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Ananas comosus-Pineapple

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Editorial

Ayurveda is getting global acceptance primarily due to its age-old therapeutic practice, profound conceptual basis and survival of its medicines for thousands of year. It is great to see that the concepts, drugs ,formulations developed in ancient times finds their relevance today albeit changes in environment, life style & culture and disease patterns. The philosophy of treating a system or body as a whole is gaining relevance during transition from reductionist approach to "systems" approach in the post genomic era. Chemical standardization like biomarker and metabolite profiling has unfolded a diverse che- mical space of safe and therapeutically relevant molecules. Exploring molecular and network phar- macology of intelligent traditional formulations to elucidate and vali- date safety, toxicity, pharmacokinetics ,metabolic stability, drug-herb interactions etc. are gaining importance. Focus on the validation of clinical traditions and practices like Panchakarma, Marmachikitsa, Ag- nikarma, Parpatichikitsa, Sarpavishachikitsa, Rasayana needs in depth scientific exploration. Ayurveda pharmaceutics needs special exploration as it presents rational multi-component formulations, safer drugs from herbs, metals and minerals, marine sources with novel pharmaceutical processes detailed in 'Bhaisajya Kalpana and Rasa Sashtra'.

Today **Ayurvedic or Herbal Neutraceuticals** may be used to improve health, prevent chronic diseases and postpone aging process or any product derived from food sources with extra health benefits. The Philosophy behind Neutraceuticals was about 2000 years ago, Hippocrates (Fathers of Medicine) correctly emphasized **"Let food be your medicine and medicine be your food"**. The term "nutraceutical" was coined from 'Nutrition' (a nourishing food component) & **'Pharmaceutical'** (a medical drug).

The term **"health supplements and nutraceuticals"** is evolving and the nomenclature for "health supplements and nutraceuticals" varies across countries. For e.g., Canada naming them 'natural and non-prescription health products' the USA calling them dietary supplements Japan naming them foods with health claims, European Union Known as Food supplements in Russia known as biologically active supplements.

The **global nutraceutical industry**, valued at US$ 182.6 bn in 2015, is one of the fastest growing industries today .Currently, the United States, Europe and Japan account for most (93%) of the total global nutraceutical market. According to a recent report, the **nutraceuticals industry in India** is worth about $ 2.2 bn and is projected to grow at 20% to $ 6.1 bn by 2019-2020.

This issue of UJPAH presents different scientific facets of Ayurvedic system.This will provide a forum for Ayurveda interest groups to exchange ideas, learn about developments and salute initiatives that hold promise for evidence based research regarding health and well-being.

UJPAH works on dissemination of knowledge in this area with the major highlights on "Globalizing local knowledge and localizing global technologies."

We express our special gratitude to all the authors for their valuable contribution and to the reviewers for their esteemed support for this issue. It would have not been possible to complete this work without the active help rendered from the research group of different universities and institutions.

Dr. S. Farooq
Chief Editor

CONTENTS

Drug Induced Haematotoxicity and It's Amelioration by Plant Products

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Abstract-Haematotoxicity refers to any kind of abnormality/toxicity present in blood. Manifestations of haematotoxicity are: aplastic anaemia, haemolytic anemia, bone marrow supression, DNA single stranded breaks, apoptosis in haematoprotective progenitor cells, leukemia, gray baby syndrome, increased myeloid/ erythroid (m:e) ratio, neutropenia, and thrombocytopenia.

Plants serve as haematoprotective agents. They actually bind to the oxidative radicals produced during the haemolysis of red blood cells. This binding restores the actual form of these cells. In this way, the degenerative effect of plants on the bloods is ameliorated by plants.

Keywords: *Saraca indica*, *Hibiscus rosa sinensis*, Anemia, phenylhydrazine, haematotoxicity.

Introduction

Hematotoxicity is induced by many drugs like chloramphenicol, phenyl-hydrazine, benzene. Phenylhydrazine (Hydrazinobenzene) is the chemical compound characterized by Hermann Emil Fischer in 1895. It is mainly used as a chemical intermediate in the pharmaceutical, agrochemical and chemical industries. Phenylhydrazine derivatives were primarily used as antipyretics but its toxic action on red blood cells made their use dangerous. Phenylhydrazine was mainly used for experimental induction of anemia in animals. Phenylhydrazine acts as a potent drug against polycythemia vera (Falconer, 1933), a disorder (Spivak, 2002), which is characterized by increase in the total number of erythrocytes in the body. Phenylhydrazine decreases haemoglobin levels, RBC (Red Blood Cell) count and CV (Packed Cell Volume) whereas increases the MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Conce- ntration) and extramedular haematopoiesis in the spleen and liver (Unami *et al.,* 1996). Phenylhydrazine induces toxicity at various levels i.e. haemolytic anemia, alter iron metabolism that lead to the splenomegaly and activate immune response. Apart from this phenylhydrazine also creates genotoxicity and interfere JAK-STAT pathway.

The *Hibiscus rosa sinensis* (Fam. Malvaceae) is a glabrous shrub available throughout India and widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers. In medicine, however, the red flowers varieties are preferred. It is native to China and also occurs in India and Philippines.

The leaves, flowers and roots of the plant were found to have medicinal values. Several articles and ancient literature have shown that the flowers of this plant possess antifertility activity like anti implantation, abortifacient, in rodents (Batta and Santhakumari, 1970). Previous studies showed that the plant possesses anti-complementary, anti-diarrhetic and anti-phlogistic (Shimizu *et al.,* 1993) activity. *Hibiscus sosa sinensis* showed remarkable haemoprotective activity at dose of 400 mg/kg body wt (Meena *et al.,* 2014).

Saraca indica showed strong antioxidant activity. The radical scavenging activity of *Saraca indica* seems to be correlated with its polyphenolic constituents though active components could play important roles in its antioxidative effects. Polyphenols act as

reducing agents and antioxidants via several mechanisms including the scavenging of free radicals, chelation of transition metals as well as the mediation and inhibition of enzymes (Chang *et al.,* 2011). Consequently, it is possible that the total phenolic constituents may contribute to antioxidant activity of *Saraca indica*.

Material and Methods

Chemicals

Methanol, phenylhydrazine were purchased from Sigma Aldrich (USA). All other chemicals used in the study were of analytical - Regrade and available locally.

Plant Materials

The fresh plant sample of *Saraca indica* and *Hibiscus rosa sinensis* were btained from a local commercial source in Lucknow (India). The plants were identified at Division of Botany, CSIR-Central Drug Research Institute, Lucknow.

Preparation of Plant Extracts

The plant samples were dried at room tempeature and grounded into powder. Dry powder (2kg) was macerated in 100% methanol and then kept for 48h at room temperature. The resulting extract was filtered. The filtrate was concentrated in an oven at a temperature of 40°C for 24 h to a constant weight. The extract was stored at –20°C.

***In vivo* hematoprotective activity**
Test animals

Charles Foster male rats (150-175 gm) were obtained from National Laboratory Animal Center (NLAC), Central Drug Research Institute, Lucknow (India) and were allowed to acclimate to animal room conditions for 7 days prior to experimentation. They were kept in a controlled environment at 25±2 °C at 30– 60% relative humidity with a 12 h light and dark cycle. The animals were fed a standard rodent pellet diet and water *ad libitum*. Animal studies were conducted according to the regulaions of the Institutional Animal Ethics Committee and the protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals, NewDelhi (India), IAEC No.- IAEC/2012/86.

Induction of Anaemia

Basal hematological profile was determined for healthy Charles Foster rats using the fully automated MS9 hematology analyser.

Anaemia was induced by phenylhydrazine in Charles Foster rats, which was as a model for the study of hematinic effects with a dose of 10mg/kg body wt for seven consecutive days. After 7 days the hematological profile was determined again and the rats with a 30% reduction in red blood cells count and hemoglobin concentration were considered anaemic and used for this study.

Experimental procedure

Rats were randomly divided into six groups, each group contained 5 male rats. The rats of group – I (Control) were orally administered with 1% gum acacia. The rats of group – II to group – VI were orally administered with phenylhydrazine once daily for 7 consecutive days at dose rate of 10mg/kg body wt. On day seven, the hematological parameters were assessed by MS9 fully automated hematology analyzer for the diagnosis of hematotoxicity.. Group – II severed as phenylhydrazine control group. Group – III, IV were administered with *Saraca indica* at dose of 200 and 400 mg/kg body wt accordingly. Also the rat of group –V and VI were administered with *Hibiscus rosa sinensis* at dose of 400 and 800 mg/kg body wt.

Food and water consumption

Monitoring of 24-hour food and water consumption of the animals in a group was done at the beginning and at end of the study by giving a measured quantity of water and pellet diet followed by estimation of the amounts

remaining at the end of 24 hours. Average food and water consumption per animal was calculated for each group.

Hematological analysis

Blood samples were collected at 0, 7 and 14 days through tail vein in EDTA coated vials. Hematological parameters were analyset using MS-9 fully automated hematology analyzer. The following parameters were estimated in blood samples i.e. erythrocyte (RBC), total and

differential leukocyte (WBC), hematocrit (Hct), hemoglobin (Hb), platelet count, volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet mean corpuscular-volume (MPV), platelet distribution width (PDW) and red distribution width (RDW).

Statistical analysis

All *in vitro* antioxidant assay were done in triplicate manner and results were expressed as mean ±S.D. Data were analyzed with one-way ANOVA. Statistically significant effects were further analyzed. The statistical significance was determined at $p < 0.05$.

Results

Body weight

There was a uniform and comparable gain in body weight among the animals of control. The animals of phenylhydrazine treated groups showed slow gain of weight as ompared to control group. After treatment with plant extract, there was significant gain in the *Saraca indica* and *Hibiscus rosa sinensis* treated groups as compared to phenylhydrazine treated animals at the end of study at day 14th in different treatment groups (Figure- 1).

Food consumption

There was no significant difference in the food consumption of control group animals, but phenylhydrazine treated animals showed decreased food consumption.

The animals treated with *Saraca indica* and *Hibiscus rosa sinensis* showed no significant difference in the consumption of food as compared to control (Figure- 2).

Water consumption- Like food uptake significant differences were observed in the

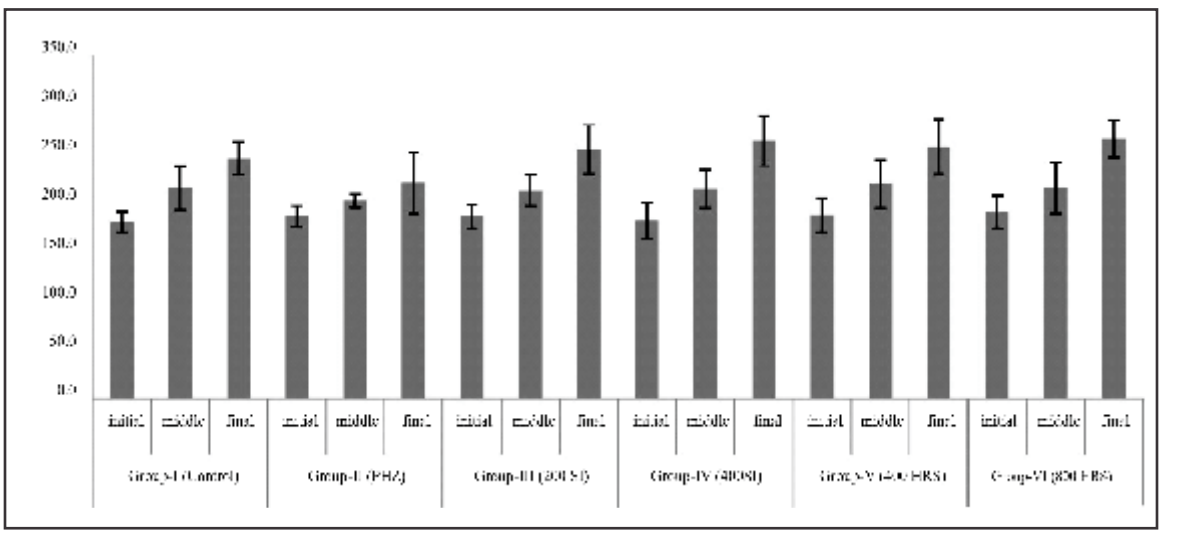

Figure – 1 Effect of phenylhydrazine on the body weight of different groups as compared to control.

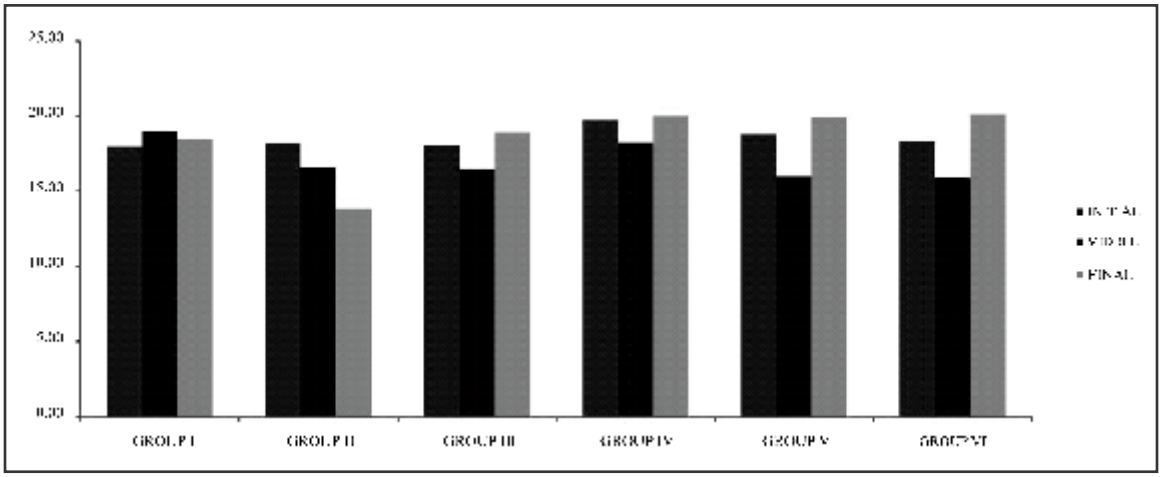

Figure – 2 Effect of phenylhydrazine on food consumption of different groups as compared to control.

consumption of water in the phenylhydrazine treated animals but reduction is recovered by the treatment of *Hibiscus rosa sinensis* and *Saraca indica* (Figure-3)

Induction of anaemia

Phenylhydrazine administration to experimental animals revealed a highly significant reduction ($p>0.001$) in RBC, haemoglobin and MCV (Figure 4,5,6), which showed anaemic condition in rats. However, there were no significant differences in haematocrit, MCH, MCHC, RDW and Hct values between the normal and anemic animals.

The values of haemoglobin were remained constant in control group but in phenylhydrazine treated group values showed significant reduction as compared to control. In *Saraca indica* and *Hibiscus rosa sinensis* treated groups here was no significant difference in he initial and final values of haemoglobin. Increased level of WBC and MCV also showing the anemic conditions.

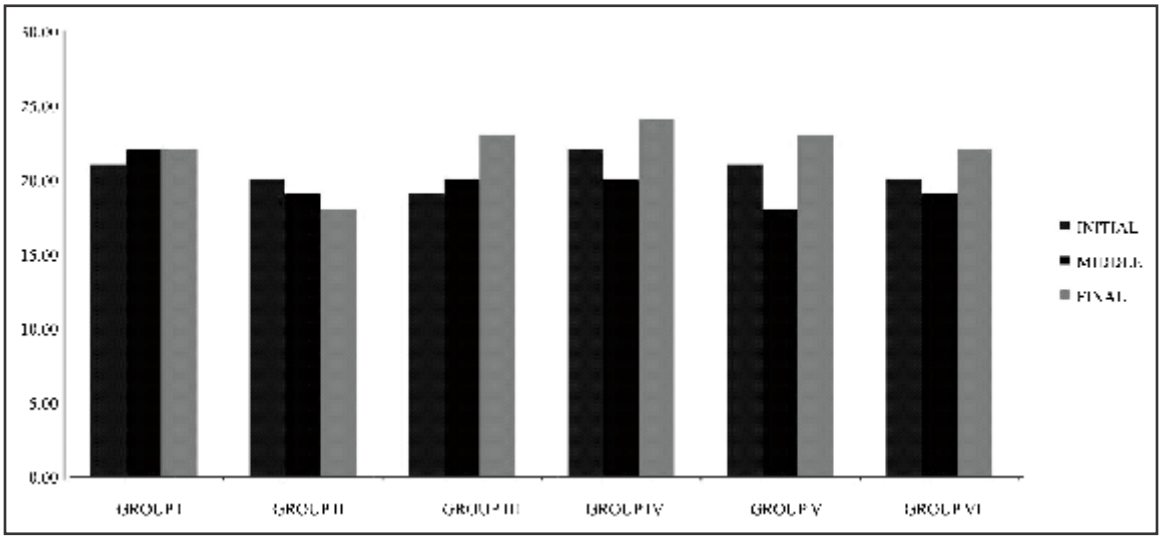

Figure – 3 Effect of phenylhydrazine on water consumption of different groups as compared to control.

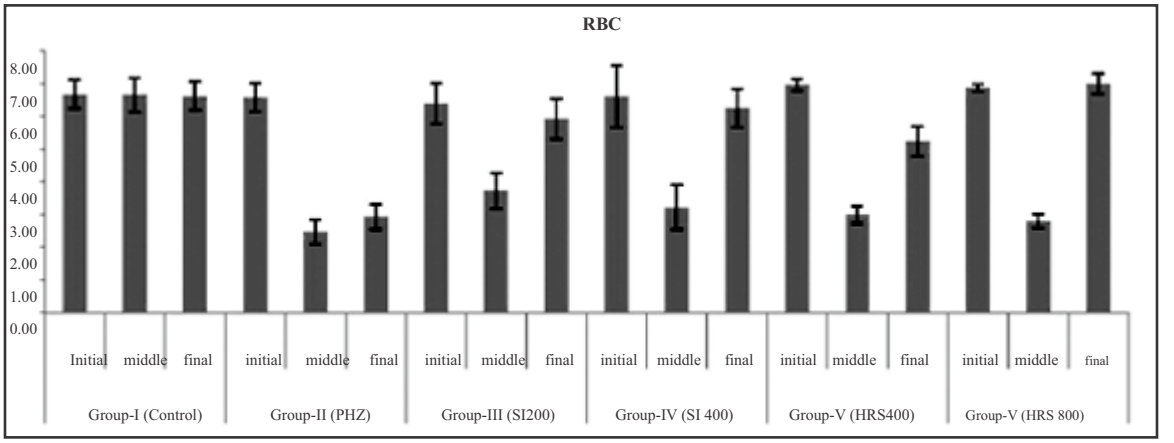

Figure – 4 Effect of phenylhydrazine on the values of RBCs in different groups as compared to control.

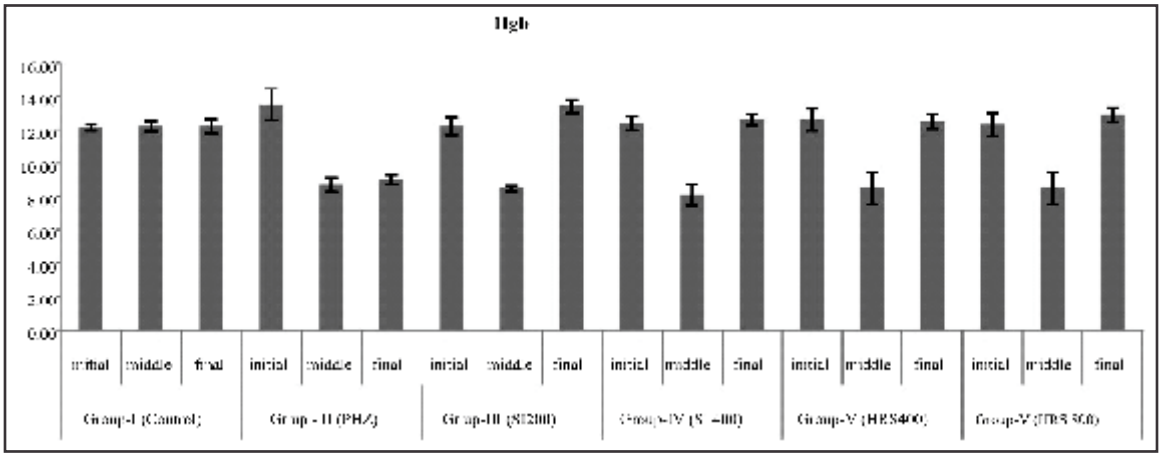

Figure – 5 Effect of phenylhydrazine on values of haemoglobin in different groups as compared to control.

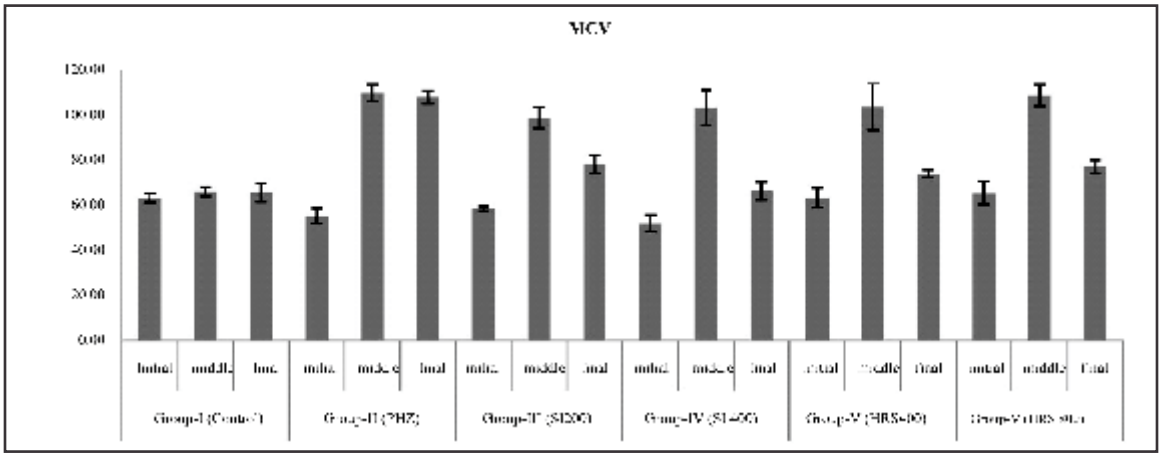

Figure – 6 Effect of phenylhydrazine on the values of MCV in different groups as compared to control.

Discussion

Phenylhydrazine caused damage in the cell membrane of red blood cells, potentially resulting in anaemia and consequential secondary involvement of other tissues, such as the spleen and liver. Phenylhydrazine induced the destruction of red blood cells by oxidation stress and many joint changes at cellular levels resulting in haemolytic anaemia. Phenylhydrazine induced toxic anaemia offers a model for research of haemolytic anaemia and the influence of anaemia on other physiological processes. Phenylhydrazine causes toxicity by the involvement of aryl and hydroxyl radical.

Pharmacological investigations of the genus *Hibiscus* have demonstrated interesting biological activities. Studies have shown that *Hibiscus rosa sinensis* possesses antispermatogenic (Reddy *et al*., 1997), antitumor (Serrame *et al.,* 1995), antidiabetic activities (Sachdewa *et al.,* 2003). In this study, a methanol extract of *Hibiscus rosa sinensis* flowers was studied to determine its haemoprotective activity. The possible haemoprotective effect of *Hibiscus rosa sinensis* may be explained by the presence of tannins, flavonoid , alkaloids, saponins and steroids, which were identified in the samples. It is already published that all the above secondary metabolites have good antioxidant activity (Wong *et al.,* 2009).

The present study provides a very good evidence for the haemoprotective activities of *Hibiscus rosa sinensis*. Like *Hibiscus*, *Saraca indica* also showed the good hemoprotective activity due to presence of secondary metabolites that showed the radical scavenging activity.

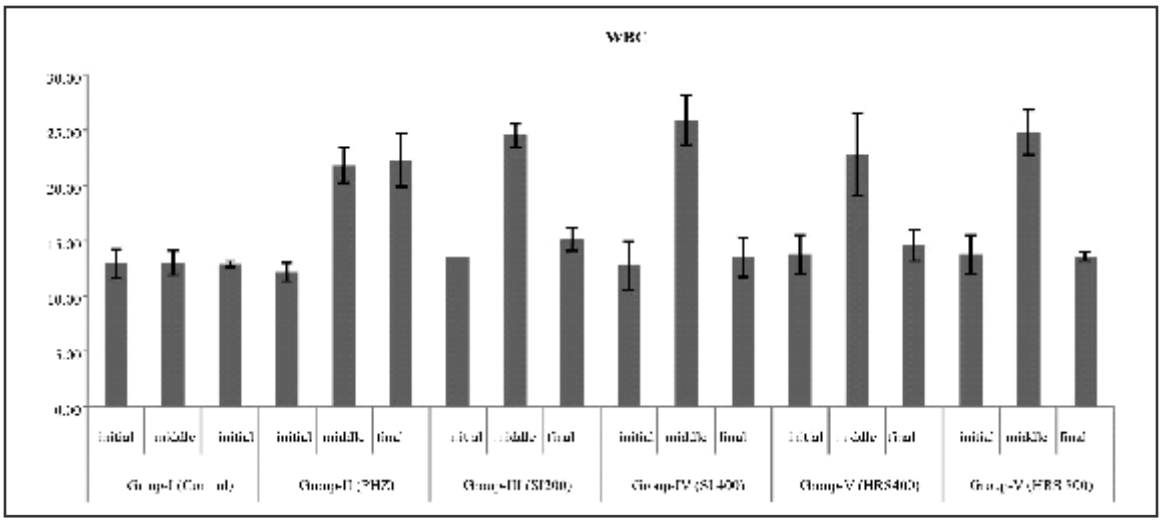

Figure - 7 Effect f phenylhydrazine on the values of WBC in different groups as compare to control.

Conclusion

Our study indicated that *Hibiscus rosa sinensis* and *Saraca indica* was remarkable haemoprotective activity which may be the result of its high phenol and flavonoid contents. This study also demonstrated that both these plants can be used as herbal haemoprotectives.

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Determination of Anti Oxidant Activity of *Ficus auriculata Leaves*

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Abstract-The present study reports formation of rapid, environment friendly and stable silver nanoparticles using aqueous leaf extract of *Ficus auriculata* without using any synthetic reagent. Silver ions were reduced by leaves extract within 5 minutes leading to formation of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-Vis-Spectroscopy, XRD, High resolution scanning electron microscopy(SEM) and High resolution transmission electron microscopy (TEM). The synthesized silver nanoparticles showed very good antioxidant activity by DPPH method.

Keywords: *Ficus auriculata,* Silver nanoparticles, Ascorbic acid,

Introduction

Green synthesis of nanoparticles is an eco-friendly approach to explore the potential of different herbs in order to synthesize nanoparticles. The plant extracts contain wide array of secondary metabolites such as flavonoids, proteins, terpenoids, tannins, polyphenols, etc. These biomolecules serve as potent reducing and capping agents for metal ion which helps to minimize the agglomeration of nanoparticles, thereby, controlling the morphology and also helping to stabilize the nanoparticles, thus improving the biological potential.

Silver is an effective antimicrobial agent which exhibits low toxicity. Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances, and medical devices[1]. Silver nanoparticles due to various properties such as catalysis, electrochemical conductivity and antimicrobial activity, can be used in different applications like biomedicine, agriculture, photo chemicals and food chemistry[2,3].

Silver nanoparticles (AgNPs) are proved to have high potential antimicrobial, antiplasmodial, and antifungal properties.So recently, they have been used for controlling harmful microorganisms such as bacteria, molds, yeasts and viruses[4,5]. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn and open wounds[6].

Antioxidants play an important role in scavenging radicals and thus providing protection against infections and degenerative diseases. Besides, natural antioxidants, some synthetic antioxidants are also reported such as, butylated hydroxytoulene and butylated hydroxyanisole. But these synthetic antioxidants possess greater risks of sideeffects.Therefore, investigations on identifying the natural antioxidants have become very important issue[7].

In the past few years, natural antioxidants have generated considerable interest in preventive medicine. Plants produce a huge amount of antioxidants and they can represent a potential source of new compounds having antioxidant properties with fewer side effects[8,9].

Plants contain bioactive constituents like alkaloids, flavonoids, tannins, phenolic compounds[10] etc.Antioxidants like vitamin C,

vitamin E, polyphenols, selenium, lycopene etc. which are present in many medicinal plants help in neutralizing free radicals. Secondary metabolites like terpenoids and flavonoids also help to defense against free radicals[11].

*Ficus auriculata***,** belongs to family *Moraceae*, is a huge tropical, deciduous and evergreen tree. It is usually found in India (Outer Himalaya ascending up to 2,000 m, Arunachal Pradesh, Assam, Bihar, Jammu and Kashmir, Jharkhand, Maharashtra, Manipur, Meghalaya, Mizoram, Orissa, Sikkim, South India, W. Bengal), Bangladesh, Malasiya, Myanmar, Pakistan to S. China, Thailand, cultivated in Taiwan[12].

Material and Methods

In the present study, silver nanoparticles prepared from leaves extract of *Ficus auriculata* are investigated for free radical scavenging activity by DPPH method.

Synthesis of Silver Nanoparticles From Leaves of *Ficus auriculata*

The leaves extract of *Ficus auriculata* was prepared and AgNPs were synthesized at room temperature by adding AgNO3solution. The colour change was observed from pale yellow to reddish brown colour. Literature study also shows that AgNPs have dark brown or dark reddish colour .This change in colour indicated the formation of silver nanoparticles[13].

Antioxidant Activity Determination by DPPH Method

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol. Decrease in the absorbance in presence of nanoparticles at different concentrations (10-100 µg/ml) was noted after 15 min. IC50was calculated from %age inhibition.

Percentage inhibition of DPPH radical by test compound was determined by the following formula.

%age Reduction = Control absorbance – Test absorbance/ Control absorbance x 100

Calculation of IC50 value was done using graphical method.

Results and Discussion

Antioxidant activity of AgNPs was assessed by DPPH scavenging assay with ascorbic acid as a positive control. The antioxidant activity was tested using DPPH and was found to increase with the concentration of nanoparticles. At the concentration of 10µg/ml silver nanoparticles from leaves of *Ficusauriculata*showed 36.72 % reduction (Table – 2). On parallel examination, it was

found to be 40.61 % (Table – 1) for standard compound ascorbic acid. Though on increasing concentration to 100µg/ml, the free radical scavenging potential increased. AgNPs from leaves of *Ficusauriculata*and standard ascorbic acid at 90µg/ml showed percent inhibition of 78.41 % and 75.31% respectively. This clearly indicates that antioxidant activity is concentration dependent. The efficiency of the antioxidant activity can be evaluated by IC_{50} value. IC_{50} can be described as the amount of sample needed for scavenging 50% of the free radicals. Lower is the IC_{50} value, better is the antioxidant property. These IC_{50} values can be calculated from the absorbance value.

From the observed figures, the calculated IC_{50} values were 39 µg/ml (Table – 2) and 25.5µg/ml (Table – 1) for AgNPs and standard ascorbic acid respectively. IC_{50} value of AgNPs is more than the standard taken but still AgNPs has very good antioxidant potential.

Conclusion

Antioxidants are of growing interest to food scientists, health professionals, and the general public owing to their protective roles in food products against oxidative deterioration and in the body against oxidative stress–mediated pathological processes.

Table – 1 DPPH Free Radical Scavenging Activity of Ascorbic Acid

S .No.	Conc. g/ml)	Absorbance of Ascorbic acid	%age Reduction	IC_{50} Value (µg/ml)
1.	10	0.291	40.61	25.5
2.	20	0.268	45.92	
3.	30	0.247	50.61	
4.	40	0.226	54.42	
5.	50	0.205	59.11	
6.	60	0.187	63.33	
7.	70	0.168	67.23	
8.	80	0.142	71.43	
9.	90	0.121	75.31	
10.	100	0.087	82.14	

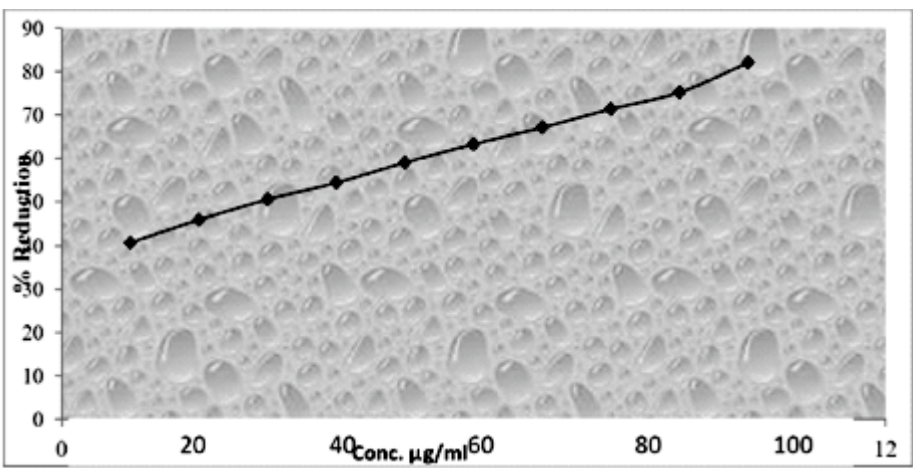

Figure – 1 Graph of DPPH Free Radical Scavenging Activity of Ascorbic Acid

Table – 2 Antioxidant Activity of AgNPs of *Ficus auriculata*

S. No.	Conc. (µg/ml)	Absorbance	%age Reduction	IC_{50} Value
1.	10	0.285	36.72	
2.	20	0.275	42.83	
3.	30	0.251	42.35	
4.	40	0.233	51.46	
5.	50	0.212	54.17	39
6.	60	0.181	61.88	
7.	70	0.146	67.14	
8.	80	0.133	72.24	
9.	90	0.115	78.41	

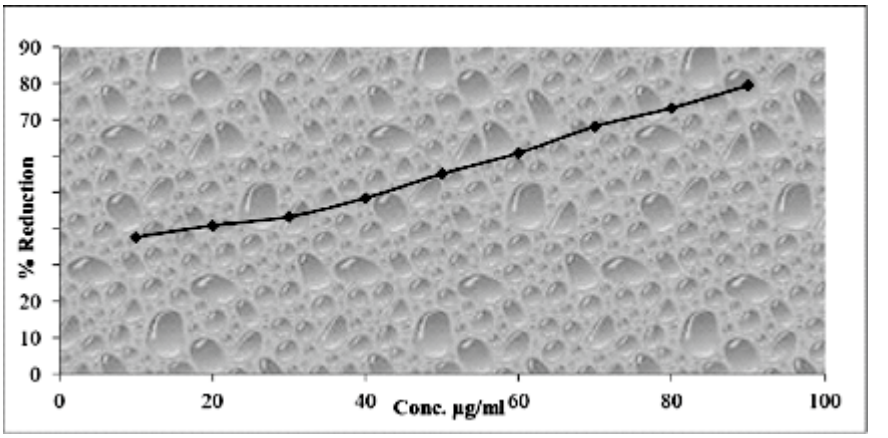

Figure – 2 Graph of DPPH Free Radical Scavenging Activity of AgNPs of *Ficus auriculate.*

In conclusion, the results from this study revealed that the aqueous extract of leaves of the *Ficus auriculate* have antioxidant activity. The medicinal properties of this plant could be due to its antioxidant potentials as evident from this present work. More studies are however needed for investigating its usefulness in the management and treatment of various diseases.

Acknowledgements

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Evaluation of Antioxidant and Antibacterial Activities of *Taxus wallichiana* Against Food Borne Pathogens

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Abstract-The present study was extended out to evaluate the phytochemical, antioxidant and antibacterial activities of ***Taxus wallichiana*** against food borne pathogens. The plant sample was taken from the Kullu region of Himachal Pradesh and the aqueous extract of the bark of the plant was organised for the phytochemical screening, which showed the confirmation of phenols, flavonoids, saponins, alkaloids, tannins, reducing sugars, glycosides and terpenoids and the absence of steroids and anthraquinones. The quantitative analyses of phytochemicals were carried out and the total phenolic count and total flavonoids were determined as 47.83±3.08 µg/ml and 113.25±2.62 µg/ml respectively. The antioxidant activity of the extract was assessed by DPPH; the antioxidant activity increased in a concentration dependent manner and was found to be 54.16%. The antibacterial activities of the plant extract were carried out against the most common food borne pathogenic bacteria and their zone of inhibition was observed. The highest zone of inhibition was observed against *E. coli* (16mm) and *salmonella spp.* (12mm), *Staphylococcus spp*. (10mm) respectively. The result suggested that traditional folk medicine could be used as a guide in our continuing search for new natural products with potential medicinal properties.

Keywords: Phytochemicals, Antioxidants, Antimicrobial

Introduction

Medicinal plants contain therapeutic potential for treatment of several ailments of humans. India has rich geographical area with high potential abilities for traditional medicines but only a few are screened pharmacologically for their medicine value (Lokhande*et al.,* 2007). These plants have a capability to fight with number of bacterial, fungal and viral infections throughout the globe. Medicinal plants are considerably useful and economically essential. They do not have any side effects and are safer than antibiotics. The forests of Himachal Pradesh said to have been the birth place of Ayurveda, were known to supply a large part of medicinal plants. Due to regular use of antibiotics, microorganisms develop a resistance to these antibiotics which increases day by day. So, there is need of look inward for synthesizing new agents by exploration of natural sources.

Taxus wallichiana, the Himalayan yew, is a species of yew, native to Himalayas and parts of South-East Asia. The species is grown in montane, temperate, and tropical montane to high montane forests. Its elevation ranges from 900m to 3700m (Khan *et al.,* 2006).This plant is used for the treatment of fever and painful inflammatory conditions. The leaves of the plant are used to make herbal tea for indigestion and epilepsy. Some literature published on this species reported the immunomodulatory, antibacterial, antipyretic, antifungal properties. (Nisar*et al.,* 2008; Chattopadhyay *et al.,* 2006; Nisar*et al.,* 2008; Quayum *et al.,* 2012). *Taxus wallichiana*is a source of the chemical precursors to the anticancer drug Paclitaxel. Its leaves and bark were found to be the prime source of taxol, a potential anticancer drug and is used in the prevention of breast and ovarian cancers (Chattopadhyay *et al.,* 2003). Extracts

of this plant show the antifungal and antibacterial properties. The methanolic extracts of *Taxus wallichiana* showed anticonvulsant and antipyretic properties (Nisar *et al.,* 2008). This plant is also used traditionally for treatment of pyrexia, acute painful conditions, indigestion, epilepsy and for the treatment of wounds and skin infections (Kaul, 1997; Baquar, 1989; Ahmed, 1997). Keeping this knowledge in view, the present study was undertaken to investigate the antioxidant and antibacterial potential of *Taxus wallichiana*.

Material and Methods

Sample collection

The bark sample of the plant was collected from the forest region of Kullu region of Himachal Pradesh, India.

Preparation of Extract

The sample was dried under shade and powdered in a mechanical grinder and stored in polythene bags. The powdered material was extracted with distilled water for 72 hours. Sonication of plant powder was done with distilled water and after filtration; filtrate was evaporated on water bath (95Ú˜C) to make a thick extract.

Qualitative Estimation of Phytochemicals

Phytochemical screening

The Phytochemical screening of crude extracts from plant species was carried out to determine the presence of active secondary metabolites. The plant extracts were screened for the presence of reducing sugars, alkaloids, saponins, tannins, flavonoids, terpenoids, anthrequinones, phlobatanin, steroids, terpenoids and cardiac glycosides according to the established procedures of Trease and Evans, 1989, Sofowora, 1993 and Harborne, 1973.

Alkaloid

30 ml of sample extract was evaporated to dryness in an evaporating dish on water bath. 5ml of 2N HCL was added and stirred while heating on a water bath for 10 minutes, cooled filtered and the filtrate was treated with a few drops of Mayer's reagent (1.36gm mercuric chloride + 5gm potassium iodide in 100ml). The sample was then observed for the presence of turbidity or precipitation.

Saponins

2gm of powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10 ml of filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed the formation of emulsions.

Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow colour in extract indicates the presence of flavonoids (Harborne, 1973).

Tannins

0.5g of each powdered sample was boiled in a 20ml of water in a test tube and then filter. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black color (Trease and Evans, 1989).

Cardiac glycoside (Keller-Kilani test)

5ml of the extract was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. Then it was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer .

Terpenoids (Salkowski test)

5ml of the extract was mixed in 2ml of chloroform and concentrated H_2SO_4 (3ml) was added to form a layer. A reddish brown

coloration of the interface showed positive results for the presence of terpenoids.

Reducing sugar

To 0.5ml of extract solution add 1ml of water and 5-8 drops of Fehling solution was been added at hot and observed for brick red precipitate.

Anthraquinones

1gm of the powdered plant material, 10ml of N/2 potassium hydroxide containing 1 ml of 3%hydrogen peroxide solution was added. The suspension was boiled for 3-5 minutes and then cooled, filtered and 5ml of the filtrate was acidified with 10drops of glacial acetic acid. This acidified mixture was extracted by shaking with 10ml of benzene. A 5ml aliquot of the benzene solution was shaken with 3ml of 10% of ammonium hydroxide solution and the 2 layers were allowed to separate. A pink to red coloration of the alkaline layer indicates the presence of anthraquinone.

Steroids

2ml of the acetic anhydride was added to 0.5gm aqueous extracts of sample with 2ml of H_2SO_4.The color change from violet to blue or green indicates the presence of steroids.

Quantitative Screening of Phytochemicals

After qualitative phytochemical estimation, quantitative phytochemical estimation was done for the total phenolic count and flavonoids content (Rashid *et al.,* 2010).

Determination of Total Phenols (Rashid *et al.*,2010)

Total phenolic count of each plant extract was determined by the Folin-Ciocalteu method. 2.5ml fresh Folin's reagent with 0.5ml of different concentrations of each extract was taken and immediately added to 2ml of Na_2CO_3 (7.5%) to each one and left for 90 minutes at 30^0 C. The absorbance was measured at 765nm.The results were expressed as Gallic acid equivalents (mg Gallic acid/g dry extract).

Total Flavonoids Determination (Rashid *et al.*,2010)

The total flavonoids content was measured by a colorimetric assay. The plant extract (0.5ml) was mixed with 2ml of distilled water and subsequently with 0.15ml of a $NaNO_2$ solution (15%). After 6 minutes, 0.15ml of an $AlCl_3$ solution (10%) was added and allowed to stand for 6 minutes, then 2ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5ml and the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Rutin was used as a standard for the calibration curve.The total flavonoid content of the extracts and fractions were expressed as mg rutin equivalents (RU) per gram of sample (mg/g).

Determination of Antioxidant Activity

After the complete preliminary phytochemical estimation, plant extracts were taken for the antioxidant studies.

DPPH assay (Suthar singh *et al.*, 2011))

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the plant extracts required to scavenge DPPH showed a dose dependent response. DPPH solution (0.004% w/v) was prepared in 95% ethanol.A stock solution of aqueous extract and standard ascorbic acid was prepared in the concentration of 10mg/100ml. From stock solution, 2ml, 4ml, 6ml, 8ml, 10ml, of this solution were taken in five test tubes respectively. Then 2ml of freshly prepared DPPH solution was added in each of the test tubes. The reaction mixture was incubated in dark for 15min and thereafter the optical density was recorded at 523nm against the blank. For the control, 2ml of DPPH solution in ethanol was mixed with 100ml of ethanol and the optical density of the solution was recorded

after 30 min. The assay was carried out in triplicates. The decrease in optical density of DPPH on the addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (% IP) of DPPH radical

The capability to scavenge the DPPH free radical was calculated using the following formula:

$(Abs_{control} - Abs_{sample})$

$$\% \text{ of radical scavenging activity} = \ldots\ldots\ldots \times 100$$
$$Abs_{control}$$

Whereas, $Abs_{control}$=Absorbance of DPPH solution

Abs_{sample} = Absorbance of extracts

Determination of In-vitro Antimicrobial Activity

The standard bacterial cultures of most common food borne pathogens *E.coli., Salmonella spp. and Staphylococcus spp.* were used in the present study. Antimicrobial assay was performed by agar well diffusion method. 10 and 20 µl of the plant extracts at a concentration of 100µg/ml were added to the each well by using the sterile micropipette and allowed to diffuse at room temperature for 2 hrs. The respective extracts were maintained and the experiment was repeated thrice, and the average values of zone of inhibition were recorded in mm for antimicrobial activity. The 50 µl of antibiotic compound ciprofloxacin (100µg/ml) was used as a standard for the antimicrobial studies.

Results and Discussion

The present study was carried out to evaluate the phytochemical and antimicrobial estimation of *Taxus wallichiana* against three most common food borne pathogens. The phytochemical secondary active metabolites of *Taxus wallichiana* were analyzed qualitatively for the bark of the plant and presented in the Table – 1.

In this screening process, the presence of alkaloids, tannins, reducing sugars, terpenoids, flavonoids, saponins and cardiac glycosides were observed, whereas anthraquinones and steroids were found to be absent in the bark extract of *Taxus wallichiana*. Muhammad *et al.,* 2016 carried out the qualitative phytochemical estimation of this species and found the presence of flavonoids, anthraquinones, alkaloids, carbohydrates and steroids and also revealed the absence of saponins. The phytochemical estimation of *Taxus baccata* leaf extract revealed the presence of glycosides, steroids, tri-terpenoids and flavonoids. Rather the absence of alkaloids, tannins and saponins was noticed in the plant sample collected from Nepal.

The total phenolic content of aqueous extract of *Taxus wallichiana*was determined by using Folin-Ciocalteu method. The phenolic content was calculated from standard curve of Gallic acid, .the total phenolic content was found to be **47.83±3.08µg/ml** Gallic acid equivalents for *Taxus wallichiana*. According to the study carried out by (Milutinovic *et al*., 2015), the total phenolic content of the aqueous leaf extract of *Taxus baccata* was found to be 33.08 µg/ml and of the seeds extracts, it was found to be 8.23 µg/ml, also the phenolic content determined from the methanolic extract of *Taxus baccata* was found to be 85.47±4.21 µg/ml and of the shoot extract, it was 27.24±1.39 µg/ml

Table – 1 Qualitative Phytochemical Screening

Sr. No.	Phytochemical Test	Result
1	Alkaloids	Positive
2	Tannins	Positive
3	Reducing sugars	Positive
4	Terpenoids	Positive
5	Flavonoids	Positive
6	Saponins	Positive
7	Cardiac glycosides	Positive
8	Anthraquinones	Negative
9	Steroids	Negative
10	Phenols	Negative

(Milutinovic *et al.*, 2015).

The total flavonoids content of aqueous extracts of *Taxus wallichiana* was determined by using aluminum chloride method. The flavonoids content was found to be **113.25±2.62µg/ml** rutin equivalents for *Taxus wallichiana*. In the studies carried out by Milutinovic in 2015, the total flavonoids content in the aqueous seed extracts of *Taxus baccata* was found to be 39.37±3.96 of this species. DPPH radical scavenging capacity in this study reported after 30 minutes of incubation was analyzed. *Taxus wallichiana* extract showed observable antioxidant activity in the concentration analyzed. Aqueous extract showed maximum activity of **54.16%** at 20µg/ml whereas ascorbic acid at the same concentration exhibited 70.56% inhibition. The studies carried out by Milutinovic *et al.,* 2015, the antioxidant activity of the ethanolic leaf extracts of *Taxus baccata* was revealed as 38% and 91% in the shoot extracts.

The in-vitro antimicrobial activity of plant extracts was observed against three most common food borne pathogens i.e. *E.coli, Salmonella spp. and Staphylococcus spp.* Observations of typical antimicrobial characteristics were considered against bioactivity of antibiotic, ciprofloxacin. Microbial inhibition spectrum for the extract of *Taxus wallichiana* was considered for fewer than two concentrations, 10µl and 20µl respectively. The bioactivity and the microbial inhibition spectrum for *Taxus wallichiana* was found highest in the concentration of 20µl in the culture of *E.coli* which was found to be 16mm, whereas for the culture of *Staphylococcus spp.* and *Salmonella spp*. was determined as 10mm and 12mm respectively.

Conclusion

Table – 2 Microbial Inhibition Spectrum

Microorganism	Microbial inhibition spectrum	
	10µl	**20µl**
E.coli	12mm	16mm
Salmonella spp.	8mm	12mm
Staphylococcus spp.	7mm	10mm

The plant screened for phytochemical analysis seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. We know medicinal plants are source of great economic importance in the subcontinent. The crude extracts of plant extracts may be used as medicaments. All the phytochemicals may have properties like anti-inflammatory, anti-diabetic and anti-analgesic activity. The plant based compounds have the effective dosage activity and minimum side effects when compared to the synthetic compounds. The results suggest that traditional folk medicine can be used as guide in continuing search for new natural products with potential medicinal properties.

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Conflict of interest

There is no conflict of interest for this work.

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Acute and Sub-chronic Toxicity Study of Itrifal Muqawwie Dimagh in Experimental Animals

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Abstract-Itrifal Muqawwie Dimagh (IMD) is a polyherbal Unani Drug / formulation. The action of this formulation is described in the NFUM as Muqawwie Dimagh (Brain tonic), Muqawwie Basar (Eye tonic) and the therapeutic use as Zofa-e-Dimagh (Weakness of Brain). Since, there is no available scientific evidence in support of safety of this formulation, the present study was designed to evaluate acute and sub chronic toxicity in *Albino* rats. The Drug (I.M.D.) was administered orally in the form of aqueous suspension at single dose 2000mg / kg for acute toxicity and observed for 14 days. The 90 days repeated oral dose sub cronic toxicity was performed at two dose levels 1000mg / kg and 2000mg / kg. b.w. given daily and observed for 90 days. Animals were observed for clinical sign of toxicity. Haematological parameters and biochemical profile did not reveal any significant difference when compared with control group. The significant decrease in serum Triglyceride in mail *Albino* rats was noted at the dose of 2000mg/ kg b.w. treatment with I.M.D. caused no significant difference in body and organ weight gain. In conclusion, the drug I.M.D. found safe at tested doses.

Key words: Itrifal Muqawwie Dimagh, Unani Medicine, Toxicity study.

Introduction

In Unani the literature, the disease in which forgetfulness is the main symptom is described under the heading of Nisyan or Humuq. Nisyan (Amnesia) / Faramoshi or Humuq (Dementia) is a condition in which memory is lost or disturbed. *Nisyan* (amnesia) occurs due to a deficit in the *quwwat - e - hafiza* (memory power) or *quwwat – e - fikr* (power of analysis), *quwwat - e - khayal* (imaginative power, cognitive power) (Adnan, 2016; Abdul, 2008; Hakeem, 2010; Sana, 2017).

Nisyan (Amnesia) has become a great medical and social problem as a result of elongation of human life. Although mild memory loss is very common in the ageing peole. It becomes abnormal when it causes impairment in activities of daily living (ADL) of a person. Traditional medicinal system in India particularly Unani and Ayurveda described neuroprotective actions of plant drugs like Brahmi (*Bacopa monnieri*), Shankhapushpi (*Convolvulus pluricaulis*), Amla (*Emblica officinalis*), Guduchi (*Tinospora cordifolia), Tulsi (Ocimum sanctum), Ashwagandha (Withania somnifera), Waj-e-Turki* (*Acorus calamus* Linn.) Sumbul-ut-Tib (*Nardostachys jatamansi* Dc.), Kalonji (*Nigella sativa* Linn.), Ispand (*Peganum harmala* Linn.), Dar-e-Filfil (*Piper longum* Linn.), Filfil Siyah (*Piper nigrum* Linn.), Bhilawan (*Semecarpus anacardium* Linn.), Haritaki / Post Halela (*Terminalia chebula* Retz.), and Zanjabeel (*Zingiber officianale* Rosc.). They help in improving the treatment for Alzheimer's disease. There are many formulations in Unani system of medicine like Majoon Barhami, Majoon Bolas, Majoon Baladur, Majoon Waj, Majoon Falsafa, Majoon Kundur, Itrifal Sagheer, Jawarish Jalinoos etc. for *Nisyan* (Adnan, 2016; Sana, 2017). *Itrifal Muqawwi Dimagh (I.M.D.)* was our choice of drug for the present study due to the reason that it is in use for many hundreds of years.

IMD is polyherbal Unani formulation. The action described in the NFUM is Muqawwi-e-Dimagh (Brain tonic), Muqawwi-e-Basar (Eye tonic) and the therapeutic use as Zofa-e-Dimagh (Weakness of Brain), Nazla (Catarrh) and Suda (Headache or cephalgia). (Anonymous, 1981, 2008a 2008b; Hakeem 2010). The drug is a semi-solid preparation made with the ingredients in the formulation given below.

Formulation composition

Ingredients	Botanical Name	Parts Used
Aamla khushk	*Emblica officinalis* Gaertn.,	Fruit
Post Bahera	*Terminalia belerica* Roxb.,	Fruit
Post Halela Zard	*Terminalia chebula* Retz.,	Fruit
Tukhm Khashkhash Safaid	*Papaver somniferum* Linn.,	Seed
Gul-e-Khatmi	*Althaea officinalis* Linn.,	Flower
Gul-e-Surkh	*Rosa damascena* Mill. ,	Flower
Kishneez Khushk	*Coriandrum sativum* Linn.,	Flower
Maghz Badam Shirin	*Prunus amygdalus*	Seed
Batsch var. Dulcis		
Ghee		
Shakar Safaid	Sugar IP	Crystals
Warq Nuqra	Silver,	Foils

The present study was carried out for evaluation of acute and sub chronic toxicity and safety of Itrifal Muqawwie Dimgah (IMD).

Material and Methods

This study was carried out in Pharmacology Research Unit, Regional Research Institute of Unani Medicine, Aligarh and conducted in accordance with the Ethical guidelines.

Experimental Animals

Albino rats of either sex weighing between 100-150 g were randomly selected and divided into three groups of 10 animals (5Male + 5Female) per group. The animals were housed 5 each, of the same sex in polypropylene cages provided with bedding of husk. CPCSEA guidelines of laboratory animal care were followed throughout the experiment. Conventional rodent laboratory diet was provided with potable drinking water *ad libitum*. The animals were acclimatized for seven days prior to dosing. The animals were maintained in standard environmental conditions at room temperature of 25 $\pm$ 2^{o}C with 12 hrs light/ dark cycles and humidity (50-55%) (OECD, 2000; 2008).

Drug Formulation and dose selection

An aqueous suspension of Itrifal Muqawwie Dimagh in 0.3% CMC (<2mL/100 gm b.w) was used for oral administration. The control animals (Group I) were administered with vehicle only. The human dose of Itrifal Muqawwie Dimagh is 10gm as prescribed in the Unani Pharmacopoeia. For acute toxicity test drug was administered with single oral dose of aqueous suspension at dose level of 2000 mg/kg b.w and for sub chronic toxicity test drug was administered at dose level of 1000 mg/kg b.w (human equivalent dose is 1028mg/kg b.w) and 2000 mg/kg b.w (limit dose) once daily for 90 consecutive days at same time each day to minimize variations (Anonymous, 2008a; 2008b).

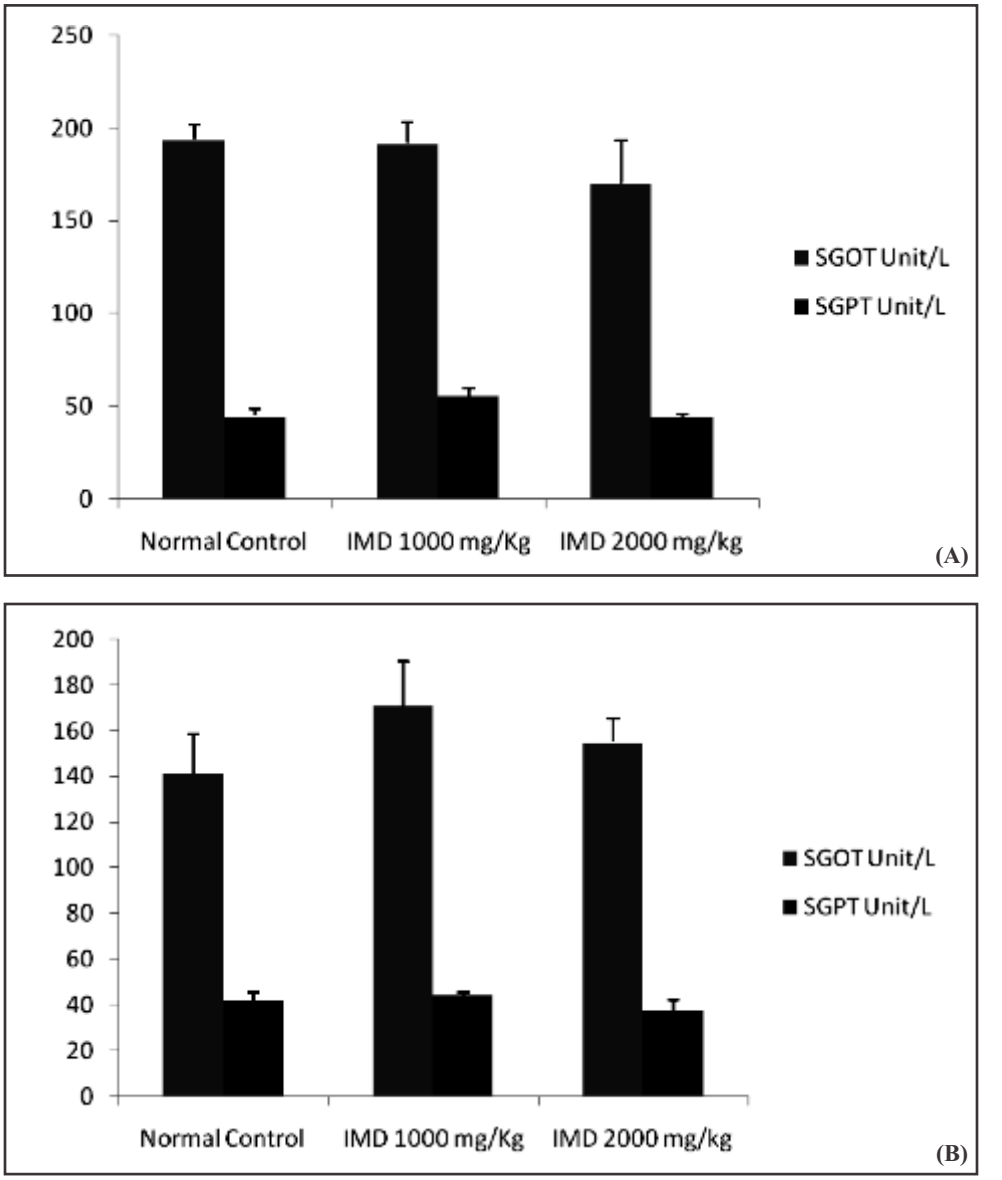


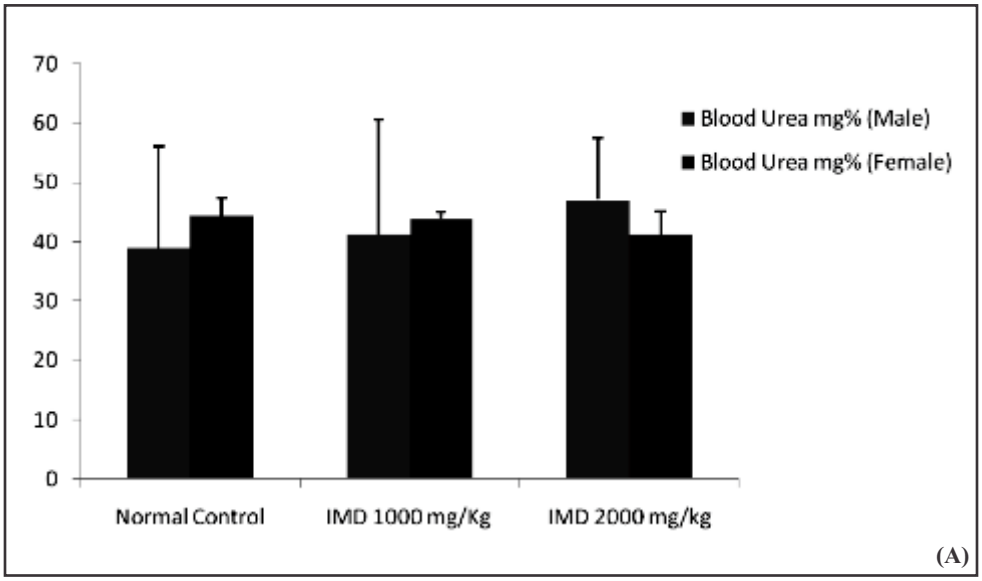
Figure – 1 Effect of aqueous Suspension of IMD on Serum transaminase Levels in Male (A) and Female (B) Albino rats

Vehicle

0.3 % CMC aqueous suspension was used as vehicle for oral administration of formulations.

Drug administration

IMD was administered in the form of 0.3 % CMC aqueous suspension prepared using mortar & pestle. Drug or vehicle was orally administered via stainless steel gavage, by calculating the dose based on the body weight of rats, for a period of 90 days - chronic toxicity study. Duration of toxicity study for IMD was decided for limit dose of 2000mg/ kg BW for



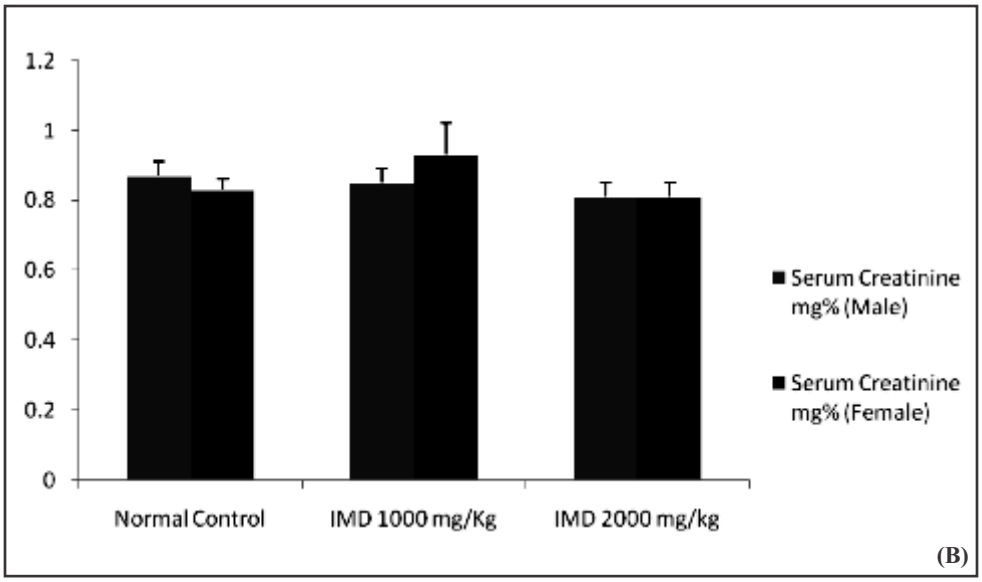


Figure – II Effect of Aqueous Suspension of IMD on Blood Urea and Serum Creatinine in male (A) and Female (B) Albino rats

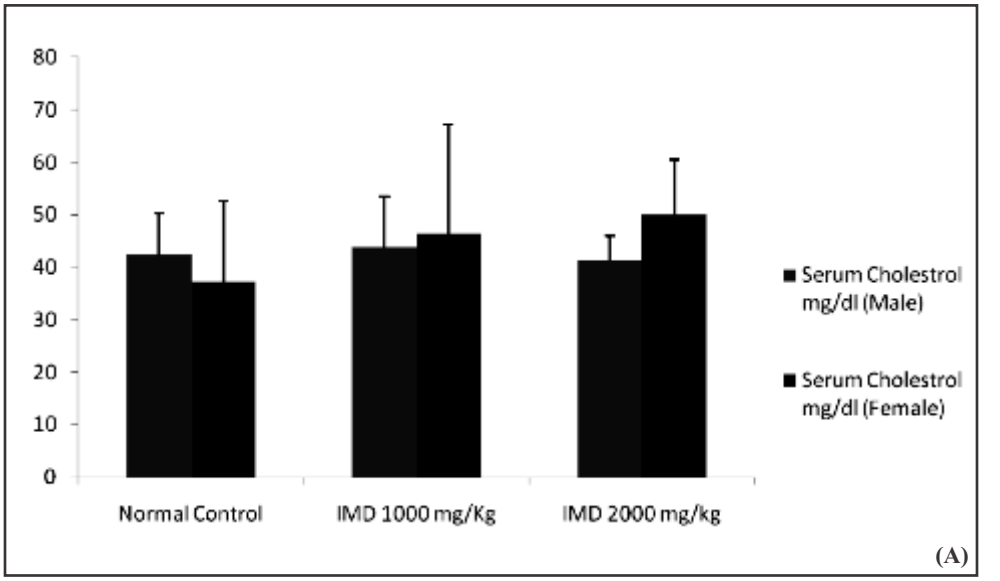
acute toxicity and 03 months (i.e., 90-days) based on its duration of clinical use in Unani system.

Experimental Design

The **14 days acute toxicity** was carried out in female Albino rats weighing 100-150 gm according to the OECD test guideline-425. The animals were divided into two groups of five animals each.

Control Group, Vehicle (0.3% aqueous CMC)

Test group 2000 mg/kg body weight. (Single aqueous suspension oral dose)



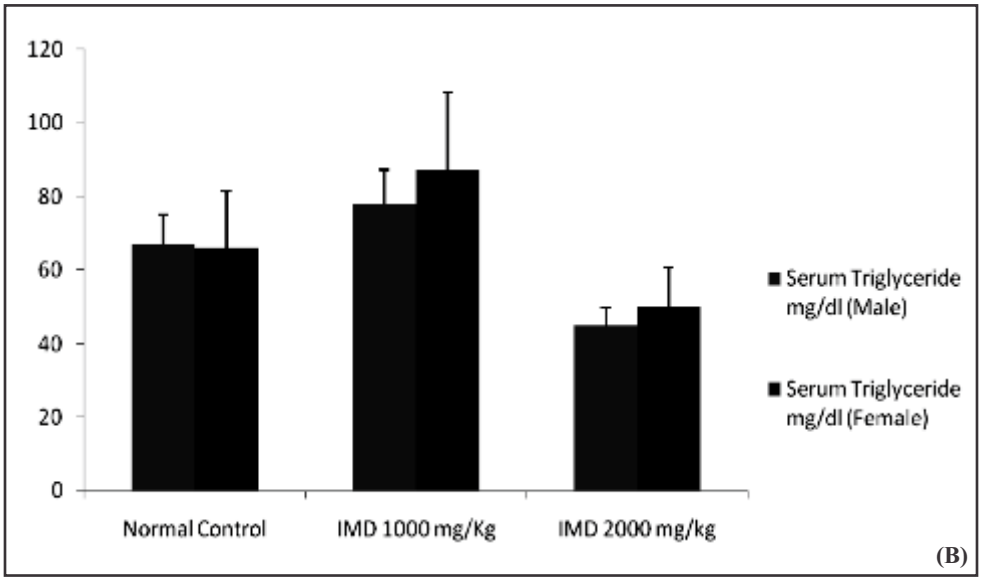


Figure – III Effect of Aqueous Suspension of IMD on Serum Cholesterol and Serum triglyceride in male (A) and Female (B) Albino rats

The animals were observed for changes in skin, fur, eyes, mucous membranes and behavior pattern. An attention was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and mortality at 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 12 hour, 24 hour and thereafter once every day up to 14 days after drug administration.

The **90-day repeated dose** oral toxicity study was performed according to the OECD test guideline-408. Male and Female rats were

Table – I Effect of aqueous suspension of IMD on Hematological parameters
I (A) Male Albino Rats

PARAMETERS ▶ GROUPS ▼	Haemoglobin gm %	TLC / Cumm	ESR mm/hr	DLC	
				% Polymorph count	% Lymphocyte count
Group I Normal Control (n = 5)	12.96 ± 0.31	14010.00 ±1925.35	1.20 ± 0.22	27.80 ± 4.09	71.80 ± 3.87
Group II Drug treated 1gm/Kg (n = 5)	14.56 ± 1.08	13680.00 ± 2178.41	1.40 ± 0.27	45.00 ± 7.89	52.20 ± 7.56
Group III Drug treated 2gm/Kg (n = 5)	13.35 ± 0.51	22175.00 ± 7954.22	1.25 ± 0.28	31.75 ± 2.02	66.00 ± 1.49

I (B) Female Albino Rats

PARAMETERS ▶ GROUPS ▼	Haemoglobin gm %	TLC / Cumm	ESR mm/hr	DLC	
				% Polymorph count	% Lymphocyte count
Group I Normal Control (n = 5)	13.52± 0.36	10240.00 ± 2452.00	1.20± 0.22	33.80± 2.36	63.60± 2.77
Group II Drug treated 1gm/Kg (n = 5)	13.00± 0.65	14525.00 ±3315.82	1.25± 0.28	38.75± 2.51	58.75± 4.19
Group III Drug treated 2gm/Kg (n = 5)	13.55± 1.44	14425.00 ± 2426.41	1.60± 0.28	31.75±6.29	66.75± 5.95

Values are Mean ± SEM, n = number of rats in a group, P* < 0.05, P < 0.01, P*** < 0.001**

divided into 3 groups with 10 animals (05 males + 05 females) in each group as follows:

Vehicle control (0.3 % aqueous CMC)

IMD 1000 mg/kg BW (1x; 1028 mg/kg BW therapeutically equivalent dose)

IMD 2000 mg/kg BW (Limit Dose)

All the experimental animals were observed for mortality and morbidity twice a day, throughout the study period. Detailed clinical observations (i.e., functional observation parameters) were made periodically to detect signs of toxicity, at the same time (1hr after vehicle or drug administration). Body weight of the animals was recorded once in a week. At the end of the treatment period, the overnight fasted (water provided *ad libitum*) rats were anaesthetized. Blood samples were collected by retro-orbital puncture in the EDTA vacutainers (for haematological) and serum vacutainers (for biochemical analysis).

Table – II Effect of Aqueous suspension of IMD on Organ Weight
II (A) Male Albino Rats

PARAMETERS ► GROUPS▼	Organ weight in gm			
	Liver	Heart	Kidney	Spleen
Group I Normal Control (n = 5)	6.07± 0.60	0.47± 0.05	1.26± 0.12	0.65± 0.04
Group II Drug treated 1 gm/Kg (n = 5)	5.70 ± 1.00	0.49± 0.07	1.29± 0.12	0.49± 0.11
Group III Drug treated 2gm l/Kg (n = 5)	5.37± 0.48	0.45± 0.05	1.20± 0.11	0.48± 0.09

II (B)Female Albino Rats

PARAMETERS ► GROUPS▼	Organ weight in gm			
	Liver	Heart	Kidney	Spleen
Group I Normal Control (n = 5)	6.41 ± 1.15	0.45± 0.02	1.28± 0.09	0.44± 0.07
Group II Drug treated 1 gm/Kg (n = 5)	5.20 ± 0.22	0.44± 0.03	1.36± 0.15	0.41± 0.02
Group III Drug treated 2 gm/Kg (n = 5)	6.37 ± 0.31	0.51± 0.02	1.29± 0.09	0.58± 0.15

Values are Mean ± SEM; n = number of rats in a group; P* < 0.05, P < 0.01, P*** < 0.001**

On 91st day blood was collected of all the three groups of rats for estimation of SGOT (Tiez., 1986), SGPT (Tietz *et. al.,*1983), blood urea (Tiffany *et. al.,* 1972), Serum Creatinine (Bowers *et. al.,* 1980), Serum cholesterol (Roeschlau *et. al.,*1974) and Serum triglycerides (Macgowan, *et. al.,* 1983), Percentage Hemoglobin, ESR, Total leukocyte count and Differential leukocyte count (% polymorphocyte count and % lymphocyte count) (Mukherjee, 1990, Latimer *et. al.,* 2003). After collection of blood the animals in all the three groups were sacrificed and liver, heart, kidney and spleen were excised out for determination of changes in organ weight as compared to control group (OECD 2000; 2008).

Statistical analyses

Statistical analysis was performed by using unpaired t test calculating p value at 5 % level. All values are expressed as Mean ± SEM (standard error of mean). *p* value less than 0.05 found to be considered statistically significant.

Results and Discussion

Acute Toxicity Study

The aqueous suspension of Itrifal Muqawwie Dimagh (IMD) single oral dose of 2000mg/kg has shown that the formulation was well tolerated and no abnormality was observed up to 14 days in the general behavior of the animals and no overnight mortality was recorded as compared to control group. The acute toxicity study of IMD suggests that it is safe.

Sub Chronic Toxicity Study

Sub-chronic (90 Days) toxicity of aqueous suspension of IMD at the doses of 1000 mg/kg body weight and 2000mg/kg body weight compared with vehicle control has shown that there were no significant statistical changes in haemotological [Table – 1 (A) and 1 (B)] and biochemical parameters observed, except significant decrease in serum triglyceride in male albino rats at the dose of 2000mg/kg body weight [Figure – 1 (A), I (B), Figure – II (A) and II (B) and Figure – III (A), III (B)]. No change was observed in gross behavior and there was no mortality noted. There were no changes observed in organ weight in both male and female rats as compared to control group, Table – II (A) and II (B). Hence these results suggest that drug is safe at the dose level of 1000 mg/kg and 2000 mg/kg body weight when administered orally.

Conclusion

The polyherbal Unani formulation (IMD) was subjected to acute (single dose toxicity) and sub-chronic toxicity (90 days repeated dose toxicity) study after oral administration. The present investigations demonstrated that no mortality occurred at test dose levels. There were no change in body weight as compared to control in both acute and sub-chronic toxicity study. There was no significant statistical change in biochemical parameters observed except significant decrease in serum triglyceride in male albino rats at the dose of 2000 mg/kg body weight. The results of hematology were normal as compared to control group. No significant differences were observed in organ weight of heart, liver, spleen, kidneys, in treatment group as compared to control group. The outcome of this study suggests that drug is safe at the tested dose levels.

Acknowledgement

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Neutraceutical Values and Phytochemical Profile of Amla Fruits with the Estimation of Ascorbic Acid (Vitamin C) and its Antioxidant Activity Evaluation

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Abstract-Recently, nutraceuticals and medicinal plants have become the focus of current medical research. The fruit or fruit pulp of *Emblica officinalis* is a reputed drug of Ayurvedic, Unani, Siddha systems of medicine and believed to increase defense against diseases. The fruit primarily contained tannins, alkaloids, phenolic compounds, amino acids, carbohydrates and other compounds especially the essential nutrients. The vitamin C content in Amla varies from place to place and from batch to batch. The comprised investigation explored physico-chemical, phyto-chemical and nutraceutical profile of this versatile and reputed ethnomedicinal fruit through basic scientific research to establish the rational scientific foundation of its utility as rejuvenating food and tonic.

Keywords :*Emblica officinalis,*Neutraceutical

Introduction

Amla is one of the most important herbs in the Indian traditional medicine system, and is becoming increasingly well known for its unusually high levels of Vitamin C which is resistant to storage and heat damage due to cooking. It has found its use in various herbal products for medicinal and nutritional value.

In Unani medicine, it is described as a tonic for heart and brain. According to the two main classic texts on Ayurved, *Charak Samhita* and *Sushrut Samhita*, Amalaki is regarded as "the best among rejuvenative herbs", "useful in relieving cough and skin disease" and "the best among the sour fruits" (Patel, S.S. and Goyal, 2012).

Amla grows in tropical and subtropical parts of India, China, Indonesia. The fruit is spherical pale yellow with six vertical furrows. The average weight of the fruit is 40-50g. The fruits of Amla are widely used in the Ayurvedic preparations and are believed to increase defense against diseases. Fresh fruit is refrigerant, diuretic and laxative. Fruit is also carminative and stomachic. Dried fruit is sour and astringent. It is an important dietary source of vitamin C, amino acids, and minerals. Keeping in view of the richness of amla the present study is designed to study physicochemical, phytochemical and neutraceutical profile in addition to the antioxidant activity of fruits.

Material and Methods

Collection and Processing of the Material

The fresh fruits of Amla were purchased from open market at Dehradun, Uttarakhand state. The fruits were processed to remove the fruit pulp for preliminary physico-chemical analysis and isolated pulp was homogenized and passed through a strainer for further, subject to drying obtaining dry powder. Fresh fruit pulp was analysed for pH, moisture content, titratable acidity, ash and total water-insoluble solids according to Association of Official Analytical Chemists (A.O.A.C.) (Williams,1984).The powder was stored in an airtight container and kept in desiccators till further use.

Loss On Drying at 105°C

About 1g sample was weighed in an LOD bottle. The sample was dried at 105°C for about 6 to 7hours, cooled to room temperature in a

desiccator and weighed till constant weight. (API,1989)

Total Ash

About 1g sample was accurately weighed in acrucible. The sample was ignited at about 550°C to 600°C for 4 to 5 hours, then allowed to attain room temperature in adesiccator and weighed till constant weight (API,1989).

Acid Insoluble Ash

The ash obtained above was dissolved in about 25ml dil. HCl, boiled for about 15 minutes and filtered through ashless filter paper No.41. Washings with hotwater were given to the residue till the filtrate was free from acid. The filter paper was dried in an oven and incinerated at 550°C to 620°C for about 3 to 4 hours. Then the residue was cooled to room temperature in a desiccator and weighed till constant weight (API,1989).

Water Soluble Extractive

About 1g of sample was soaked overnight, in 100 ml water and slightly warmed. Next day, this solution was filtered and 25 ml of filtrate was dried at 105°C, to constant weight, in a petri dish(API,1989).

Alcohol Soluble Extractive

About 1g of sample was soaked overnight, in 50 ml alcohol. Next day, this solution was filtered and 25 ml of filtrate was dried at 105°C, to constant weight, in a petri dish(API,1989).

pH of 1.00% w/v solution

About 1 g of sample was dissolved in 100 ml water, slightly warmed, cooled and the pH was recorded on a suitable, previously calibrated pH meter(API, 1989).

Determination of Glycosides

Glycosides were confirmed by subjecting the acid hydrolysed extract to Legal's test, Borntrager test and Libermann-Burchard's test (Sachan *et al*, 2010).

a) ***Legal's test***

Hydrolysate was dissolved in pyridine and sodium nitro-prusside solution, added sodium hydroxide; a colour change result in presence of glycosides.

b) ***Borntrager's test***

A few milliliters of hydrolysate treated with chloroform, decanted off chloroform layer, added equal quantity of dilute ammonium solution. A pink colour is produced in ammonical layer in presence of glycosides.

c) ***Libermann-Burchard's test***

Hydrolysate treated with chloroform, to this added Libermann-burchard reagent; a colour change result in presence of glycosides.

Determination of Carbohydrates Extracts were dissolved separately and were tested with Molisch reagent, Fehling's reagent, Bendict solution, and Barfoed's test for detection of carbohydrates (Sachan et al,2010).

a) Molisch's test

To the filtrate, added few drops of alcoholic alpha nepthol and 2ml of concentrated sulfuric acid slowly through the side of test tube; presence of carbohydrate produce a violet colour ring at the junction of two layers.

b) ***Fehling's test***

A little fraction of filtrate treated with Fehling's solution I & II and then heated on a water bath. A brick red precipitate is indicator for reducing sugars.

c) ***Bendict's test***

Small quantity of filtrate treated with equal quantities of Bendict's reagent, heated subsequently on a water bath result to formation of a brown precipitate in presence of reducing sugars.

d) ***Barfoed's test***

The different extracts were treated with

Barfoed's reagent. Monosaccharides, presence, produced a brick red precipitate.

Determination of Fixed oil and Fats (Sachan et al, 2010; Gupta and Kohli, 2010).

a) ***Spot Test***

A small quantity of sampler was separately pressed between two filter papers and was observed for appearance of oil stain on paper which will indicate presence of fixed oils or fats.

b) ***Saponification test***

Few drops of 0.5N potassium hydroxixe along with one or two drops of phenolphthalein were added to various extracts, heated on a water bath for 1-2 hours. Saponification or no saponification indicates the presence or absence of oil and fats.

Detection of Protein & Amino acids

The spay dried extract was subject to Million's test reagent, Biuret test reagent, and Nin-hydrin test reagent, presence of amino acids and proteins is indicated by production of red, violet and blue colour respectively(Sachan *et al*, 2010).

Detection of Phenolic compounds & Tannins

All the dry extracts were dissolved in minimum amount of water, filtered and subject to Ferric chloride test, Gelatin test. Filtrate on addition of few drips of ferric chloride produce a violet colour precipitate in presence of tannins. A white precipitate is resulted in presence of tannins on addition of 1ml 1% solution of gelatin to the filtrate (Sachan *et al*, 2010; Gupta Kohli, 2010 and Kalaiarasan, 2010).

Detection of Phytosterols

Small quantity of the dry extracts dissolved in about 5ml of the chloroform and subjected to Salkowski's test and Libermann-Burchard's test (Sachan *et al*, 2010; Gupta Kohli, 2010 and Kalaiarasan, 2010).

a) ***Salkowski's test***

One ml of the chloroform solution, prepared as above was added with few drops of concentrated sulfuric acid; green colour is the indicative of phytosterols.

b) ***Libermann-Burchard's test***

The chloroform solution, prepared as above was treated with few drops of concentrated sulfuric acid followed by one ml of acetic anhydride. Presence of phytosterols is confirmed by the production of a bluish green colour.

Determination of Alkaloids

Small fractions of solvent free extracts were separately stirred with a milliliters of dilute hydrochloric acid and filtered, the filtrate is tested with Mayer's reagent, Wagner's reagent, Hanger's reagent, Dragendroff's reagent to confirm the presence or absence of alkaloids as indicated by production of cream, reddish brown, yellow or brown colour respectively with these reagents in presence of alkaloidal substances (Sachan e*t al*, 2010; Gupta and Kohli, 2010 and Kalaiarasan, 2010).

Determination of Flavonoids Shinoda's test

Small quantity of the extract was dissolved in alcohol, to those pieces of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids Gupta and Kohli, 2010 and Kalaiarasan, 2010).

Determination of Gums and Mucilage

Small quantity of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilages. Precipitate tested for the swelling and presence and carbohydrate (Sachan *et al*, 2010).

Estimation of Ascorbic Acid and Gallic acid by HPLC

HPLC conditions required for analysis

1. Column : C18
2. Mobile phase: 10mM hexane-1-sulfonic acid salt containing 1% acetic acid and 0.13% triethylamine.
3. Flow rate:1ml/min
4. Detection : 254 nm
5. Volume of injection: 20µl of standard an sample solutions.

Standard Ascorbic acid solution

Weighed accurately 10mg of standard Ascorbic acid in 100 ml of demineralized water and dissolved.

Standard Gallic acid solution

Weighed accurately 50mg of standard Gallic acid in 100ml of demineralised water and dissolved. (Filter the solution through 0.20 µm syringe filter).

Sample solution

Weighed accurately about 500mg of the fresh Amla juice in 100ml water. Sonicated the solution to ensure complete solubility of ascorbic acid and gallic acid, but did not heat the solution. (Filtered the solution through 0.20µm syringe filter).

Method of analysis

Stabilized the instrument with the above-mentioned mobile phase and inject 20µl of working standard solutions. Recorded the chromatogram. Injected 20 µl of sample solution and record the chromatogram. Calculated the AUC of the Standard Ascorbic acid peak, gallic acid peak and corresponding peak in the sample.

Calculation

% Content of ascorbic acid/gallic acid=

$$\frac{\text{AUC of sample peak}}{\text{AUC of standard peak (Individual standard)}} \times \frac{\text{Standard concentration}}{\text{Sample Concentration}} \times \%\ \text{Purity of Individual standards}$$

Note: Ascorbic acid is highly unstable in solution form. Weigh and keep the sample in dry flask. Dissolve it in water, just before injection.

Estimation of Tannins(Diaz,1987)

Reagent

Potassium ferricyanide reagent

Weighed accurately about 1 g of Potassium ferricyanide [$K_3(Fe(CN)_6$] IN 100ml volumetric flask. Added about 50 ml of purified water, dissolved by sonication for 10 minutes and finally make up to the mark.

Ferric chloride reagent

Weighed accurately about 1 g of Ferric chloride[$Fecl_3$] in 100ml volumetric flask. Added about 50 ml of purified water, dissolved by sonication for 10 minutes and finally make up to the mark.

Sample Preparation

Weighed accurately about 0.1g of finely powdered sample in to 250ml flat bottomed flask and 100 ml purified water and reflux at 100± 2 using water bath for 1 hour , cool and allow the residue to settle for 5 minutes. Decanted the dissolved extract into 500 ml volumetric flask and washed the residue with purified water and made the volume up to the mark with same solvent. Filtered the extract through Whatman No.1 filter paper. Discarded first 25ml of filtrate and used the subsequent filtrate for analysis.

Standard Preparation (0.01mg/ml)

Weighed accurately about 100mg of standard tannic acid in 100 ml volumetric flask, dissolved with purified water and made up to volume with

purified water (standard stock solution). Pipetted out 1 ml from this solution and make up to 100ml with purified water (working standard solution).

Preparation for optical density reading

Standard Solution

Took 1 ml of working standard solution in 10 ml Volumetric flasks. Add 1 ml of potassium ferric cyanide and 1 ml of ferric chloride reagent. Mixed well and make the volume up to 10 ml with purified water. Exactly 30 Minutes after addition of the reagents read the Optical density (S) at 720 nm against reagent blank.

Reagent blank

Reagent blank was prepared by diluting 1ml of potassium ferric cyanide and 1 ml of ferric chloride to 10 ml with purified water.

Test Solution

Take 0.2 ml sample preparation in 10 ml Volumetric Flasks. Add 1 ml of potassium ferric cyanide and 1 ml of ferric chloride reagent. Mix well and make the volume up to 10 ml with purified water. Exactly 30 Minutes after addition of the reagents read the Optical density (S) at 720 nm against reagent blank.

Test blank

Take 0.2 ml of sample solution or volume of sample solution taken to prepare the test solution and make volume to 10 ml with purified water and measured the absorbance(TB) against water.

Note

a. All the optical density readings should be taken exactly 30 min after addition of the reagents.

b. All the reagents and standard solution should be prepared freshly anad used immediately.

Calculation

Subtracted the reading of test blank from test solution and calculated the content of tannic acid from the standard absorbance, expressed as %w/w of total tannins=

$$\frac{\text{Absorbance of sample (T-TB)}}{\text{Absorbance of standard (S)}} \times \frac{\text{Weight of standard (mg)}}{100} \times \frac{1}{100} \times \frac{\text{Volume of standard taken for reaction (ml)}}{\text{Volume of sample taken for reaction (ml)}} \times \frac{\text{Total volume of sample (ml)}}{\text{Weight of sample (mg)}} \times \text{\% Purity of standard}$$

T=Test solution absorbance

TB-Test Blank

S= Standard absorbance

Note: All chemicals used are of AR grade

Evaluation of free radical scavenging activity DPPH Radical Scavenging Activity

The free radical scavenging activity was determined according to the method of Shimada *et al*. 1992. The extract was dissolved with methanol to prepare various sample solutions at 10, 20, 40, 60, 80 and 100 u g/ml. 2 ml of extract solution was mixed with 1 ml of 0.2 mm DPPH in ethanol. The mixture was shaken vigorously and maintained for 30 min in the dark. The absorbance was measured using at 517nm.The % reduction in absorbance was calculated from the control and sample absorbance at each level by using the following formula :% Reduction = (Ac As)/Ac×100

Ac = Control Absorbance

As = Sample Absorbance

Results and Discussion

The Physicochemical studies of *Emblicaofficinalis* (amla) fruits are tabulated in Table -1. The fruits of *Emblicaofficinalis*are rich in tannins. The fruits have 30% of the total tannins distributed in the whole plant. *Emblica* fruit is a rich source of vitamin C (ascorbic acid) and gallic acid (Table – 2 Figure -1, 2 & 3).

The tests for aminoacids have been identified positive; it is reported to have variety of amino acids *viz.* glutamic acid, proline, aspartic acid, alanine, and lysine *etc.* Qualitative Phytochemical chemical examination of the dried fruit powder of *Emblic a officinal is* revealed the presence or absence of various plant constituents. The observations were recorded in + (present) or – (absent) and are summarized in the Table – 3.

The percentage reduction in absorbance of

Table – 1 Physicochemical analysis

S.No.	Test Parameters	Results
1.0.	Loss on Drying	9.320% w/w
2.0.	Total Ash	4.22 % w/w
3.0.	Acid insoluble ash	0.855% w/w
4.0.	Water soluble extractive	96.990% w/w
5.0.	Alcohol soluble extractive	34.334
6.0.	pH of 1.00% w/v solution	4.02

Table – 2 Estimation of Bioactive markers

S.No.	Bioactive markers	Value
1.0.	Ascorbic acid(Vit.C)	0.24%
2.0.	Gallic acid	0.024%
3.0.	Tannins	30.27%

Table – 3 Phytochemical analysis

S.No.	Constituents	Observations
1.0	Alkaloids	+
2.0.	Carbohydrate	+
3.0.	Glycosides	+
4.0.	Proteins and Amino acids	+
5.0.	Phenolic compounds	+
6.0.	Saponins	+
7.0.	Phytosterols	+
8.0.	Flavonoids	+
9.0.	Fixed oils and Fats	-
10.0.	Gums and mucilages	+

Table – 4 Percentage reductions in absorbance of DPPH at 517 nm by methanolic seed extract of *Emblica officinalis* (Amla).

S.NO.	Concentration(µg/ml)	% Reduction
1.0.	10	48.14
2.0.	20	54.12
3.0.	40	62.13
4.0.	60	72.12
5.0.	80	77.14
6.0.	100	86.23
7.0.	IC_{50}(µg/ml)	16

HPLC CHROMATOGRAPHS

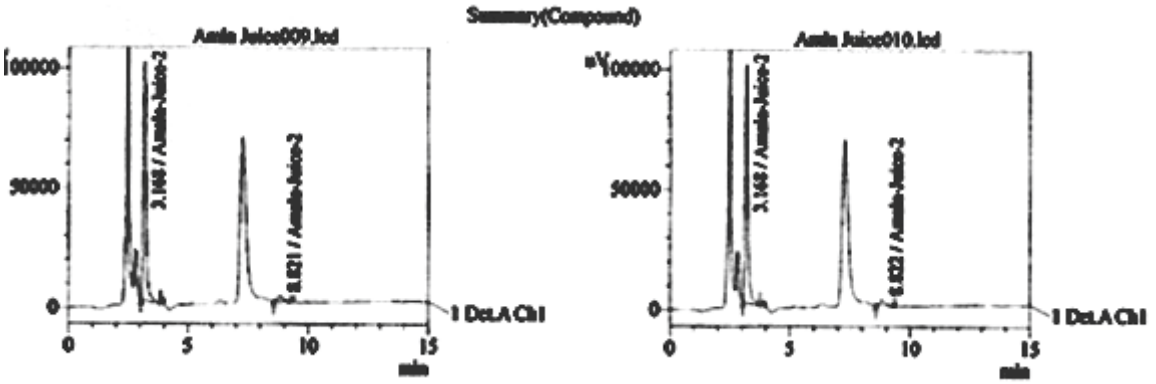

Figure – 1 HPLC analysis

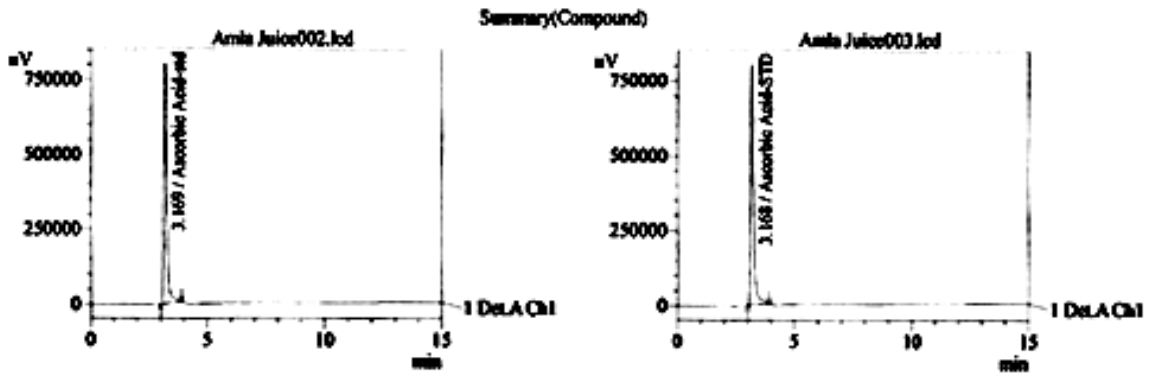

Figure – 2 Estimation of Ascorbic acid through HPLC analysis

DPPH by methanolic extract was revealed in Table – 4 indicating its antioxidant activity.

The observations in present investigation indicated that the emblic fruit is highly nutritious. It servs as the packets of vitamin C wrapped with essential minerals amino acids and other vital nutrients.

The study has explored nutrient and non-

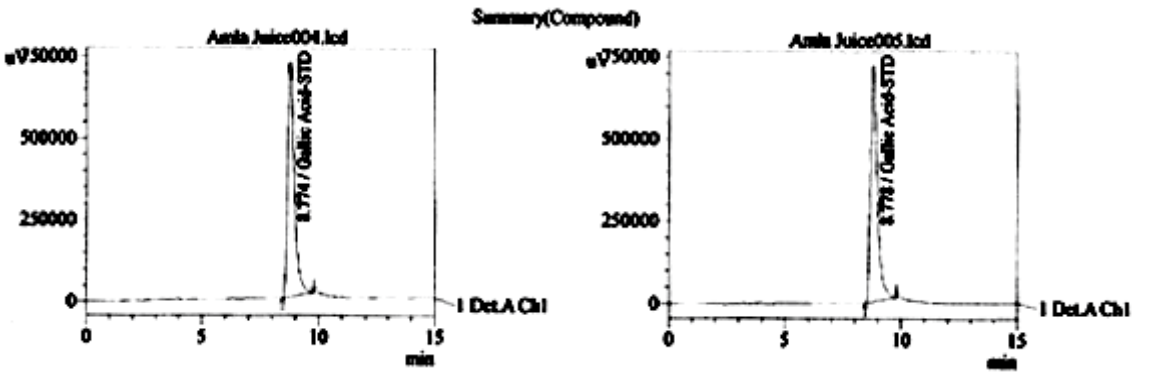

Figure – 3 Estimation of Gallic acid through HPLC analysis

nutrient components of *Emblicaofficinalis*. As the results revealed that the Amla has important nutritional factors contained with minerals, amino acids and vitamin. Its proven nutraceutical potential will serve the popularity of fruit in the world wide educated population. The vitamin C in Amla is remarkably stable even after prolonged cold storage or cooking, probably due to the presence of phenolic compounds tannins and phytophenols which retarded the oxidation of ascorbic acid present in the fruit. Amla has been and remains a product of substantial interest in India and south asia. It is particularly noteworthy that amla is reported as an essential component in rasayanas such as triphala and chyawanprash to assist the body attain a state of improved nutrition that prevents aging and enhances longevity (McQuate *et al*, 2009)..

Free radicals damage cellular membranes, proteins and DNA and cells and produce a range of diseases in body which could be prevented with amla fruits consumption. Phenols, flavonoids, anthocyanins and carotenoids are some of the important antioxidant found in fruits and vegetables. The antioxidant activity of amla is reported in Table – 4.

Conclusion

Amla fruits have potent antioxidant activity due to the presence of tannins, vitamin C, gallic acid and flavonoids. The observations in present investigation indicated that the *Emblica officinalis* (Amla) fruit is highly nutritious.

From the above results and discussions, we can conclude that amla, a reputed herbal medicines with the significant nutritive study results (it is proved that, they) can also be used as dietary supplements (neutraceutical), to enhance our daily diet and therefore, it's a rejuvenating food and a tonic.

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Chromatographic Analysis and Antioxidant Activity of Bark of *Acacia catechu*

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Abstract-*Acacia catechu* is an important medicinal plant used in Ayurveda for so many diseases and commonly for mother and child healthcare. The drug is used as a medicine for several common ailments like skin diseases, ulcers, diabetes etc. These medicinal properties may be due to their antioxidant properties. Hence, a preliminary study on the antioxidant activity of methanol, hexane and aqueous extract was carried out by evaluating the free radical scavenging activity on TLC silica gel plate by the method of DPPH radical scavenging assay. The methanol and aqueous extract gave very good antioxidant activity. An attempt was also made to estimate the catechin present in the methanolic extract of *Acacia catechu*. The detection and estimation of catechin and epicatechin were carried out by HPTLC and HPLC method. These studies on *Acacia catechu* are being reported for the first time.

Keywords:*Acacia catechu*, Antioxidant activity, DPPH radical scavenging, HPLC and HPTLC.

Introduction

Acacia catechu Wild. (Fam: Mimosaceae, Hindi Khair) is widely used in Ayurveda for many diseases and mainly for skin diseases[1]. It is highly valuable for its powerful astringent and antioxidant activities. It is useful in dental oral, throat infections and as an astringent for reducing oozing from chronic ulcers and wounds. The extracts of *Acacia catechu* exhibit various pharmacological effects like antipyretic, anti-inflammatory, antidiarrhoeal, hypoglycaemic, hepatoprotective, antioxidant and antimicrobial activities. [2,3]

The phytochemical constituents present in *Acacia catechu* includes catechin, catechutannic acid, epicatechin, catechin tetramer,dicatechin, gallocatechin, kaempferol, taxifolin, isorhamnetin, (+)afzelechinn, L arabinose, D galactose, D rhamnose and aldobiuronic acid[4]., It also contains active principles like cyanidol, tannins, Flavonoids and polyphenols.[5]

Epicatechin is present in many plants. High quantities can be found in cocoa, tea and grapes. Pure epicatechin is an odorless white powder. Epicatechin is a flavonol belonging to the group of flavonoids and it is a powerful Antioxidant agent. Flavonoids are a group of polyphenolic compounds which possess many biochemical effects like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, and anticancer, antihepatotoxic, protection of cardio vascular system.[6] This study aimed at the investigation of the antioxidant activity of the methanol, hexane and aqueous extracts of bark of *A. catechu* Wild. The extracts were also studied for their possible chemical constituents present in it via High Performance Thin Layer Chromatography (HPTLC) which may possess the antioxidant activity. Hence, an effort was made to assess the presence of catechin and epicatechin present in methanol bark extract of *A. catechu* by HPLC method.

Material and methods

Extraction

The barks of Acacia catechu were found in good quality in the desert region of Rajasthan

(Jobner), India. The plants were identified using their morphological identification keys and confirmed by the local forest officers. Shade dried bark were ground mechanically using a mixer grinder, the obtained powder was added with different polar solvents like hexane, methanol and water ie.10g of bark powder in 100ml of solvent in a 250 conical flask and kept overnight for 24h. Samples were filtered dried, weighed and stored in refrigerator at 4◦C till use. The dried samples were used with appropriate solvent before study [7].

Thin Layer Chromatography

HPTLC finger printing performed for standardization of the drug. CAMAG HPTLC system equipped with Linomat-5 sample applicator, TLC scanner-3, Reprostar-3 and wINCATS software was used to analyse the methanolic, hexane and aqueous bark extract of *Acacia catechu* (Sethi[8], Stahl[9] and Wagner *et al*[10]).

Sample application

The samples (5µL) and standard epicatechin (5µL) were spotted in the form of bands of width 12 mm using a 100µL Hamilton syringe on precoated silica gel GF254 plates (20cm×10cm) with the help of Camag Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through wINCATS software. Developed the plate in the solvent system in a twin trough chamber to a distance of 8 cm.

Standard solution

Dissolved 5.0ml of epicatechin in 5ml of methanol.

Development of chromatogram

The mobile phase for the detection of epicatechin was Toluene : Ethyl acetate : Glacial acetic acid (5:5:1) and was used for chromatography run. The plates were developed in a solvent system in a (10cm×10cm) twin through glass chamber saturated previously for 10 min with the mobile phase.

Visualization

The plate was kept in photo-documentation chamber (CAMAG Reprostar-3) and captured the images under UV light at 254 and 366 nm, respectively. The Rf values and finger print data were recorded by wINCATS software.The developed plate was then sprayed with 10 percent ethanolic potassium hydroxide solution and dried at 105ºC in hot air oven for 3min.Now, the plate was observed under white light and recorded the Rf value and color of resolved bands.

HPLC Analysis of *Acacia catechu* Bark Extracts

HPLC Instrument

The methanolic extract was concentrated and analyzed using HPLC of *Acacia catechu* was analysed as per standard method[11] with some modifications. The extract was filtered through membrane syringe filter (0.20 m) and 20 µl of filtrate was used for analysis in the HPLC.

HPLC conditions

Column (250×4.6 mm 5µ)	:	C18 phenomenex luna
Mobile phase (0.1%) in water	: :	orthophospheric acid Acetonitrile (40:60)
Flow rate	:	1 ml/minute
Wavelength	:	210 nm
Injection	:	20 µl
Temperature	:	35°C

Procedure

Stabilized the instrument with the mobile phase till the baseline is satisfactory then injected the standard solution of three times and then recorded the chromatogram. The % RSD between the results should be less than 2 % and then injected the sample solutions and recorded the chromatogram.

Preparation of standard solution

A stock solution of standard of catechin and epicatechin were prepared by accurately weighing about 5.0 mg catechin (1.0mg/ml) and 5.5 mg epicatechin (1.1mg/ml) standard in 5 ml of methanol in a volumetric flask. It was then sonicated for 15-20 min and made the volume up to the mark with methanol.

Sample preparation (5.0mg/ml)

500 mg *Acacia catechu* bark sample was accurately weighed and dissolved in 100 ml methanol in a volumetric flask. It was then sonicated for 15-20 minutes and made the volume up to the mark with methanol. filter through 0.2µ syringe filter.

TLC DPPH Bio-autography for Antioxidant Activity

The filtrate (methanolic, hexane and aqueous) were loaded on to the activated Silica gel G (Merck) plate. The antioxidant compounds were separated using solvent mixture of Toluene: Ethyl acetate: Glacial acetic acid (5:5:1). Once the run was completed, plates were air dried for 15 min and were sprayed by 0.04% DPPH solution in methanol using a spray gun for 5 sec. The image was observed under visible light at exactly 2 min after spraying using a white light illuminator. The bright yellow bands against the purple background confirmed the antioxidant molecule[12]. The Rf value of the samples were calculated.

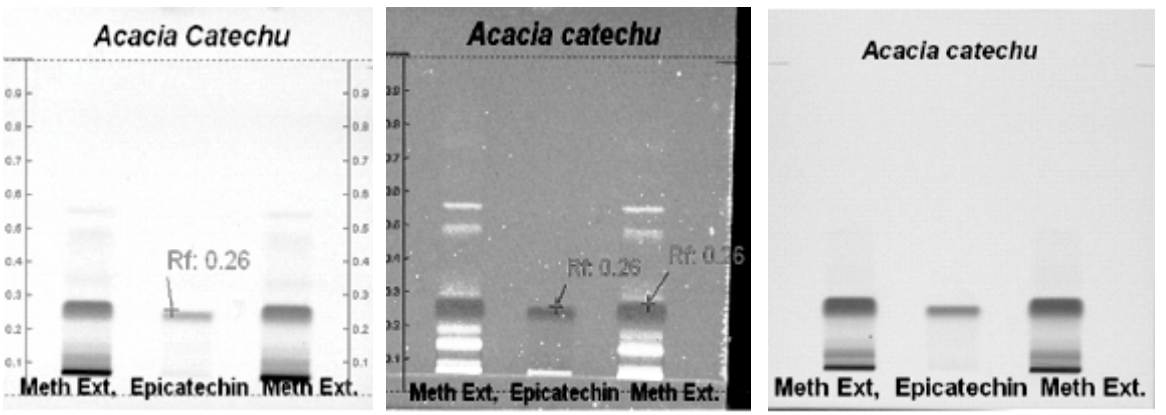
Results and Discussion

HPTLC analysis

The HPTLC chromatogram was run for *Acacia catechu* bark extract along with standard of epicatechin. In this study HPTLC fingerprinting of methanol bark extract and aqueous extract showes the presence of epicatechin at Rf (0.26) in *Acacia catechu*. TLC profile under UV 366 nm and 254 nm is shown in the Figure-1 and the corresponding HPTLC chromatogram of standard epicatechin is also shown in Figure-1. The bark extracts revealing more than four peaks with different Rf values in the range between 0.04 to 0.84 confirmed the presence of epicatechin in Acacia *catechu* along with other unknown components.

TLC based antioxidant activity

In the present study, methanolic, hexane and aqueous extracts are monitored by a TLC bioautography method which gives quick access for the detection and localization of the active compounds in a complicated plant extract[13].



at 254nm at 366nm After Derivatization with KOH reagent

Figure-1& 2 HPTLC chromatogram of bark extracts of *Acacia catechu*.

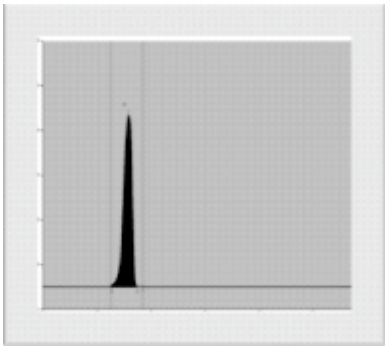


Figure-3 HPTLC chromatogram of standard peak of epicatechin.

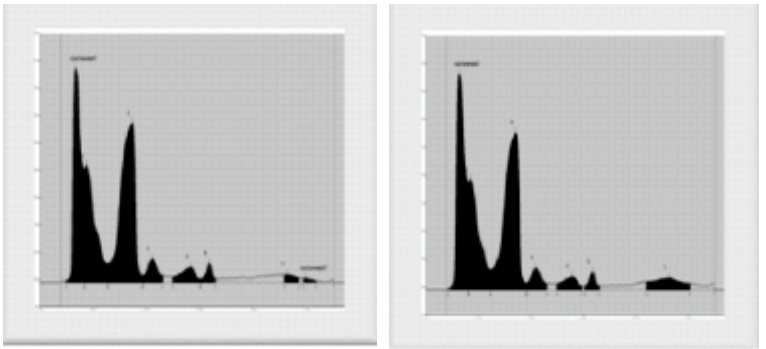
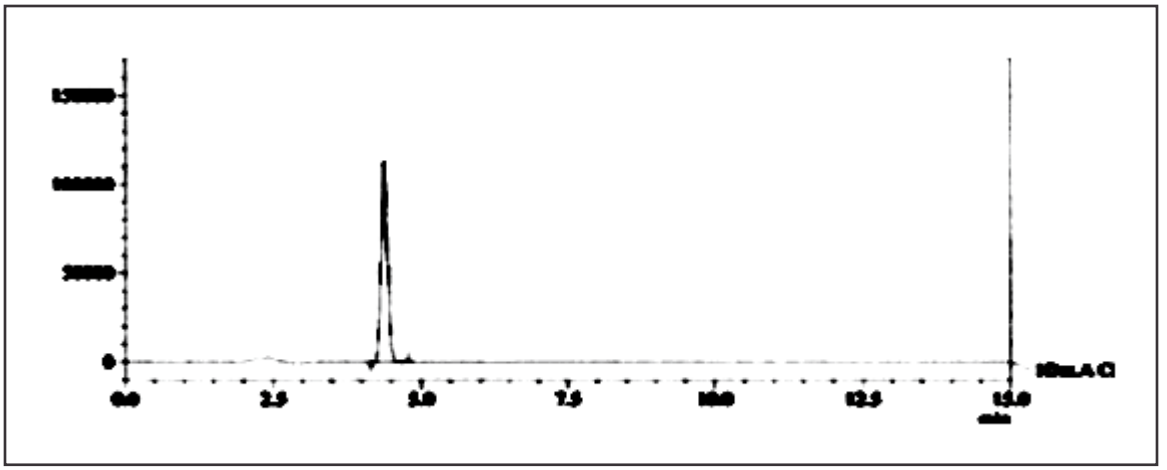


Figure-4 HPTLC chromatogram ofmethanol and aqueous extract of Acacia catechu showing different phytoconstituents.



Graph-1 Estimation of standard (Epicatechin)

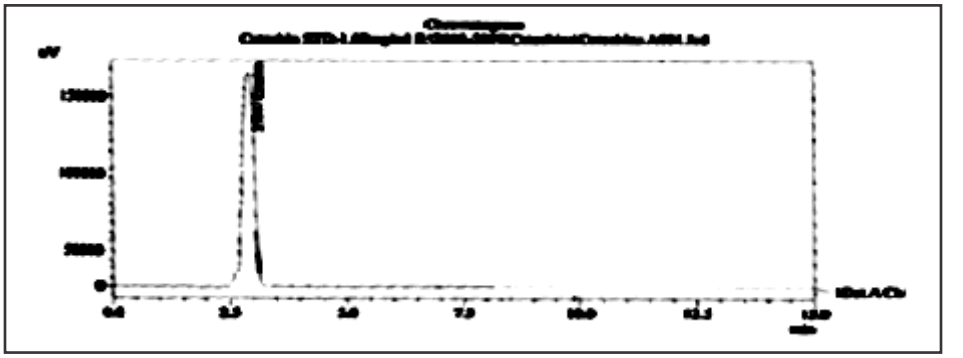
PeakTable

Detector A Ch1 210nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1	Epicatechin	4.301	12245243	1107709	100.000	100.000
Total			12245243	1107709	100.000	100.000

The results of TLC antioxidant bioautography assay showed yellow coloured spots when sprayed with DPPH solution showing the plant extract to be antioxidant.When the plates were sprayed with DPPH solution in methanol (0.4mM), the regions where substances with antioxidant capacity occurred stained yellow in the purple background.

The TLC of the methanolic, hexane and aqueous plant extracts conducted using (toluene: ethylacetate: glacial acetic acid, 5:5:1) as mobile phase and DPPH as spray reagent. The methanolic and aqueous extracts shows yellow colour on purple surface indicating that the epicatechin present in both the extracts shows the antioxidant activity, while hexane extract does not show any antioxidant bands.

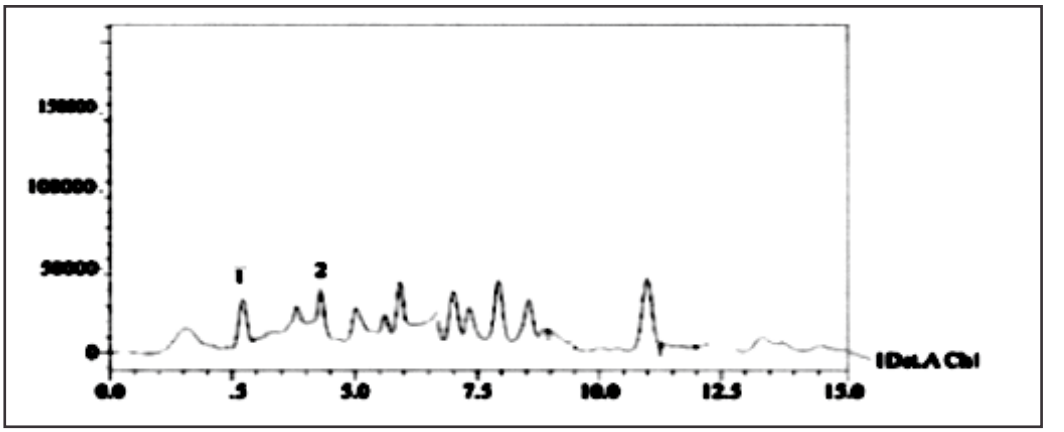


Graph-2 Estimation of standard (Catechin)

PeakTable

Detector A Ch1 210nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1	Catechin	2.904	14483722	1066980	100.000	100.000
Total			14483722	1066980	100.000	100.000



Graph-3 Estimation of Epicatechin and Catechin content in *Acacia catechu* bark extract

PeakTable

Det.A Ch1 / 210

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1	1	2.955	1021445	82637	39.226	45.722
2	2	4.385	1582575	98102	60.774	54.278
Total			2604019	180738	100.000	100.000

HPLC Analysis

Our HPLC studies revealed that the plant extracts are rich in catechin (1.37%) and Epicatechin (2.75%w/w), the assay was calculated according to the Equation 1 .The retention times observed for the authentic sample of catechin (RT-2.904)and epicatechin are 2.904 and 4.301 respectively. The retention time of those of Acacia catechu methanolic bark extract are

given in graph 3. Study conducted by Shen D et al proved that the leaves and heartwood extract exhibits predominant amount of catechins[14]

Equation 1: Calculation of Assay of Epicatechin (Std Wt / Sample Wt) x (Sample Area / Std Area) x Assay of Standard = Assay of Sample

Assay of Epicatechin in test sample:

(1.1 /5.0) x (1582575 /12245243) x 97% = 2.75% [w/w]

Assay of Catechin in test sample:

(1.0 /5.0) x (1021445 /14483722) x 97% = 1.37% [w/w]

Conclusion

On the basis of this preliminary study, it is concluded that the identification of antioxidant compound through TLC is a simple and fast method for screening of large number of extracts. The result of this study revealed that *Acacia catechu* contains pharmacologically active components which have antioxidant activity. These attribute may provide the rationale for the use of *Acacia catechu* in the management of various diseases as a traditional herbal medicine. The results of this study also showed that the bark extract of *Acacia catechu* contain many phytoconstituents. In conclusion, the results of this investigation revealed that methanol bark extract of *Acacia catechu* contains pharmacologically active components like catechin and epicatechin with antioxidant properties, thus justifying its widespread use by the local population for the treatment of various diseases.

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Phytochemical Evaluation and Microbial Inhibitory Activities of *Stevia rebaudiana*

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Abstract–The present study was aimed to evaluate the phytochemical screening and microbial inhibitory activity of dry leaves of ***Stevia rebaudiana***. Fresh matured leaves of *stevia* were collected, air dried and then aqueous, methanol and hexane extracts were prepared. For phytochemical screening, the crude extract was tested in order to reveal the presence of Bioactive markers compounds. The result of phytochemical screening indicate that methanol extract was the best solvent with the maximum Bioactive markers compounds than the other two extracts. For the microbial inhibitory activities, the most active extract was found to be aqueous extract with the maximum diameter of zone of inhibition against *E.coli* (13mm) followed by methanol extract against *S.aureus* (12mm). The result indicates that the inhibitory activity of *stevia* is more towards gram negative *E.coli* and shows the moderate antimicrobial activity.

Keywords: Valuate, Phytochemical, Screening, Bioactive, Microbial, Inhibition, *E.Coli, S.Aureus.*

Introduction

Stevia rebaudiana is a plant species in the genus stevia of the sunflower family Asteraceae which is indigenous to Brazil and Paraguay (Uddin *et al.,* 2006; Alhady *et al*, 2011). It is perennial and endemic medicinal herb (Sivaram et al, 2003). It is commonly known as candy leaf, sugar leaf, honey leaf due to its sweetness, which is estimated to be 300 times sweeter than the sugarcane (Chalapathi *et al*, 1997). It is semi-humid and subtropical plant that can grow like any other vegetable crop even in a kitchen garden.

Stevia rebaudiana is a natural alternative to artificial sweetener is found to contain over hundred phytochemicals including the well characterized active compounds, stevioside and rebaudioside. A (Bhimba *et al*, 2010). *Stevia* is well known for its applications in treatments of any disease like diabetes, high blood pressure and weight loss as mentioned in the various traditional systems of medicine(Abu Bakar Siddique *et al*, 2014). It is well known for some notable health promoting properties due to the presence of some bioactive markers compounds such as alkaloids, steroids, flavonoids, saponins, glycosides, terpenoids and carbohydrates which creates definite physiological action on human body by treating different diseases such as hypertension, diabetes, cancer, neural disorder, cardio vascular disease and renal function (Abu Bakar Siddique *et al*, 2014). Presence of these compounds in the plant provide anti-oxidant, anti-microbial and anti- fungal properties (Gupta *et al*, 2014). Plants can possess antimicrobial natural products to protect themselves from microbial infection and deterioration (Cowan et al,1999).

The major objectives of the present study is to evaluate the phytochemical screening in order to identify its medicinal bio-active substances and the determination of antimicrobial potential of methanolic, hexane, and aqueous extracts of *S.rebaudiana* leaves against two pathogens *S.aureus* and *E.coli.*

Material and Methods

Collection and Preparation of the plant sample

The fresh matured sample of *stevia* were collected from the Herbal garden of Himalaya drug company, Dehradun. The leaves were removed from the plant, then washed thoroughly in running tap water and spread on tray and dried in oven at 40°C. After drying, the fine powdered (15gm) of dried leaves of *stevia* was prepared by using mixer grinder and then stored in the polythene bag until used.

Evaluation of Phytochemical of *Stevia rebaudiana*

Preparation of plant extract

The powdered *Stevia rebaudiana* was extracted by taking 5gm of sample powder after weighing in 50ml respective solvent like methanol(A) , hexane(B) and aqueous(C) in iodine flask to prepare extracts A,B and C. The extracts were, then shaken for 24hrs by using Wrist action shaker. After 24 hours, the crude extracts were filtered through the Whatman no.1 filter paper. The filtrate was collected, filtration was done twice, as there were particulates present in extract, the extract was concentrated on the water bath at 80^0C for one hour. After which, the solutions were ready for carrying out phytochemical screening.

Test for the Preliminary Phytochemical Screening (Sheikh *et al*, 2016)

The extracts were tested for the detection of the presence or absence of the bioactive markers compounds like flavonoids, alkaloids, carbohydrates and the others.

Test for Tannins

Ferric chloride reagent test

1ml of each extract was allowed to react with 3-4 drops of 5% ferric chloride solution, gave intense green/greenish black/ brownish green color, indicated presence of tannin.

Test for Flavonoids

Ferric chloride test

1ml of the extract mixed with the few drops of ferric chloride solution gave green color, indicated presence of flavonoid.

Test for alkaloids

Wagner′s test

To find out the presence of alkaloids, a few drops of Wagner's reagent was added in 1ml of each extract. A brownish flocculent/ reddish-brown precipitate confirmed the test as positive.

Test for Carbohydrates

Fehling's test

Few drops of extract were heated with Fehling's A and B solution, appearance of orange red precipitation indicated the presence of carbohydrates.

Test for Saponins

Froth test

1ml of extract was shaken with little quantity of water. The foam persisted for 10minutes. It confirmed the presence of saponin.

Test for Cardiac glycosides

Keller-kilani test

2ml of Glacial acetic acid containing 1-2 drops of 2% solution of ferric chloride was added to 1ml extract, followed by the addition of 1ml concentrated sulpuric acid. A brownish ring was formed at the junction of two layer indicating the presence of cardiac glycosides.

Test for Steroids

Salkowaski′s test

1ml of chloroform was added to 2ml of each extract followed by the careful addition of concentrated sulphuric acid on the wall of test tube to form a lower layer. A reddish brown ring produced immediately at the interface indicated the presence of steroid.

Test for Terpenoids

Salkowaski's test

1ml of chloroform was added to 2ml of each extract followed by the careful addition of concentrated sulphuric acid on the wall of test tube to form a lower layer. A golden yellow ring produced immediately at the interface indicated the presence of terpenoid.

Microbial inhibitory activity of *Stevia rebaudiana*

The microbial inhibitory activity was determined by the diameter of the zone of inhibition (ZOI) which was measured with the help of zone reader in micrometer.

Microorganism

Two bacterial culture i.e.*Escherichia coli* (gram negative) and *staphylococcus aureus* (gram positive) were used in the microbial inhibitory activities.

Antimicrobial Activity of the Extracts

To check the antimicrobial activities, the different prepared extracts of the leaves of *stevia* were tested for antimicrobial activity against the test organism using agar well diffusion method (Navarro et al, 1996). (The nutrient agar media were added to sterile petri dishes. The inoculum of the test pathogen was luoculated in the prepared media plates. The plates were kept undisturbed for 10 to 15 minutes in order to set the medium properly. Then, used a sterile cork-borer with the internal diameter 5mm to make well on the medium. 100µl of the various extracts concentration were dropped into each appropriate well by using micropipette (Atata *et al*, 2003 & Bonjar, 2004). Then the plates were incubated at 37^0C for 24 hours (Maunyr balouiri. *et al.*, 2016). The Antimicrobial inhibitory activity was determined by measuring the diameter of zone of inhibition (mm) produced after incubation.

Results and Discussion

Evaluation of Phytochemical of *Stevia rebaudiana*

The preliminary phytochemical screening tests are helpful in qualitative estimation of various bioactive chemical constituents that are pharmacologically active chemical compounds which perform different activities for examples, antimicrobial, antifungal, antidiabetic, antioxidant, etc. The phytochemical screening conducted on methanol, hexane, and aqueous extract of *stevia* revealed the presence of active chemical constituents such as alkaloids, flavonoids, glycoscides, tannins, saponins, steroids and terpenoids(Table – 1).

Table – 1 Result of various phytochemical test

Name of the Test	Reagent Test	Aqueous Extract	Methanol Extract	Hexane Extract
Tannins	Ferric chloride	+	++	++
Flavanoids	Ferric chloride	++	++	-
Alkaloids	Wagner's	++	-	-
Carbohydrates	Fehling's	++	++	++
Saponin	Froth	-	++	-
Glycosides	Keller-kilani	++	++	-
Steroids	Salkowaski's	++	++	-
Terpenoids	Salkowaski's	++	-	++

Here, (-) sign indicates absence of constituents in the plant.
(+) sign indicates moderate presence of constituents in the plant and
(++) sign indicate high presence of constituents in the plant.

Results of antimicrobial inhibitory activity

In antimicrobial inhibitory activity, the presence of different phytochemical constituents present in these extracts may be responsible for the antimicrobial activity of plants. The most active extract was found to be aqueous extract with the maximum diameter of zone of inhibition against *E.coli* (13mm) followed by methanol extract against *S.aureus* (12mm). The result indicates that the inhibitory activity of *stevia* more towards gram negative *E.coli* and shows the moderate antibacterial activity. The results are shown in Table – 2

Conclusion

From the recent study, it has been concluded that, the methanol extract show significant amount of phytochemical constituents of *Stevia rebaudiana*. So, methanol solvent proved to be the most effective among the others solvents. Since the plant contains maximum amount of bioactive markers compounds, it is reliable to possess large number of medicinal values like antifungal, antibacterial, etc. The results also showed that the aqueous extract of *stevia rebaudiana* had moderate amount of microbial inhibitory activity with the 13mm diameter of zone of inhibition.

Table – 2 Zone of inhibition of the extracts

S. No.	Name	Type of Extract	Zone of inhibition agianst *S.aureus* (mm)	Zone of inhibition against *E.coli* (mm)
1.	Stevia	methanol	12	10
2.	Stevia	hexane	12	10
3.	Stevia	aqueous	10	13
4.	+ve control		34	31

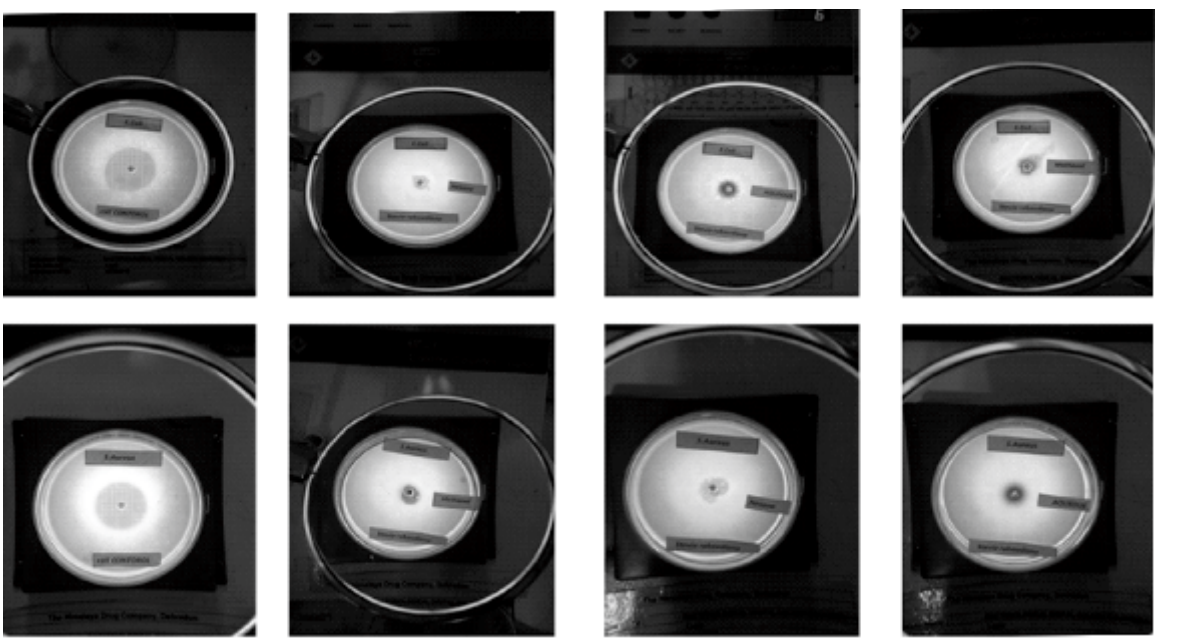

Figure – 1 Zone of inhibition of the 3 extracts of *stevia* leaves

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Comparative Solvent Extraction of *Ocimum sanctum* and *Terminalia arjuna* with their Antimicrobial Inhibitory Activity Towards Intestinal Pathogens

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Abstract-The human intestine is the most preferred location for the thriving pathogens inside the human body. The presence of this complex community of microorganism inside the gastrointestinal tract comes with its own pros and cons, the relationship should not be commensal but more mutualistic between the host and the microbes present. But an overabundance of some bacteria may contribute to inflammatory disorders and diseases like cholera and diarrhoea.

Hence, there arises a need to invest in certain herbal drugs which would have inhibitory properties against intestinal pathogens. The plant species taken for the present study are *Terminalia arjuna* (dried grounded bark) and *Ocimum sanctum* (dried grounded leaves). The study was conducted with the help of extraction of the respective plant parts in the solvents namely acetone, hexane, chloroform, aqueous, methanol and ethanol.

The inhibitory effects of the selected plant extracts were studied against two bacteria, a gram-negative,*E coli* and a gram-positive *Staphylococcus aureus.*The solvent extracts showed higher inhibitory effects against gram-positive bacteria than gram-negative.The leaves of the *Ocimum sanctum* extracted with hexane and acetone have shown more activity against both the gram positive and gram negative bacteria. The methanolic and the ethanolic extracts of *Terminalia arjuna* were found to be more effective against the pathogenic bacterial stain.

Introduction

Gorbach, S.L. stated that the human body is a complex ecosystem containing over 400 species of microorganism in an individual. The presence is found sparsely in the stomach but abundantly in lower bowel of the gastrointestinal tract. Bacteria occur both in the lumen and attached to mucosa but does not penetrate the bowel wall. The upper intestinal tract is characteristically colonized by infectious pathogens like *Vibrio cholera* and *E coli.* Usually, anaerobes like *Streptococci* outnumber the facultative anaerobes like *E coli* by the factor of 1000.These intestinal pathogens contribute their major share in the occurrence of diseases like cholera and diarrhea, which sometimes can cause a heavy toll upon human health. Commonly found intestinal pathogens are Viruses- Rotavirus; Norwalk agent; *Vibrio chloreae; Shigella; E coli; Campylobacterjejumi; Salmonella species; Protozoans.*

Many tree and herb species have important pharmacological properties which can be used to inhibit the microbial activities towards intestinal pathogens. These naturally occurring microbial inhibitors have been recovered from a wide range of floral species. Two species undertaken for the study of their inhibitory activity towards intestinal pathogens are Arjuna and Tulasi.

***Terminalia arjuna*; Vern**.

Arjuna belongs to the family Cobretaceae. *Terminalia arjuna* is a large deciduous tree, commonly found in the greater parts of the country. The bark of the tree is used as a drug.

The bark is available in pieces, flat, curved recurved, channeled to half quilled, 0.2-1.5 cm thick, market sample up to 10 cm in length and up to 7 cm in the width, outer surface smooth and grey, inner surface somewhat fibrous and pinkish, transversely cut smoothened bark shows pinkish surface, fracture, short in inner and laminated in the outer part; taste, bitter and astringent. (API; Part1,vol.2)

***Ocimum sanctum*; Vern**.

Tulsi belongs to the family Lamiaceae. *Ocimum sanctum* is an erect, 30-60 cm high, much branched, an annual herb found throughout the country. Leaves are used in the manufacture of drugs. Leaves are 2.5-5 cm long, 1.6-3.2 cm wide, elliptic-oblong, obtuse or acute,entire or serrate, pubescent on both sides; petiole thin, about 1.5-3 cm long-hairy; odor, aromatic; taste, characteristic. (API; Prt1, vol.2)

With the given rationale, the present study was aimed to screen above the medicinal plants for antibacterial inhibitory activity against *Streptococcus* and *E coli.* The study was conducted by the solvent extraction of the dried grounded bark of *T arjuna* and dried grounded leaves of *Ocimum sanctum*. For the purpose of extraction, six different solvents were used namely hexane, acetone, methanol, aqueous, ethanol and chloroform.

Material and Methods

Collection of Test Material

The dried bark of *arjuna* as well as the dried leaves of Tulsi was provided by the Himalaya drug company, Dehradun (Uttarakhand state, India) and authenticated by pharmacognist. The plant material was crushed, powdered and henceforth used in the solvent extraction.

Preparation of Solvent Extracts

20 grams of the powdered material was extracted with 100 ml of methanol, hexane, ethanol, chloroform, acetone and chloroform water separately for 24 hours. The extracts were then filtered with Whatman filter paper No.1 into a clean conical flask. These conical flasks were then transferred to water-bath having a constant temperature of 80 °C for the evaporation of the solvent. The evaporated sample was then measured and stored in air tight bottles for further use.

Culture Media Preparation

The microbiological media was prepared according to the standard instructions provided by the Himalaya drug company laboratories, Dehradun, Uttrakhand, India. 18.2 grams of nutrient Agar was added to 650 ml of water for the preparation of the media. The media prepared was then transferred to the autoclave for sterilization at 120 °C at 15lbs for 15-30 minutes.

Plate Preparation

After taking the flasks out of the autoclave they were transferred to laminar Air flow chamber to cool down and UV sterilization. 25ml of pre-autoclaved Nutrient Agar was poured in 90mm diameter pre-sterilized Petri dish and was allowed to solidify at room temperature for 10 minutes. Four Petri plates were prepared for each plant extract. The different prepared extracts of the leaves of *Ocimum sanctum* and *Terminalia arjuna* were tested for antimicrobial activity against the tested organism using the well agar diffusion method of Mavarro *et al.* 1996.

Cup or HoleWell Diffusion Method

After solidifying the plate, 24hrs microbial broth culture suspension about 3ml was spread over the nutrient media under aseptic conditions using laminar air flow. Then, one well was made in each plate with the help of the borer of 8mm diameter. In these wells, about 0.1ml of each plant extract were loaded. All tests were made in duplicate sets. This method depends on the diffusion of the plant's extract from the vertical hole through the solidified agar layer of the petri dish to that extent till the growth of the

added microorganism is inhibited in a definite zone around the well containing the extracted solvents.

Incubation

The petri plates were incubated for 24 hours at 37°C in an incubator.

Measurement of the Zone of Inhibition

After the incubation, the diameter of the clear zone produced around the well is measured in mm and diameters of inhibition by the extracted solvents were compared with reference antibiotics.

Results

Observation Tables

Extracts obtained from selected plants were observed for the inhibitory effects against selected bacterial pathogens namely *E coli* and *S. aureus.*The Comparative data of the two plants for their anti microbial inhibitory activity is shown in Table-1 and Table-2

The inference drawn from the data in Table-1 shows that all six solvents except Chloroform and Hexane showed a considerable amount of inhibitory property against both the bacteria.The Ethanolic extract was found to be most effective against *S.aureus* (18mm), followed by E. coli (16mm). The Zone of inhibition zone produced by methanolic, acetonic, aqueous extracts against *S. aureus* was 16mm, 11mm, 15mm respectively while for *E.coli* 15 mm, 10mm, 13mm respectively.

The data of Table-2 concluded that the only the aqueous extract of the Ocimum sanctum was not showing any inhibitory effect against the bacteria from all six solvents. The acetone extract was found to be most effective against *S.aureus* (16mm), followed by E coli (15mm). The inhibition zone produced by methanol, hexane, ethanol, and chloroform against S.

Table-1 Antimicrobial Activity of *Terminaliaarjuna*

S.no.	Name of the sample	Extract type	Zone of inhibition against *S. aureus* (mm)	Zone of inhibition against *E coli*(mm)
1	*Terminaliaarjuna (TE)*	Methanol	16 mm	15 mm
2	*Terminaliaarjuna (TH)*	Hexane	Not detected	Not detected
3	*Terminaliaarjuna (TM)*	Ethanol	18mm	16mm
4	*Terminaliaarjuna (TC)*	Chloroform	Not detected	Not detected
5	*Terminaliaarjuna (TAC)*	Acetone	11 mm	10mm
6	*Terminaliaarjuna (TA)*	Aqueous	15 mm	13mm

Table-2 Anti microbial Activity of *Ocimum sanctum*

S. no.	Name	Type of extract	Zone of inhibition against *S. aureus* (mm)	Zone of inhibition against *E.coli* (mm)
1	Tulsi (TLM)	Methanol	13mm	12 mm
2	Tulsi (TLH)	Hexane	16mm	12mm
3	Tulsi (TLE)	Ethanol	14 mm	11mm
4	Tulsi (TLC)	Chloroform	10mm	10mm
5	Tulsi (TLAC)	Acetone	16mm	15mm
6	Tulsi (TLA)	Aqueous	Not detected	Not detected

aureus was 13mm, 16mm, 14mm, 10mm while for *E coli*was 12mm,12mm, 11mm, 10mm respectively.

Positive control produced a significantly sized inhibition zone against tested bacteria, however, negative control produced no observable inhibitory effect against any bacteria.

Discussion

The extracts of *Ocimum sanctum* and *Terminalia arjuna* were subjected to antimicrobial screening against the pathogens such as *S. aureus* and *E. coli*. Both the medicinal plants were found to posses was equipotent zone of inhibition against the selected pathogens. Kamal Rai Aneja et al. found the similar results against pathogens where ethnomedicinal plants were taken for antimicrobial study.

The bark extract of *Terminalia arjuna* has shown considerable antimicrobial activity against the intestinal pathogens *S.aureus* and *E.coli*. The major difference in the inhibitory property is found in the type of solvent that is used for extraction. The manufacture of drugs if ever so is conducted for the anti-microbial treatment against intestinal pathogens then the solvents considered should be ethanol, methanol and aqueous respectively, on the basis of the diameter of the inhibition zone.

The dried leaf extracts of *Ocimum sanctum* have shown anti-microbial property in almost all the solvents extracts. The hexane and acetone extracts were found to be more effective against *E.coli* and *S.aureus*. These solvents would be preferred for extraction of drugs due to good results against intestinal pathogens.

Conclusion

The results now allow us to conclude that *Ocimum sanctum* has comparatively broad spectrum anti-microbial activity against intestinal pathogens as compared to *Terminalia arjuna*. All tested extracts of *Terminalia arjuna* and *Ocimum sanctum* have shown good activity against both gram +ve and gram –ve bacteria, this explains the indigenous importance of these plants in the drug manufacturing. The antibacterial activities can be enhanced if the active components are purified and adequate dosage determined for proper administration. At last, the need of the hour is the development of an effective compound into an exploitable herbal product devoid of side effects and drug resistance problem.

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Chemical Variability of *Artemisia vulgaris* L. Essential Oil, Dehradun Region of Uttarakhand Himalaya, India

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Abstract-*Artemisia vulgaris* L. growing in Uttarakhand Himalaya has been studied. The wild growing plants were collected from three distinct populations at the full flowering stage in month of July 2017. Oils were extracted by hydro distillation of aerial parts and characterized by GC-MS. α-thujone has been found as the first principal component in population I & II (14.40- 21.66 %), However, in population III, *Artemisia* ketone (29.38%) was determined as a major constituent. All the populations were dominated by monoterpenoids hydrocarbons fractions. *Artemisia* ketone has been reported first time in *Artemisia vulgaris*, which need to be isolated for commercial utilizations.

Keywords: *Artemisia vulgaris,* Essential oil, GC-MS, α-thujone, *Artemisia* ketone

Introduction

The genus *Artemisia* is one of the largest in the Asteraceae family, consisting of more than 800 species which are widespread over the world. Many of *Artemisia species* grow in Eurasia, North and Central America and Northern Africa [1]. *Artemisia vulgaris* L., known as common mugwort, is a pesty weed frequent over the entire country. The plant is perennial, 50–150 cm in height, flowering by August and setting seeds by September. This species grows in open fields, roadsides and waste ground, often forming dense colonies. *A. vulgaris* has been known not only as an edible plant (mostly as a spice) but also as a folk medicine resource. Mugwort essential oils are used for their insecticidal, antimicrobial and anti-parasitical properties[2, 3, 4]. Authors have reported that *A. vulgaris* essential oils have a significant fumigant and repellent effect on *Musca domestica*[5]. The investigations of mugwort extracts indicated a hepatoprotective activity and validated the traditional use of this plant for various liver disorders[6]. In Oriental medicine, *A. vulgaris* has been used as an analgesic agent and in acupuncture therapy[7]. The emmenagogic properties of this plant are related to estrogenic flavonoids[8]. Mugwort leaves and stems contain traces of alkaloids. Essential oils make a major contribution into the plant's biological activity as well. For that reason, the chemical composition of mugwort oils has been investigated in several studies. It has been determined that *A. vulgaris* growing in different European countries is dominated mostly by the monoterpene fraction. German mugwort oil is rich in sabinene (16%), myrcene (14%) and 1, 8-cineole (10%) [9], the oils from Italy contained camphor (47%) alone[10] or camphor (2–20%) together with myrcene (9–70%), 1, 8–cineole (1–27%) or borneol (3–18%) as the major constituents [11]. The amounts of monoterpenes varied in camphor from 1 to 13%, 1, 8-cineole 1–23% and terpinen-4-ol 1– 19% in the leaf oils investigated in France [12]. α-Thujone or thujone

isomer and camphor were determined as the main components in *A. vulgaris* from India[13]. The oils from Morocco were also rich in thujone/isothujone and camphor [14]. Oxygenated monoterpenes (1, 8-cineole, camphor, and α-terpineol) dominated in the essential oils of *A. vulgaris* of Vietnamese origin[15]. The plants cultivated under Indo-gangetic plain conditions produced leaf essential oil with 1,8-cineole (2.2–12.2%), α-thujone (0– 11.4%), camphor (15.7–23.1%) and isoborneol (9.3–20.9%) as predominant compounds, while flower oil rich in camphor (38.7%)[16]. The sesquiterpene fraction dominated in the mugwort oils from Cuba[17], where caryophyllene oxide (31%) was the predominant component, and from Vietnam with β-caryophyllene (24%), β-cubebene (12%) and β-elemene (6%) as the major constituents. The aim of the present study was to explore the chemical composition of *A. vulgaris* essential oils from Uttrakhand region. At present, there is no data on the essential oil composition of these populations.

Material and Methods

Plant material

The flowerings plants of *A. vulgaris* were collected during the full bloom stage from the three population sites in month of July 2017. Tapkeshwar, Rishikesh and Chakrata region from Dehradun district of Uttarakhand, India. Voucher specimens deposited at the University Herbarium, Uttarakhand, after identification by Botanical Survey of India, Dehradun Uttarakhand (India).

Isolation of Essential oil

200 gm aerial parts of sample subjected to hydro-distilled in using Clevenger apparatus for 6 hours. The essential oil was extracted and dried by anhydrous sodium sulphate. Oil was stored in a refrigerator before GC/MS studies till its analysis.

Gas Chromatography-Mass Spectrometry

The gas chromatograph (GC) analyses of the oil samples was carried out by using HP7890 GC manufactured by Agilent equipped with a flame ionization detector (FID) detector and a HP-5 fused silica column (30 m x0.32 mm x0.2µm film thickness). The sample was injected directly into the column. Nitrogen was used as a carrier gas during analysis. The injector and detector temperature was maintained at 210°C and 230°C, respectively. The column oven temperature was programmed from 60°C to 220°C with an increase in the rate of 3°C/min. The injection volume was 0.2µl. The gas chromatography-mass spectroscopy (GC-MS) analysis of the oil was performed out on an Agilent mass spectrometer (Model 5957). The sample was injected directly into the column. Helium was used as the carrier gas (flow rate 1 ml/min). The oven temperature was programmed from 60°C to 220°C at 3°C/min. Other conditions were the same as described under GC. The identity of the constituents of the oils was established on the basis of GC retention indices by comparing their 70 eV mass spectra with those reported in literature[18] and by computer matching with NIST and WILEY libraries as well as where possible by co-injection with authentic compounds available in our laboratory.

Result and Discussions

The essential oil yield of *Artemisia vulgaris* collected from three regions varied from 0.40%-0.50% v/w. Maxiumum oil content was found in Chakrata (0.50%) and minimum in Tapkeshwar, (0.40%). Total 40 constituents were identified in Tapkeshwar region. In both Tapkeshwar, and Rishikesh region, α-thujone (14.40 – 21.66%) was found as the major component, this data set resembles with the previous reports from Nilgiri hills, India[13] and the contradictory reports available on flower oils of Gangotri plane sample that detected camphor as the major component[16]. Crotia samples showed β-thujone[19] and Turkey

Table- 1 Essential oil constituents of *Artemisia vulgaris*

S.N	Components	RI	Percentage		
			Population-I (Tapkeshwar)	**Population-II** (Rishikesh)	**Population-III (Chakrata)**
1.	α-Pinene	926	1.10	0.78	3.51
2.	camphene	950	1.01	0.66	3.57
3.	sabinene	970	2.53	2.45	0.64
4.	β-pinene	982	1.03	0.89	1.82
5.	β-myrcene	987	1.02	0.60	0.18
6.	yomogi alcohol	998	0.60	0.49	5.48
7.	α-terpinene	1014	0.52	0.35	0.31
8.	p-cymene	1017	1.74	2.10	7.60
9.	limonene	1032	1.12	1.01	4.82
10.	1,8 cineole	1027	5.13	4.75	3.44
11.	β-ocimene	1045	0.38	0.15	-
12.	g-terpinene	1053	1.06	0.96	-
13.	*Artemisia* ketone	1062	6.77	8.64	29.38
14.	trans sabinene hydrate	1107	0.96	0.48	1.31
15.	*Artemisia* alcohol	1082	1.80	1.66	-
16.	α -thujone	1098	14.40	21.66	3.10
17.	β-thujone	1108	4.11	4.60	0.74
18.	cis-sabinene hydrate	1074	0.41	0.27	-
19.	chrysanthenone	1267	2.37	1.91	0.86
20.	thujyl alcohol	1291	0.30	0.17	-
21.	trans pinocarveol	1158	0.77	0.67	0.23
22.	camphor	1132	-	-	3.52
23.	myrtenol	1221	0.22	0.48	-
24.	borneol	1170	1.83	1.68	2.21
25.	4-terpineol	1187	3.19	2.82	0.97
26.	thymol	1282	-	-	0.39
27.	α-terpineol	1194	0.39	0.36	0.37
28.	carvone	1249	-	-	0.38
29.	myrtenal	1195	0.32	0.16	-
30.	geraniol	1250	-	0.84	-
31.	chrysanthenyl acetate	1264	0.19	0.23	0.95
32.	geranyl acetate	1361	3.76	3.44	2.31
33.	α -ylangene	1369	0.31	0.21	0.35
34.	β-elemene	1431	-	-	0.15
35.	trans caryophyllene	1421	6.22	6.94	-
36.	trans β-farnesene	1448	0.88	0.61	-
37.	α-humulene	1457	0.78	0.66	-
38.	β-selinene	1483	-	-	0.45
39.	α-murolene	1485	-	-	0.12
40.	germacrene D	1478	8.37	7.42	0.21
41.	α-farnesene	1512	1.67	1.57	-
42.	davanone	1567	0.18	0.15	-
43.	caryophyllene oxide	1581	0.64	0.78	3.68
44.	β-eudesmol	1649	-	-	4.20
	Total identified		**88.14**	**93.30**	**89.09**

oil α- thujone,[20] Bashkortostan and New York samples showed α- pinene[21,22], North Lithuania samples showed Chrysanthenyl acetate, artemesia ketone, 1,8 cineol, β- pinene and sabinene[23] and Iran samples showed Isobornyl isobutyrate, β-pinene as major components[24].

In our investigation, trans-caryophyllene (6.22-6.94%), 1,8-cineole (4.75-5.13%), β-thujone (4.11-4.60%),g-curcumeme (2.82-3.46%), germacrene-D (4.60-9.04%), geranyal acetate (3.44-3.76%), 4- terpineol (2.82- 3.19%) and other components are shown table–1, our samples were collected from three different locations. It was found that in all the locations, the amount of monoterpenoids hydrocarbon was found to be the highest i.e. in Tapkeshwar, (51.75%), in Rishikesh (59.36%) and in Chakrata (56.84%). Oil from Chakrata population showed artemisia ketone, (29.38%) and 1,8 cineol (3.44%) as major components whereas these components were also found in samples collected from Kanditalla and Jhanjhar but in lesser quantity as compared to Chakrata populations. Other components such as trans – carophyllene, α- farnescene, g-curcumeme trans- β farnescene, α- humulene g- terpinene, β-ocimene, myrtenol, geraniol, cis sabinene hydrate and davanone are not detected in the Chakrata population, however camphor (5.23%), thymol (0.39%), β-selinene(0.45%), β-eudesmol (3.0%), calamenene (0.18%) are completely absent in Kanditalla and Jhanjahar populations. A review of the published literature emphasized that, independent of geographical origin, other compounds appear to be always present e.g. sabinene, and myrcene α-humelene, α- muurolene and α- cadinene or β-cubebene [24]. Study recommended that it may be a new chemo type of *Artemisia vulgaris* and need to be further investigated before giving such judgement. The chemical variation of *Artemisia* species populations from different geographical origins may be due to environmental influences, but the existence of chemo variation under the same conditions determined by genetic factors[25].

Conclusion

Our results show that geographical origin of a particular place plays an important role in the chemical composition and constituents of *Artemisia vulgaris* essential oil and suggest that it is difficult to distinguish any chemotype for this species. Study also concluded that the new components (*Artemisia* ketone) reported from population III need to be warranted for further careful investigation of micro and marco habitat features soil nutrients and associated species influence in production of essential oil.

Acknowledgement

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Biological Activity and Phytochemical Analysis of *Roylea cinerea Leaves*

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Abstract-The present study was investigated to determine the antioxidant activity and antibacterial activity of methanolic extract of R. *cinerea* leaves. The result showed the percentage inhibition of the DPPH radical by the methanolic leaf extract of *R. cinerea* and Ascorbic acid at 100 mg/ml was 96.16± 1.10 % and 97.011 ± 0.34% while the IC_{50} values were 29.86 mg/ml and 21.82mg/ ml respectively. The scavenging activity of the methanolicleaf extract of *R. cinerea* was found slightly higher than ascorbic acid. The antibacterial activity of methanolic extract does not show activity against all the tested strain except *Bacillus cereus*.

Keywords: Antioxidant activity, Antibacterial, *Roylea cinerea*

Introduction

Indian system of traditional medicine now getting so much popularity these days and researcher are in continuous search of new therapeutic agents from medicinal plant. Our old scripture such as Sushruta Samhita and Charak Samhita have description of many medicinal herbs which have potential to prevent disease or even cure the disease. People of India and other countries now recognizing the benefits of medicinal herbs. The trend of people to visit Allopathic practitioner has been changed now due to the side effect associated with antibiotic (Yadav*et al*., 2018) and increasing resistance to common antimicrobial agents (Chandra *et al*., 2017)

The medicinal plants from Himalyan region of Garhwal have great input in most of the Ayurvedic preparation. *Roylea cinerea* belongs to family-Lamiaceae which is commonly known as Karui, Titpatti and Patkarru, widely distributed in Himalyan region of India and Nepal. The effect of extract of *R. cinerea* was reported to have good antiplasmodial activity (Dua *et al*., 2011). The ethanolic extract of aerial part of *R. cinerea* have cytotoxic activity SK-Mel 41, U-87 MG, Hela, MDA-MBA-231 cell lines and also reported to have good antioxidant activity (Bahuguna *et al*., 2015). The methanolic extract of aerial part of *Roylea cinerea* was found to exhibit potent antidiabetic activity *In vitro* as well as *In vivo*. The methanolic extract showed maximum reduction (41.20% *In vivo* and 80% *In vitro*) in blood glucose levels. The present study was conducted to evaluate antibacterial and antioxidant potential of methanolic extract of *R. cinerea* leaves.

Material and Methods

Collection and processing of Plant Materials

The leaves of *R. cinerea* were collected from local area of Srinagar Garhwal. The collected leaves was hand sorted and washed 2-3 times with tap water followed by washing with double distilled water. The washed leaves were then shade dried in room temperature.

Preparation of Methanolic extract

The powdered leaves of *R. cinerea* were soaked in 250 mL methanol for overnight with agitation at 1 hour of interval

Phytochemical Evaluation of the Crude Extracts

Phytochemical investigations on *Roylea cinerea* extract samples were performed as described by Trease and Evans (2002); Harborne (1984). The methanolic crude extracts of the different concn. samples were subjected to various chemical tests in order to determine the secondary metabolites present by employing the use of various methods as follows.

Test for Flavonoids

A sample of 1 ml of each of the extracts and a few drops of 10 % ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus.

Test for Amino Acids

A sample of 0.5 ml of each of the extracts was added treated with few drops of ninhydrin reagent, heated in water bath, a purple colour indicated the presence of amino acids.

Test for Alkaloids

A sample of 0.5 ml of each of the extracts was to 0.2 ml of 36.5 % hydrochloric acid and 0.2 ml Dragendroff's reagent. Production of orange precipitate denoted the presence of alkaloids.

Test for Steroids

Sample of 0.5 ml of each of the extracts were evaporated and dissolved in 2 ml chloroform. This was followed by the addition of 2 ml of concentrated sulphuric acid carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of steroids.

Test for Saponins

Sample of 1 ml of each of the extracts was diluted with 2 ml of double distilled water. This was followed by the addition of few drops of olive oil. Formation of soluble emulsion on agitation of the mixture indicated the presence of saponin.

Test for Tannin

Lead acetate test: The extracts were treated with 10% lead acetate and observed for the formation of white precipitate.

Evaluation of Total Phenolic Content

Total phenolic content was evaluated according to the method described by Taga*et al.* (1984). Briefly: A 100 µL of Folin-Ciocalteau reagent (2N wrt acid Fluka Chemic AG-Ch-9470 BUCHS) was added to each sample (20 µL) and well mixed after addition of 1.58 mL of water. After 30 seconds, 300 µL of 2% sodium carbonate solution was added and the sample tubes were left at room temperature for 2 h. The absorbance (A) of the developed blue colour showed the total flavonoids content.

DPPH Radical Scavenging Activity

The DPPH radical scavenging assay is a standard procedure to determine the antioxidant activity of plant extracts. The free radical scavenging activity of methanolic leaf extract was measured using the method described by Blois with some modifications. Methanolic DPPH solution (0.001M) was added to 500 µl of leaf extract at different concentrations (10-100 µg/ml) or to 500 µl of methanol was used as the control. After 30 min, the absorbance was measured at 517 nm . The radical scavenging activity of DPPH was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [A_0 - A_1)/ A_0] \times 100$$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of methanolic leaf extract.

Result

Qualitative phytochemical analysis

Preliminary phytochemical screening of *R. cinerea* extract revealed the presence of various components such as phenolic content, tannins, flavanoid ,saponins, steroids, and alkaloids among which phenolic content, flavanoid and tannins were the most prominent ones and the results are summarized in Table – 1.

The presence of phenols, saponin, flavonoids, alkaloids, steroid and terpenoids were also reported by Sharma *et al.* (2017).

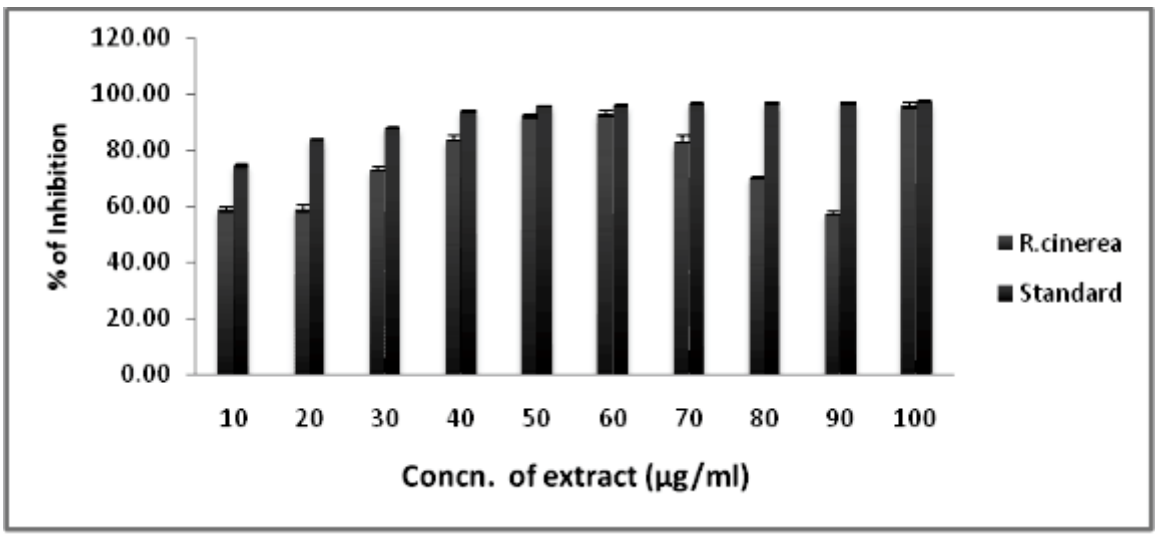
DPPH Free Radical Scavenging Activity

The DPPH assay is a simple method to measure the ability of antioxidants to trapfree radicals. The scavenging effects of the methanolic leaf extract of *R. cinerea* at different concentration(10, 20, 30,40, 50, 60, 70, 80, 90

Table 1–Phytoconstituents in methanolic leaf extract of *R. cinerea.*

Phytoconstituent	Presence/Absence
Flavanoid	+++
Tannin	+ + +
Saponins	+ + +
Phenolic content	+ + +
Alkaloid	+ +
Steroids	+++
Amino acid	+++

Note: +presence, - absence .



Figure– 1 DPPH radical scavenging activity of the Methanolic leaf extract of *R.cinerea* in comparison with standard. Each value represents means ± SD (n=3).

Table – 2. Inhibition of DPPH radical by *R. cinerea* and standards.

Compound	IC50 (mg/ml)
Control	21.82
R.cinerea	29.86

Table – 3 Antibacterial Activity of Methanolic extract of *R. Cinerea*

S.No.	Name of Microorganism	Gram Reaction	Zone of inhibition in mm		
			DMSO	Chloramphenicol	MeOH Extract
1	*Salmonella Typhi*	GNB	NA	26.0±0.0	NA
2	*Pseudomonas aeuroginosa*	GNB	NA	NA	NA
3	*Escherchia coli*	GNB	NA	30.0±0.14	NA
4	*Morganella*	GNB	NA	27.0±0.2	NA
5	*Staphylococcus aureus*	GPB	NA	NA	NA
6	*Bacillus cereus*	GPB	NA	25.0±0.0	15.0 ±0.1

NA- No Activity

and 100 mg/ml) was studied and showed in figure – 1.

The results of DPPH radical scavenging activity of the methnolic leaf extract of *R. cinerea* and the standard Ascorbic acid are presented in figure – 1. The percentage inhibitory activity of free radicals by 50 % has been used widely as a parameter to measure antioxidant activity. In this study, both of the plant extract and the standard ascorbic acid significantly scavenged the DPPH radical with increasing concentrations.

The percentage inhibition of the DPPH radical by the methnolic leaf extract of *R. cinerea* and ascorbic acid at 100 mg/ml was (96.16± 1.10) % and (97.011 ± 0.34)% while the IC_{50}values was 29.86 mg/ml and (21.82mg/ml) respectively. The scavenging activity of the methanolic leaf extract of *R. cinerea* was found slight higher than ascorbic acid .

Sharma *et al*. (2017) investigated the different solvent extract for antioxidant activity and reported that butanol extract have highest antioxidant activity among different extract of aerial parts of R. cinerea

Antibacterial activity of Methanolic extract of *R. cinerea*

The antibacterial activity of methanolic extract of *R. cinerea* was investigated against five uropathogens and one soil isolated. The methanolic extract does not show any activity against all the tested uropathogens. However, it showed significant activity against *B. cereus* i.e. 15.0±0.1 mm.

The literature on antibacterial activity of *R. cinerea* is limited. However, antiprotozoal activity against Plasmodium was reported by Dua*et al*. (2011).

Conclusion

The findings of the present study reported that methanolic leaf extract of *Roylea cinerea* has strong antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stresses inducing diseases which would be beneficial to the human health. This may be related to the high amount of phenolic, flavonoid and tannin compounds present in this plant extract**.**

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About Flowers (Shown on the cover page)

Cocos nucifera*-(*Coconut)

Kingdom:	Plantae - Plants
Subkingdom:	Tracheobionta - Vascular plants
Super division:	Spermatophyta - Seed plants
Division:	Magnoliophyta - Flowering plants
Class:	Liliopsida - Monocotyledons
Subclass:	Arecidae
Order:	Arecales
Family:	Arecaceae - Palm family
Genus:	*Cocos* L. - coconut palm
Species:	*Cocosnucifera* L. - coconut palm

The **coconut tree** (*Cocosnucifera*) is a member of the palm tree family (Arecaceae) and the only living species of the genus*Cocos*. The term "**coconut**" (or the archaic "**coconut**") can refer to the whole **coconut palm**, the seed, or the fruit, which botanically is a drupe, not a nut. The term is derived from the 16th-century Portuguese and Spanish word *coco* meaning "head" or "skull" after the three indentations on the coconut shell that resemble facial features.

Coconut oil contains 2.6 percent fewer calories than other fats. It has been said to provide various health benefits.

Here are a few of them:Increasing "good" cholesterol: A component in coconut oil has been found to give "good" HDL cholesterol "a nudge."Controlling blood sugar: It appears to preserve insulin action and insulin resistance in mice. Reducing stress: It has antistress and antioxidant properties, which could make it useful as an antidepressant, according to research in rodents.Shiny hair: It makes hair shinier, because it penetrates better than mineral oils.Healthy skin: It has been found to enhance

protective barrier functions and have an anti-inflammatory effect on skin in humans.Preventing liver disease: It has reversed hepatosteatosis, a type of fatty liver disease, in rodents.Reducing asthma symptoms: Inhaling coconut oil has helped reduce asthma symptoms in rabbits. Fighting candida: Coconut oil has reduced colonization with *Candida albicans* in mice, suggesting it could be a treatment for candida. Improving satiety: One argument has been that coconut oil leaves people feeling "fuller" after eating, so they will not eat so much. However, other research has shown that this is not the case.Weight loss: It has reduced obesity and promoted weight loss in mice.

***Ananas comosus*-(Pineapple)**

Kingdom:	Plantae - Plants
Subkingdom:	Tracheobionta - Vascular plants
Superdivision:	Spermatophyta - Seed plants
Division:	Magnoliophyta - Flowering plants
Class:	Liliopsida - Monocotyledons
Subclass:	Zingiberidae
Order:	Bromeliales
Family:	Bromeliaceae - Bromeliad family
Genus:	*Ananas* Mill. - pineapple P
Species:	*Ananascomosus* (L.) Merr. - pineapple

The pineapple is a tropical plant with an edible multiple fruit consisting of coalesced berries, also called pineapples, and the most economically significant plant in the family Bromeliaceae.

The **pineapple** (*Ananascomosus*) is a tropical plant with an edible multiple fruit consisting of coalesced berries, also called pineapples, and the most economically significant plant in the family Bromeliaceae. Pineapples may be cultivated from the offset produced at the top of the fruit, possibly flowering in five to ten months and fruiting in the following six months. Pineapples do not ripen significantly after harvest. In 2016, Costa Rica, Brazil, and the Philippines accounted for nearly one-third of the world's production of pineapples.

Fresh pineapple is low in calories. Nonetheless, it is a storehouse for several unique health promoting compounds, minerals, and vitamins that are essential for optimum health.

100 g fruit provides just about 50 calories; equivalent to that of apples. Its flesh contains no saturated fats or cholesterol. Nonetheless, it is a rich source of soluble and insoluble dietary fiber like *pectin*.

Pineapple fruit contains a proteolytic enzyme **bromelain** that digests food by breaking down protein. Bromelain also has anti-inflammatory, anti-clotting and anti-cancer properties. Studies have shown that consumption of pineapple regularly helps fight against arthritis, indigestion and worm infestation.

Fresh pineapple is an excellent source of antioxidant vitamin; **vitamin-C**. 100 g fruit contains 47.8 or 80% of this vitamin. Vitamin-C required for the collagen synthesis in the body. Collagen is the main structural protein in the body required for maintaining the integrity of blood vessels, skin, organs, and bones. Regular consumption of foods rich in vitamin-C helps the body protect from scurvy; develop resistance to combat infectious agents (boosts immunity) and scavenge harmful, pro-inflammatory free radicals from the body.

***Carica papaya*-(Papaya)**

Kingdom:	Plantae - Plants
Subkingdom:	Tracheobionta - Vascular plants
Superdivision:	Spermatophyta - Seed plants
Division:	Magnoliophyta - Flowering plants
Class:	Magnoliopsida - Dicotyledons
Subclass:	Dilleniidae
Order:	Violales
Family:	Caricaceae - Papaya family
Genus:	*Carica* L. - papaya P
Species:	*Caricapapaya* L. - papaya

The **papaya** (from Carib via Spanish), **papaw,** (or **pawpaw** is the plant***Carica papaya***, one of the 22 accepted species in the genus *Carica* of the family Caricaceae. Its origin is in the tropics of the Americas, perhaps from southern Mexico and neighboring Central America.

The possible health benefits of consuming papaya include a reduced risk of heart disease, diabetes, cancer, aiding in digestion, improving blood glucose control in people with diabetes, lowering blood pressure, and improving wound healing.

Papayas are a soft, fleshy fruit that can be used in a wide variety of culinary ways. Here we will explore more on the health benefits, uses, how to incorporate more of them into your diet, and what nutritional value papayas have. Lowers **cholesterol**. Papaya is rich in fibre, **Vitamin C** and **antioxidants** which prevent **cholesterol** build up in your arteries.Helps in **weight loss**..Boosts your **immunity**. Good for diabetics. Great for your eyes.Protects against **arthritis**.**Improves digestion**. Helps ease menstrual **pain.**

Emblica officinalis-Amla

Kingdom:	Plantae - Plants
Subkingdom:	Tracheobionta - Vascular plants
Superdivision:	Spermatophyta - Seed plants
Division:	Magnoliophyta - Flowering plants
Class:	Magnoliopsida - Dicotyledons
Subclass:	Rosidae
Order:	Euphorbiales
Family:	Euphorbiaceae - Spurge family
Genus:	*Phyllanthus* L. - leafflower
Species:	*Phyllanthus emblica* L.

Phyllanthus emblica, also known as emblic, emblicmyrobalan, myrobalan, Indian gooseberry, Malacca tree, or amla from Sanskrit amalaki is a deciduous tree of the family Phyllanthaceae. It has edible fruit, referred to by the same name.

Emblica officinal is Gaertn. or Phyllanthus emblica Linn, commonly known as Indian gooseberry or Amla, is perhaps the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Several parts of the plant are used to treat a variety of diseases, but the most important is the fruit. Many ailments are treated by the fruit which is used either alone or in combination with other plants. These include common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive. E. officinalis possesses antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, cardioprotective, gastroprotective, antianemic, antihypercholesterolemic, wound

healing, antidiarrheal, antiatherosclerotic, hepatoprotective, nephroprotective, and neuroprotective properties as demonstrated in numerous preclinical studies. Furthermore, experimental studies have reported that E. officinalis and some of its phytochemicals also exhibit anticarcinogenic properties. E. officinalis is also reported to possess radiomodulatory, chemomodulatory, chemopreventive, free radical scavenging, antioxidant, anti inflammatory, antimutagenic and immunomodulatory activities. These properties are efficacious in the treatment and prevention of cancer. This review summarizes the results related to these properties and also emphasizes the aspects that warrant future research establishing its activity and utility as a cancer preventive and therapeutic drug in humans.

Forth Coming Events

1. International Conference on Nanobiotechnology for Agriculture 2018 - Detection, conservation and Responsible use of natural resources
   13-14 December 2018, GualPahari, Gurugram
   http://www.teriin.org/events/nanoforagri/

2. HealthMedicon-2019:"International Conference on Advances in Medicine, Technology & Holistic Health"organized by Odisha University of Agriculture and Technology, Bhubaneswar.
Date : 21st and 22nd January, 2019.
http://www.liveayurved.com/2019/upcoming-ayurveda-events-2019.shtml

3. 19th World Congress on Medicinal and Pharmaceutical Chemistry
   January 28-29, 2019 Osaka, Japan
   Theme: Research & Application of Medicinal and Pharmaceutical Chemistry
   https://medicinal-chemistry.pharmaceuticalconferences.com/

4. 30th International Conference on Nutraceuticals and Natural Medicine
   January 28-29, 2019 Osaka, Japan
   Theme: Current Concepts and Prospects of Nutraceuticals and Natural Medicine
   https://nutraceutical.nutritionalconference.com/events-list/pharmacognosy-phytochemistry

5. ICPPP 2019 : 21st International Conference on Phytochemistry and Pharmacy Practice
   February 4-5, 2019 Bangkok, Thailand
   Theme :Phytochemistry and Pharmacy Practice
   https://waset.org/conference/2019/02/bangkok/ICPPP

6. Florida Herbal Conference
   22-24 Feb 2019
   Camp La Llanada, Lake Wales,USA
   https://10times.com/herbal

7. 7th Edition of International Conference on Pharmacognosy and Medicinal Plants
   March 11-12 2019, London,UK
   Theme: Medicinal Plants as A key for Disease Hindrance
   https://pharmacognosy.euroscicon.com/

8. 2nd World Congress on Pharmacology & Chemistry of Natural Compounds
   March 13-14, 2019 London,UK
   Theme: Accelerating Innovations & fostering advances in Natural Drug Research
   https://natural-compounds-chemistry.pulsusconference.com/

9. 19th Global Chemistry Conference
   March 20-21, 2019 New York, USA
   Theme: Chemistry Federation: Frontiers in chemistry and Therapeutic Drugs
   https://globalchem.conferenceseries.com/

10. 10th European Organic Chemistry Congress
March 21-22, 2019 Rome, Italy
Theme: Advanced Perspective in Organic Chemistry and Research Excellence Framework
https://organicchemistry.chemistryconferences.org/

11. 5th World Congress on Medicinal Plants and Natural Products Research
April 15-16, 2019 Hong Kong
Theme: New Frontiers in Transforming Future of Medicinal Plants
https://medicinalplants.pharmaceuticalconferences.com/
https://medicinalplants.pharmaceuticalconferences.com/registration.php

12. 5th International Meeting on Traditional & Alternative Medicine
April 22-23,2019 Rome,Italy
Theme: Exploring new Horizons in Traditional & Alternative Medicine
https://www.meetingsint.com/conferences/traditional-medicine

13. 6th International Conference and Exhibition on Naturals Products and Medicinal Plants Research
June 24-25, 2019 Vienna, Austria
Theme: "Advances and Innovations In Natural Product Research"
https://naturalproducts.pharmaceuticalconferences.com/

14. 5th Global Summit on Herbals and Traditional Medicine
August 14-15, 2019 Auckland, New Zealand
Theme: Unwinding Nature's Recipes for Health
https://herbal.global-summit.com/

15. 2nd World Congress on Traditional and Complementary Medicine
September 16-17,2019 Dubai, UAE
Theme: Understanding the wisdom of Pharmacy and Nature Care, encouraging new innovations in Pharmacy and Traditional Medicine
https://traditionalmedicine.pharmaceuticalconferences.com/events-list/phytochemistry-and-traditional-medicines

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Council Initiative for promotion of reverse pharmacology in Ayurvedic drug development

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

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

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

Dr. Rajendra Dobhal
Director General

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