Nautiyal et al., 2003) due to over- exploitation of rhizomes for medicinal use, habitat degradation and other biotic interference. Rhizome of *N. jatamansi* is used in Perfumery products, Tonic, Stimulant, Laxative, Diuretic, Anti spasmodic and Stomach ache. It promotes the growth of hair and imparts blackness (Anonymous, 1997; Kirtikar et al 1993; Nadkarni, 1954). Traditionally, Jatamansi is used as tonic, stimulant and antiseptic

and also used for the treatment of epilepsy, hysteria, convolutions, heart palpitation, intestinal colic and antiarrhythmic activities (Anonymous, 1985) and also is the active components of many Ayurvedic formulations such as Tapaswiniwati, Jestalabangadi, Chandanadi churna and Rachhogna ghrit (Adhakari, 1998) etc. Extensive work on the chemical constituents as well as on the composition of the essential oils of *Nardostachys* is reported in literature (Mahesh et al., 2012; Vijendra and Ali 2002; Bagchi et al., 1990 and Sun et al., 1985). Chemical profiling studies on this species have revealed its great pharmaceutical importance for the mankind e.g. the oil of spikenard possesses antiarrhythmic activity with possible therapeutical usefulness for auricular flutter (Anonymous 1997), Anxiolytic effect (Razack and Khanum 2012), Sedative effect (Takemoto et al., 2008). Therefore, it is necessary to access the quality of the oil obtained from the Jatamansi from its natural habitats. In this paper we report the variation of chemical composition between two populations collected from different location in Garhwal Himalaya.

Material and Methods

Plant Material

Fresh rhizomes of *N. jatamansi* were collected during the month of October, 2012 from two naturally growing locations of Garhwal Himalaya of Uttarakhand (India); Kedarnath (Rudraprayag); 30°73′27″ N and 79°07′74″ E; altitude 3400 m and Hansabugiyal, Ghesh (Chamoli); 30°08′51″ N and 079°57′35″ E; altitude 3100 m. The specimens were identified by Prof. R.D. Gaur, Department of Botany, H.N.B. Garhwal University, Srinagar Garhwal. The voucher specimens have been deposited at herbarium of HAPPRC (Acc. No.: HAPPRC- AG/G SBB-1, 2).

Isolation of Essential oils

The shade dried rhizomes (250 gm) were chopped into small pieces and subjected to hydro-distillation for 5 hours using a Clevenger apparatus. The isolated essential oils were dried over anhydrous sodium sulphate and stored carefully in dark vial at low temperature until analysis.

Gas Chromatography (GC)

GC analyses of the oil samples was carried out using Agilent (HP7890 GC) gas chromatograph equipped with a Flame Ionization detector (FID) and a HP-5 fused silica column (30 m×0.32 mm, 0.25 μm film thickness). The sample was injected directly into the column. Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed from 60° to 220° with an increase in rate of 3°/min. The injection volume was 0.2μL.

Gas chromatography-mass spectrometry (GC-MS)

Analysis of the oil was performed out on Agilent mass spectrometer (Model 5975C) coupled to an Agilent gas chromatograph with a 60 m×0.32 mm, 0.25 μm film thickness column (DB5). The sample was injected directly in split less mode. Helium was used as the carrier gas (flow rate 1 mL/min). The oven temperature was programmed from 60° to 220° at 3°C/min. Other conditions were the same as described under GC. The mass spectrum

was taken with a mass range of 40-600 Daltons.

Identification of components

The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes, C_8-C_{24} with co-injection of standards (Sigma Aldrich, USA) under same analytical conditions and by matching their recorded mass spectra with the MS library (NIST/Pfleger/Wiley) and available literature (Adams, 2009).

Results and discussion

The essential oils obtained from *N. jatamasnsi* rhizomes were slightly viscous and pale yellow in colour with strong spicy odour. The yields were ----% for Kedarnath population and ----% for Hansabugiyal (Ghes) population.

The composition of the essential oils obtained from *N. jatamasnsi* rhizomes is presented in Table 1. Altogether, 22 compounds were identified by GC and GC/MS representing 98.4% (Kedarnath oil) and 97.6% (Ghesh oil). Both the oils were dominated by oxygenated sesquiterpenes, representing 58.7% and 46.0% in Kedarnath and Ghesh oils, respectively. The sesquiterpene hydrocarbons were found to be 35.8% (Ghesh oil) and 31.5% (Kedarnath oil).

The GC and GC/MS analysis of essential oils of *N. jatamansi* allowed the detection of 24 components indentify in accounting 98.22% occurred in Kedarnath and 96.41% in Hansabugiyal (Ghes) of the oil (Table 1). Quality assessment of the essential oils from *Nardostachys jatamansi* obtained from Kathmandu valley market (Paudyal et al., 2012) identified 31 compounds with total 63.41% of essential oil which is quantitavely less in amount as compared with two populations of present study.

The major common compound of *N. Jatamansi* in both the location were characterised as Patchouli alcohol (54.11% in Kedarnath and 46.0 % in Hansabugiyal Ghes) and Calarene () (15.6% in Hansabugiyal Ghes and 9.10% in Kedarnath) with high degree of variation between the location. It yields up to 1.9% of a pale yellow essential oil (spikenard oil) with a pleasant odour, suggestive of patchouli or valerian and oil also possesses antiarrhythmic activity with possible therapeutical usefulness for auricular flutter; it is less effective than quinidine but has the advantage of being less toxic (Anonymous, 1997). Pachouli alcohol (54.11%), Caryophyllene oxide (10.25%), Calarene (9.10%), Elemene (4.56%) and Formic acid (4.4%) were the major compounds in the sample collected from Kedarnath, a totals of 24 compounds detected including other minor compounds like Caryophyllene (3.3%), Gurjunene (2.5%) and H-epi-cubedol (2.20%) found greater than 2% of peak area of FID response. Sample collected from Hansabugiyal (Ghes) showed Pachouli alcohol (46.0%), Calarene (15.6%), Caryophyllene (5.8%), p- Myrecene (5.6%) and H-epi-cubedol (5%) as the major compounds whereas other minor compounds like Calarene oxide (3.5%), p-Myrecene (2.33%), 1,8-Cineole and α-pineene (2.1%) found greater than 2% of peak area of FID response. Study of volatile constituents of the rhizome of *Nardostachys jatamansi* (DC.) by Mahalwal and Ali 2002 showed n-Hexane (0.2%), α-Pinene (0.1%), β- Pinene (0.4%), p-Cymene (0.4%), 1,8-Cineole (0.2%), Terpinene-4-ol (0.1%), Copaene (0.30%) and Caryophyllene (3.3%)

compounds are the same with our result sample collected from Kedarnath population.

Table 1. Volatile constituents of *Nardostachys jatamasnsi* rhizomes from two locations of Uttarakhand Himalaya (India)

Constituents	**RI**	**Composition (%)**	
		Kedarnath	Hansabugiyal
formic acid	406	4.4	1.6
propionic acid	446	1.4	0.8
α-pinene	939	0.1	2.1
β-pinene	981	0.4	0.9
β-myrcene	992	0.2	2.3
p-cymene	1027	0.4	5.6
1,8-cineole	1031	0.2	2.3
terpinen-4-ol	1179	0.1	tr
γ-terpineol	1199	tr	0.2
α-copaene	1377	0.5	0.9
β-elemene	1393	4.6	1.9
α-gurjunene	1412	4.1	tr
β-caryophyllene	1418	3.3	5.8
calarene	1435	15.1	21.6
α-patchoulene	1456	1.9	0.7
α-humulene	1459	0.3	3.5
β-guaiene	1495	1.4	0.9
α-selinene	1498	0.3	0.5
cubebol	1516	2.2	5.0
caryophyllene oxide	1586	10.3	1.3
patchoulol	1656	46.8	39.1
valeranone	1676	0.4	0.6
monoterpene hydrocarbons		1.1	10.9
oxygenated monoterpenes		0.3	2.5
sesquiterpene hydrocarbons		31.5	35.8
oxygenated sesquiterpenes		59.7	46
others		5.8	2.4
Total identified (%)		**98.4**	**97.6**

RI: Retention index relative to n-alkanes (C_8-C_{24}) calculated on a non-polar HP-5 capillary column; tr: trace (<0.05)

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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About flowers on the cover page

Hemidesmus indicus (Anantamool)

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Gentianales
Family: Asclepiadaceae
Genus: *Hemidesmus*
Species: *Indicus*

Hemidesmus indicus, Indian sarsaparilla, is a species of plant that is found in South Asia. It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody and aromatic. The stem is numerous, slender, terete, thickened at the nodes. The leaves are opposite, short-petioled, very variable, elliptic-oblong to linear-lanceolate. The flowers are greenish outside, purplish inside, crowded in sub-sessile axillary cymes.

It is Tonic, Diuretic, Demulcent, Disphoretic and Blood purifier. Employed in Nutritional disorders, syphilis, chronic rheumatism, gravel and other urinary diseases and skin afections

Anantmool is one of the Rasayana plants of ayurveda as it is anabolic in effect. It is used for veneral diseases, herpes, skin diseases, arthritis, gout, epilepsy, chronic nervous didorders, abdominal distention, debility etc. It'ssaponin content is considered to have a steroidal effect that enhances the production of testosterone.

Sesamum indicum *(Til)*

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Pedaliaceae
Genus: *Sesamum*
Species: ***S. indicum***

Sesamum indicum L. (Pedaliaceae) is an annual plant, which has been domesticated for well over 5000 years. It is widely cultivated for its seeds and is one of the oldest known oilseed crops. Traditionally, its seeds, seed oil, and different organs of the plant have been used to treat various diseases or conditions like ulcers, asthma, wound healing, amenorrhea, hemorrhoids, inflammations, etc.*Sesamum indicum* has great importance in traditional Indian medicine, which is further supported by modern pharmacological studies, especially in hepatoprotection, inflammation, and cancer. Several researchers have suggested that *Sesamum indicum* extracts and isolated compounds could have a wide therapeutic potency range.

Punica granatum (Anar)

Kingdom: Plantae
Phylum: Tracheophyta
Family: Punicaceae
Kingdom: Plantae
Order: Myrtales
Genus : *Punica*
Species: *Granatum*

The pomegranate is a fruit-bearing deciduous shrub in the family Punicaceae, subfamily Punicoideae, that grows between 5 and 10 m tall. The pomegranate was thought to have originated from Afghanistan and Iran before being introduced and exported to other parts of Asia, Africa, and Europe.

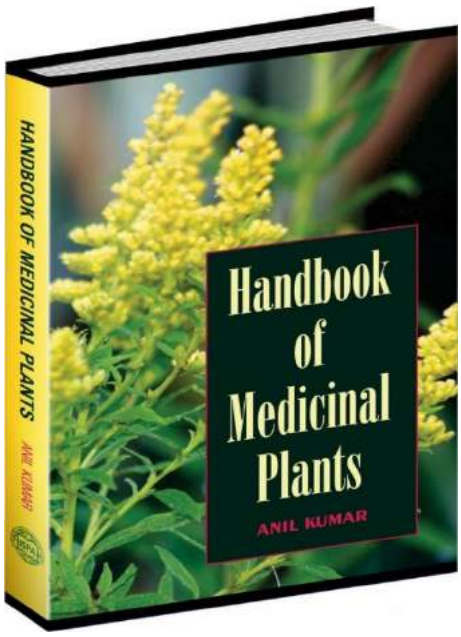
Punica granatum, commonly known as pomegranate is a member of the monogeneric family, Punicaceae, and is mainly found in Iran which is considered to be its primary centre of origin. *Punica granatum* possess is various pharmacological and toxicological properties including antioxidant, anti-inflammatory (by inhibiting pro-inflammatory cytokines), anti-cancer and anti-angiogenesis activities.

*Trachyspermum ammi (*Ajowain*)*

Scientific name: Trachyspermum ammi
Family: Apiaceae
Kingdom: Plantae
Order: Apiales
Genus:*Trachyspermum*
Species: *ammi*

Ajwain or ajowan —also known as ajowan caraway, omam, thymol seeds, bishop's weed, or carom—is an annual herb in the family Apiaceae. Both the leaves and the seed-like fruit of the plant are consumed by humans. The name "bishop's weed" also is a common name for other plants. Ajwain is a bitter, aromatic warming herb with a thyme-like aroma used internally for colds, coughs, influenza, asthma, diarrhoea, cholera, colic, indigestion, flatulence, oedema, arthritis and rheumatism.Externally for vaginal discharge and rheumatism, used mainly in Ayurvedic medicine as a stimulating decongestant for the respiratory and digestive systems.
Ajwain oil is given to expel hookworms.

REVIEW



Handbook of Medicinal Plants

Authored by: **Anil Kumar**

The Book entitled **'Hand Book of Medicinal Plants'** authored by **Anil kumar** and published and printed by International Scientific Publishing Academy 4594/9 Daryaganj, New Delhi will appeal to professional in the field of medicinal plants.

The publishers and its authors make no representation or warranties with respect to accuracy or completeness of contents of the book. The contents of the book has been divided into six chapters **1.** Medicinal Plants: An introduction, **2.** Medicinal Plant- and their Traditions, **3.** Health Matters in a changing community, **4.** Acquiring Herbal Knowledge, **5.** Herbs as Medical Intervention, **6.** Practices of treating the ailments and symptoms alongwith Glossary, Bibliography and Index.
It covers descriptions specially (a) Heyday and Decline of Indigenous Remedies. (b) Safety and Efficacy of Herbal Medicine (c) Pharmacology and Actions (d) Atherosclerosis (e) Toxicity (f) Cognitive Functioning
(g) cytotoxic activity (h) Chemistry (i) Phytochemical Characteristics (j) Folk Medicines (k) Economics (l) cultural studies.

Herbs obtained from (1) Roots and other underground parts, barks, stems, leaves, flowers, fruits, seeds, lower part of plants and insecticides and raticides have been mentioned.

Many conventional drugs, which originate from plant sources: some of the most effective drugs are plant based, such as aspirin derivedfrom bark of willow, digoxin derived from foxglove, quinine derived from the bark ofcinchona and morphine derived from picuum poppy. The development of drugs from plants by drug companies encourage large scale of pharmacological screening of herbs as well as Chinese herbalism as the most dominant of the ancient herbal traditions have been described. The book may act as an essential reference guide for well being

Dr. I. P. Saxena
(Editor)

Forthcoming Events

- **International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB), New York, United States of America**
  2024-01-01
- **International Conference on Medical, Pharmaceutical and Health Sciences (ICMPH), Male, Maldives**
  2024-01-02
- **International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB), Dehradun, Uttarakhand, India**
  2024-01-02
- **World Conference on Pharma Industry and Medical Devices (WCPIMD), Rajkot, Gujarat, India**
  2024-01-02
- **1690th International Conference on Medical, Biological and Pharmaceutical Sciences (ICMBPS), Dublin, Ireland**
  2024-02-01
- **International Conference on Medical, Pharmaceutical and Health Sciences (ICMPH), Chennai, Tamil Nadu, India**
  2024-02-04
- **International conference on Medical Health Science, Pharmacology & Bio Technology (ICMPB), Salem, Tamil Nadu, India**
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- **International Conference on Medical, Pharmaceutical and Health Sciences (ICMPH), Thrissur, Kerala, India**
  2024-03-31
- **International Conference on Medical, Pharmaceutical and Health Sciences (ICMPH), Goa, India**
  2024-03-28
- **International Conference on Medical, Pharmaceutical and Health Sciences (ICMPH), Kawasaki, Japan**
  2024-03-21
- **1582nd International Conference on Pharma and Food (ICPAF)Moscow, Russian Federation**
  2024-04-10
- **International Conference on Pharmaceutical Science and Drug Manufacturing (ICPSDM),Kyoto, Japan**
  2024-04-12
- **International Conference on Medical, Pharmaceutical and Health Sciences (ICMPH), Fukuoka, Japan**
  2024-04-26
- **International Conference on Pharmaceutical Science and Drug Manufacturing (ICPSDM),Bangkok, Thailand**
  2024-05-02
- **1746th International Conference on Medical, Biological and Pharmaceutical Sciences (ICMBPS), Malacca, Malaysia**
  2024-05-03
- **International Conference on Pharmaceutical Science and Drug Manufacturing (ICPSDM), Kuala Lumpur, Malaysia**
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- **1752nd International Conference on Medical, Biological and Pharmaceutical Sciences (ICMBPS), Bali, Indonesia**
  2024-05-13
- **1613th International Conference on Pharma and Food (ICPAF), Hong Kong, Hong Kong**
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- **International Conference on Pharmaceutical Science and Drug Manufacturing (ICPSDM), Paris, France**
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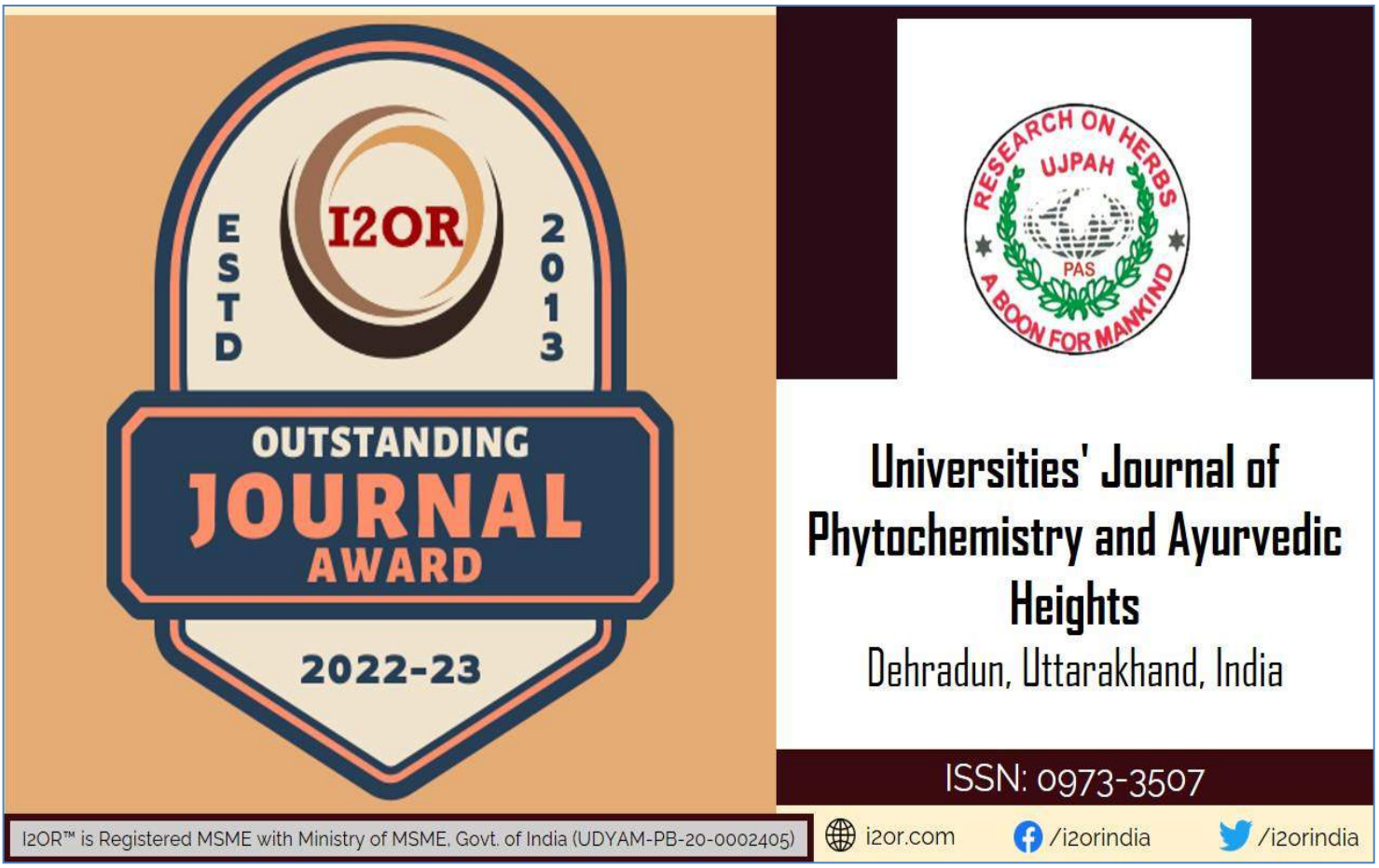
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Initiatives for promotion of reverse pharmacology in Ayurvedic domain

Uttarakhand State Council for Science and Technology (UCOST) was established in Uttarakhand in 2005 after a new state was carved out of Uttar Pradesh. Due to difficult terrain, the hill state has seen limited technological intervention which could otherwise have propelled technology-led development in the state. UCOST is playing a crucial role in developing a science and technology enabled ecosystem in the state to encourage Research & Development activities, Identification and support to Scientific talent at all the levels, Science dissemination, Entrepreneurship development in the state. UCOST is driving collaborative innovation through Science & Technology leveraging state of art infrastructure at Dehradun. Council aims to forge conducive result-oriented partnerships between central and state government, Civil society organizations, R&D Institutions, Academia and Industry. The regional science center within the UCOST campus has been drawing large crowds from schools and colleges. This Science center has an innovation lab sponsored by National Innovation Council.

UCOST has initiated an ambitious "Ayurvedic Drug Development" initiative within the ambit of reverse Pharmacology and the guidance laid down by WHO for the development of natural herbs and other products. We have recently reviewed promising therapeutic effects of Herbo-Mineral formulations for prophylaxis of chronic pancreatitis and migraine, Faltrika dikwath for prophylaxis of Hepatitis B and started to facilitate advanced R&D following reverse pharmacology. We are committed to promote and support Ayurvedic drug development for some of the tropical diseases in near future. We intend to work with Vaidyas or traditional Ayurvedic healers to preserve and maintain meticulous record of their clinical work and harvest this important traditional knowledge in the state.

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

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