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Editorial

Geographically India is the seventh largest country in the world. When there was acute shortage of food in the country. Late Prime Minister Lal Bhadur Shastri gave a slogan "*Jai Jawan Jai Kissan*" with this urge country became surplus in wheat production .Today Chandrayaan-II and CARTOSAT-3 an indian satellite launched recently is another success story which narrates potential of India. On the other hand we have to introspect our dependence on China, India today imports from China the equivalent of 6,000 rupees worth of goods for every Indian, which has doubled from 3,000 rupees in 2014. Now, we have to wake up and say "*Jai Ayurveda*".

Ayurveda is getting global acceptance primarily due to its age-old therapeutic potential.Today Ayurvedic or Herbal Nutraceuticals may be used to improve Health, prevent chronic diseases and postpone aging process or any product derived from food sources with extra health benefits and used as nutraceuticals. The Philosophy behind Nutraceuticals 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). 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 2020-2021 which will lead to pharma sector growth.

Iam happy to present December 26th issue of biannual Universities Journal of Phytochemistry and Ayurvedic Heights(UJPAH) released today on this symposium of UJPAH.

Scientific Knowledge now reaching journal, UJPAH is gaining popularity amongst eminent scientists, research scholars, students of the scientific institutions who contribute their research papers, articles reviews on therapeutic properties, pharmacognostic characteristics, Phytochemistry, pharmacology and microbiological aspects etc. of herbs.

My best wishes to all those scientists, Research scholars, students and teachers who contributed for bringing out this issue and also express my sincere gratitude to all board members who make this issue a memorable for science fraternity of the uttarakhand and the people of science at large.

Dr. S. Farooq
Chief Editor

CONTENTS

Characterization of Soil Actinomycetes Against Food Borne Gram-positive and Gram-negative Bacteria

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Abstract-In the present investigation, thirty-five food borne bacteria (fifteen Gram-positive and twenty Gram negative) were isolated from ninety-one food samples. Morphological and biochemical characteristics showed that all isolates belong to *Escherichia, Pseudomonas, Salmonella, Staphylococcus* and *Bacillus* group of bacteria. To check the susceptibility and resistance pattern of these food borne isolates, 57 soil samples were collected from various depths (beneath the upper surface to 1m depth) at different places of Dehradun, Haridwar district, Uttarakhand, India. Seven actinomycetes strains viz, *Streptosporangium* sp. (AI-4), *Actinomycete* (AI-2), *Streptomyces* sp. (AI-18), *Actinomadura* sp. (AI-15), *Nocardia* sp. (AI-19), *Streptosporangium* sp. (AI-21) and *Micromonospora* sp. (AI-24) were identified based on their morphological and biochemical characteristics. Antimicrobial potential of soil Actinomycetes were observed against both food borne Gram-positive and Gram-negative bacteria except extracts of *Micromonospora* sp. (AI-24) where no activity was observed against any food borne isolates. In our study, extracts of Actinomycete AI-2 showed varied zone of inhibitions, ethyl acetate extract was found more active as compared to hexane and methanol extracts. The highest activity in terms of zone of inhibition was observed against Gram-positive isolate *Staphylococcus* sp. The extracts of *Streptosporangium* sp. (AI-4) showed varied activity against food borne bacteria. The ethyl acetate extract and methanol extract showed activity against *Staphylococcus* sp. However, these extracts were found inactive against other food isolates. Hexane extracts and mycelium did not exhibit any sort of antibacterial activity against any food borne isolates. In the case of *Actinomadura* sp. AI-15, the ethyl acetate extract and methanol extract showed activity against *Staphylococcus* sp. Maximum zone of inhibition was observed (22.13 mm) against FBI-17. MIC values of this extract was recorded 512 µg/ml. Ethyl acetate extract of *Streptomyces* sp. AI-18 were found effective against Gram-positive isolates (*Staphylococcus* sp. and *Bacillus* sp.). Methanolic extract of these actinomycetes was found effective against *Staphylococcus* sp. only with maximum zone of inhibition 24 mm. MIC values of both the extracts were recorded in the range of 512-2048 µg/ml. Extracts of *Nocardia* sp. (AI-19) were found inactive against all food borne isolates. In the case of *Streptosporangium* sp. AI-21. only ethyl acetate extract exhibited activity against *Staphylococcus* sp. and *Escherichia* sp. as compared to *Pseudomonas* sp., *Bacillus* sp. and *Salmonella* sp. isolates in which no activity was observed. The MICs of this extract was recorded in the range of 512 µg/ml to 2048 µg/ml. No zone of inhibition was observed when all food borne isolates were tested against the extracts of *Micromonospora* sp. (AI-24). The present results suggest that the soil, sediments and water samples are rich source of actinomycetes, which exhibit a wide spectrum antimicrobial agent.

The study not only targeted food borne isolates but also suggested how soil could be explored for the production of antimicrobial compounds by actinomycetes. This study could be a platform for new drug discovery.

Keywords: Food Borne Bacteria, Soil Actinomycetes, Antimicrobial Compounds and Drug Discovery

Introduction

Actinomycetes are Gram-positive, aerobic and thread like bacteria with high DNA G+C contents, free-living, saprophytic, and abundant in soil, water, and colonizing nodulating plants. Actinomycetes assume a significant job in reusing squanders in the earth and they are additionally the makers of thousands of metabolic items, which display organic action. After the discovery of broad-spectrum antibiotic named *Streptomycin* by Waksman and Schatz, more consideration was paid towards the actinomycetes for detachment of a lot more anti-toxins.

They are tremendous sources of innovating novel microbial compounds with many therapeutic applications and hold a prominent stand due to their ubiquitous and proven ability to produce versatile novel bioactive compounds including antibiotics, immune modulators, anticancer drugs, antiviral drugs, bio pesticides, herbicides, and insecticides. So far, more than 22,000 known microbial secondary metabolites, 70% of which are produced by actinomycetes, 20% from fungi, 7% from *Bacillus* spp. and 1–2% by other bacteria. *Streptomycetes* produce 50–55% antibiotics out of the approximately more than 10,000 known antibiotics from *Actinomycetes* group. Presence of number and types of actinomycetes in soil affected by several factors such as soil type, organic matter content, soil temperature, aeration, moisture content, soil pH and cultivation. Actinomycetes populations are relatively lower than other soil microbes and contain a predominance of *Streptomyces* that are tolerant to acid conditions. They also play an important role in recycling wastes in the environment.

For isolation and characterization of bioactive molecules, several factors must be remembered such as source of screening, initial treatment of samples, growth medium both selective and non-selective, culture conditions and identification of colonies on Petri-plates[1], using pretreatment of soil, drying and heating, stirred the isolation of rare Actinomycetes. Isolation procedure must be more selective by adding certain chemicals[2].

As it is very well known, actinomycetes are most important group of bacteria studied extensively and known to produce a wide range of bioactive compounds with diverse biological activity. Secondary metabolites obtained from various potential strains of actinomycetes are very effective against Gram-positive and Gram-negative bacteria. Many researchers both nationally and internationally isolated this potential group of microorganisms from soil, water, sediment etc. and checked their potential antimicrobial activity. Actinomycetes are also explored against various spp. of Candida albicans and against various pathogenic fungi. Bioactive compounds/antimicrobial compounds/antibacterial agent/molecules discovered from this group of microorganism are used in medicine and agriculture. They are also known to produce branching mycelium of two types first one is substrate and second one is aerial mycelium. They are extensively dispersed in natural and manmade environments, and reported by researchers their important role in the degradation of organic matter. The genus Streptomyces is extensively study for antimicrobial potential and for the production of bioactive molecules (the biggest producer of antibiotics).

Due to the outstanding history of actinomycetes in the production of bioactive molecules for human interest, a large number of effort have been made on the isolation, characterization and

identification from terrestrial sources in the past half-decade. Presently, the rate of exploring new compounds from soil actinomycetes has fallen down, whereas the amount of isolating commercialized bioactive molecule has inflated. Thus, new strain of actinomycetes must be isolated from unexplored or underexploited habitats for the development of innovated antimicrobial compounds for the benefits of society and humankind.

Amongst all Actinomycetes strains, Streptomyces remains unique producer of antibiotic. Streptomyces had produced 500 species of this genus, which have been reported to produce antimicrobials[3]. This may be because as for non-Streptomycetes species requires new and innovative techniques for isolation from soil, sediment and other natural environment. In addition to this, their complex conservation and cultural procedures, which commonly need some precise and scarce conditions. This is the reason these organisms are called rare organisms. Most of the species recovered on plates containing selective media through basic conventional isolation techniques (serial dilution, pour plate, spread plated and pre and post-enrichment using highly selective media). Non-Streptomycetes or Rare actinomycetes have observed, whose occurrence is minor than that of strains of Streptomycete. This had increased physiology, habitat and efficiency of bioactive compounds from rare actinomycetes. Ecologically important potential of actinomycetes expected worth which lead to screen sources to develop into unusual surroundings.

Actinomycetes are explored for diverse group of antimicrobial compounds notably polyenes, polyketides, aminoglycosides glycopeptides, beta-lactams, macrolides, Actinomycins and tetracyclines. Most of the antibiotics (approximately two third of the naturally occurring) are obtained from extracts/fractions of Streptomycetes. The Streptomyces species isolated from various natural environments showed moderate to strong antibacterial and antifungal activities. *Streptomyces* sp. strain US80 isolated from Tunisian oasis soil, investigated for antifungal and antibacterial substances[4]. The isolate was evaluated against various bacteria. The results showed that US80 is the only defined strain, which yields all these three active molecules at once. Similarly, Coringa mangrove forest soils from Andhra Pradesh were studied to isolate actinomycetes for enzyme production and antimicrobial activity[5]. Terrestrial samples from Western Ghat, forest rock, Tamil Nadu, India were analyzed for the isolation, characterization and antimicrobial activity of ERI-3, a Streptomyces spp.[6]. Thirty-seven soil samples were investigated for isolation of species of Actinomycetes from agriculture field in Vengodu, District Thiruvannamalai, Tamil Nadu, India. Jeffrey et al[7] assessed the Malaysian soil for the isolation, characterization and antimicrobial potential enzymatic activities. The investigated report by Gurung et al have focused on antimicrobial potential of extracts of actinomycetes isolated from soils of Kalapatthar11[8] Mount Everest. El-khawaga et al[9] have reported the antibacterial and insecticidal potential of crude extract of twenty Actinomycetes isolated from sandy soil of Cairo, Egypt. Marine actinomycetes are also a significant source of antimicrobials as the environmental conditions of the sea are completely diverse from the terrestrial conditions[10]. Many interrogated research have also obtained novel antibiotics/ antimicrobials/ crude extracts from the marine environment[11]. Mohanraj et al[12] explored actinomycetes isolated from marine sediments for their potential to produce bioactive molecules to combat the disease caused by pathogenic microorganisms. Five isolates were selected for antimicrobial spectrum of ethyl acetate extract. Minimum inhibitory concentration of active

extract was also determined. Study concluded with the statement that naturally occurring actinomycetes have a potential to produce antimicrobial compounds against dermatophytes supporting the discovery of new antibiotics[13].

Although abundance of antibiotics has been obtained from actinomycetes, these represent only a pie of fraction of the repertoire of bioactive compounds produced. Therefore, there is an urgent need for isolation of new species from natural resources and their characterization for secondary metabolites is a valuable endeavor. A few investigations have been done so far using Dehradun and Haridwar, Uttarakhand soils, sediments and lake water to screen for novel actinomycetes for new bioactive molecules. Present research was designed and investigated to study antibacterial activity of isolates of actinomycetes against food borne bacterial isolates.

Antimicrobial compounds isolated for Actinomycetes

Actinomycetes are extensively studied by national and international researchers for the isolation of bioactive compounds with diverse biological activity. Great amount of antimicrobial molecules have been screened and characterized. Numerous lead molecules from this collection of bacteria have developed a status as potential antimicrobial agents in medical field because of their antimicrobial effect against pathogenic microorganisms[14]. Arasu et al[15] 2013 discovered polyketide antibiotic from the culture of Gram-positive bacteria named *Streptomyces* sp. AP-123 isolated from marine environment in Andra Pradesh, India. The isolates exhibited varied antimicrobial potential. 64.3% (against Gram-positive bacteria) 48.5 % (against Gram-negative bacteria) and 38.8% against both group of bacteria while 80.85% activity observed against fungi. The microorganisms studied for

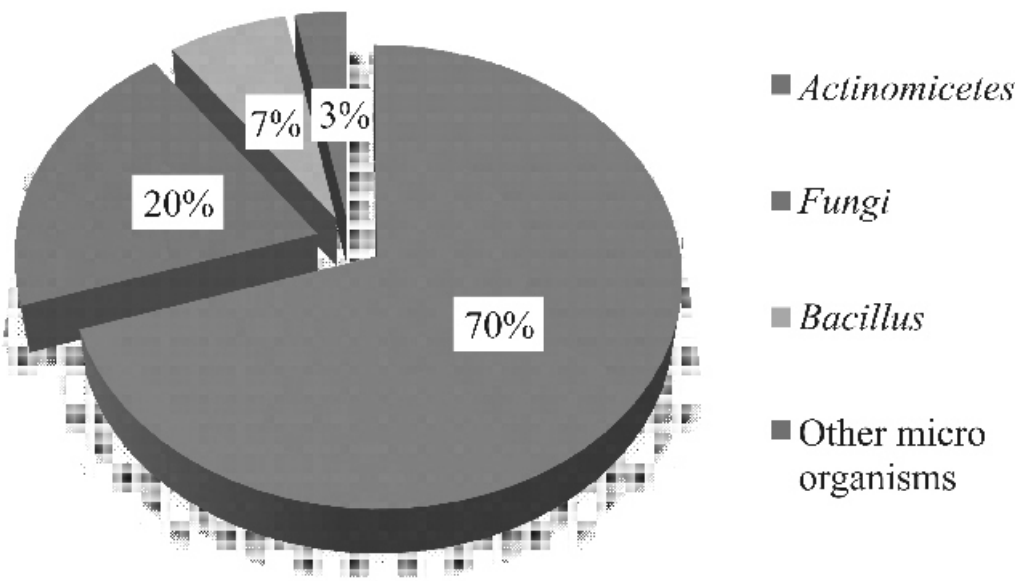

Fig.-1 Bio-active Compounds Isolated from Microorganisms

the production of antibiotics were from the groups of *E. faecalis* ATCC 29212, *B. subtilis* MTCC 441, *S. aureus* ATCC 25923, , *E. coli* ATCC 25922, *S. epidermidis* MTCC 3615, *K. pneumoniae* ATCC 15380, *P. aeruginosa* ATCC 27853, *P. vulgaris* MTCC 1771, *Erwinia* sp. MTCC 2760, *T. rubrum* 57/01, *T. simii* 110/02, *Scopulariopsis* sp. 101/01, *T. rubrum* MTCC 296, *T. mentagrophytes* 66/01, *E. loccosum* 73/01, *A. niger* MTCC 1344, *B. cereus, C. albicans* MTCC 227 and *C. lunata* 46/01.

and twenty Gram-negative bacteria isolated from food samples. Antibacterial activity of all actinomycetes isolates were observed against both food borne Gram-positive and Gram-negative bacteria except extracts of *Micromonospora* sp. (AI-24) where no activity were observed against any food borne isolates. Extracts of Actinomycete AI-2 showed varied zone of inhibitions and ethyl acetate extract was found to be more active as compared to hexane and methanol extracts. The highest activity in terms of zone of inhibition (24.10 mm) was

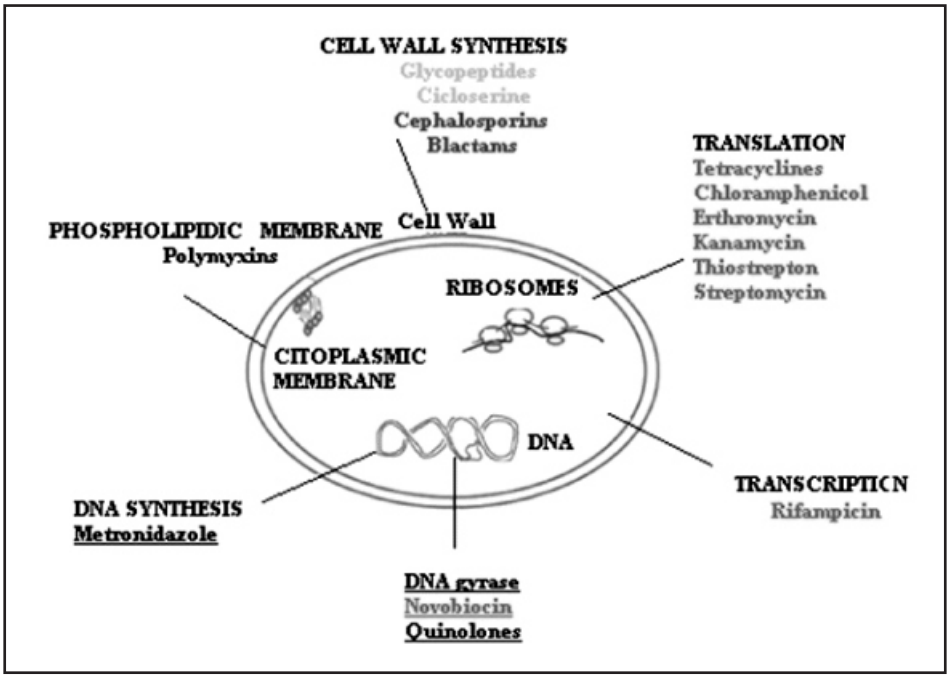

Fig.-2 Principal Target of Antibiotics Produced by *Actinomycetes*[16]

Conclusion

Seven spp. of actinomycetes namely *Streptosporangium* sp. (AI-4), *Actinomycete* (AI-2), *Streptomyces* sp. (AI-18), *Actinomadura* sp. (AI-15), *Nocardia* sp. (AI-19), *Streptosporangium* (AI-21) and *Micromonospora* sp. (AI-24) were identified. All actinomycetes isolates were tested for their activity pattern against fifteen Gram-positive observed against Gram-positive *Staphylococcus* (FBI-11). The extracts of *Streptosporangium* sp., (AI-4) showed varied activity against food borne bacteria. The ethyl acetate extract and methanol extract showed activity against *Staphylococcus* isolates. However, these extracts were found inactive against other food isolates (*Escherichia* spp. FBI-1 to FBI-10, *Salmonella* spp. FBI-18 to

FBI-21, *Pseudomonas* spp. FBI-22 to FBI-27, and *Bacillus* spp. FBI-28 to FBI-35. Hexane extracts and mycelium did not exhibit any sort of antibacterial activity against any food borne isolates. MIC values of ethyl acetate extracts of *Streptosporangium* sp., (AI-4) were recorded in the range of 512 µg/ml to more than 2048 µg/ml against *Staphylococcus* isolates. However, MIC values of methanol extract were recorded more than 2048 µg/mL against FBI-11 to FBI-16 except against FBI-17 with 2048 µg/mL MIC values. No any zone of inhibition was observed when pure solvents were analyzed for antibacterial assay. In the case of *Actinomadura* sp. AI-15, the ethyl acetate extract and methanol extract showed activity against *Staphylococcus*. Maximum zone of inhibition was observed 22.13 mm against FBI-17. MIC values of this extract were recorded 512 µg/mL. Ethyl acetate extract of *Streptomyces* sp. AI-18 were found effective against Gram-positive isolates *Staphylococcus* and *Bacillus*. Methanolic extract of these actinomycetes was found effective against *Staphylococcus* spp. only with maximum zone of inhibition 24mm. MIC values of both the extract were recorded in the range of 512-2048 µg/mL. Extracts of *Nocardia* sp. (AI-19) were found inactive against all food borne isolates. In the case of *Streptosporangium* AI-21, only ethyl acetate extract exhibited activity against *Staphylococcus* spp. and *Escherichia* spp. compared to *Pseudomonas* spp., *Bacillus* spp. and *Salmonella* spp. in which no activity was observed. Ethyl acetate extracts were found more active as compared to methanolic, hexane extracts. Maximum antibacterial activity was shown against *Staphylococcus* followed by *Escherichia* spp., *Bacillus* spp., *Pseudomonas* spp. and *Salmonella* spp. was found to be least susceptible against all tested actinomycetes extracts. In comparison with the standard ciprofloxacin, all the extracts exhibited low antibacterial activity. The above findings obtained indicate a spectrum of antimicrobial activity that show that actinomycetes have a potential use in pharmaceutical sciences.

Recommendation

Further investigations are needed to identify the food borne bacterial isolates and actinomycetes isolates at molecular level. The active ethyl acetate and methanolic crude extract of Actinomycetes isolates may possibly be taken up further to identify the active principle by various techniques like GC-MS, LC-MS, IR and NMR, the efficacy of such active compound may also be tested with animal model. Thus more intensive screening of Actinomycetes from extreme to be done as to overcome chemically synthesized drugs. The study not only targeted food borne isolates but also suggested how soil could be explored for the production of antimicrobial compounds by actinomycetes. This study could be a platform for new drug discovery.

Acknowledgement

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Pimenta racemosa Essential Oil Found Stronger Inhibitory Effect than Antibiotics

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Abstract-The primary objective of this comparative study was to assess the antibacterial effect of essential oil of an Indian herb *Pimenta racemosa* with some synergistic antibiotic drugs against *Escherichia coli* and *Staphylococcus aureus*. The essential oil was obtained using Clevenger apparatus. The antibacterial activities of essential oil was evaluated using the disk diffusion method. The inhibitory zones were recorded in millimeters. The minimal inhibitory concentration (MIC) of the essential oil against *E. coli*, *Staph. aureus* and *Pseudomonas aeruginosa* were assessed using microdilution method. The synergistic effect between plants and antibiotics was assessed using disc diffusion method. Our results indicate the possibility of using essential oil in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects *in vivo*. These results revealed the importance of plant essential oil when associated with antibiotic drugs in control of bacteria.

Keywords: Essential oil; Antimicrobial activity; Minimum inhibitory concentration

Introduction

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semisynthetic derivatives of natural products and used in traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products.

In the present time, drug resistance in microbes is a very serious problem. Hence, plant origin herbal medicines are considered as safe alternatives of synthetic drugs. Currently, Aurveda is considered as a vital system of medicine and governs the worldwide recognition and having non-toxic substances.

However, newly discovered non-antibiotic substances such as certain essential oils (Sonboli *et al*. 2006) and their constituent chemicals (Chavan *et al*. 2006) have shown good fighting potential against drug resistant pathogens (Cowan, 1999; Ahmad and Beg, 2001).

Essential oils are aromatic oily liquids which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots by steam distillation. Scientifically, these oils have been proved highly potent antimicrobial agents in comparison to antibiotics. These plant essential oils are rich source of scents and used in food preservation and aromatherapy.

These possess multiple antimicrobial i.e., antibacterial (Ozcan *et al*. 2006), antifungal (Cafarchia *et al*. 2002), anticancer, antiviral and antioxidant properties (Salehi *et al*. 2005; Vardar-Unlu *et al*. 2003), against viruses, bacteria and fungi (Kalemba and Kunicka, 2003).

Pimenta racemosa (Mill.) J. W. Moore. (syn. *Pimenta acris* Kostel,) is cultivated for the production of essential oil exploited in industry. The oil is commonly called "bay oil" or "Myrcia oil". This spice is used to aromatize the food (Leung et Foster, 1996)**.** The culinary uses are the same as those of the "bay-tree sauce" (*Laurus nobilis*). A decoction of bark, taken out of infusion, is used against hypertension. The essential oil extracted from the leaves presents the disinfectants and astringent properties; it is moderately toxic by oral way because of its high

percentage of phenol, but as a matter of principle it does not give place to allergic reactions at the man (Opdyke, 1973). It is used in the manufacturing of creams, lotions, detergents, or in the shampoos. It is also used in perfumery (Opdyke, 1973) like febrifuge (Ayedoun *et al.* 1996). It possesses anti-inflammatory and analgesics properties (Duke, 1986; Robineau, 1991). Essential oil intervenes in the patented formula of a capillary cosmetic composition (Orenga, 2003). The most asserted biological properties are the antimicrobial effects which are explained by the high percentage of phenols. In the present study antimicrobial potential of essential oil was screened against pathogenic bacterial strains.For antimicrobial susceptibility of essential oil MIC values and growth inhibition zone diameters were determined.

Material and Methods

Extraction of essential oils

Essential oils used in this study were extracted from Pimenta *racemosa*. These were separately grounded and powdered in domestic mixi and hydrodistilled in a Clevenger's apparatus by the technique of Guenther (1948) to obtained essential oils. Before application, solubility of essential oil was tested. Dilution was made by adding fresh solvents and stored at 5ºC till used.

Bacterial cultures

Cultures of of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923*),* were maintained in SCD Broth (2% w/v) a constant at 37ºC in the laboratory. For inoculation, a portion (100: 1) of overnight culture of each bacterial strain was mixed in 15 ml of media for each test and control separately. For activity testing, bacterial cultures were stored at 4ºC and sub cultured after every 8th day in solid agar plates.

Screening of antibacterial activity

Antimicrobial activity of essential oils on bacterial growth was accessed. For this purpose, essential oils were diluted by using serial micro dilution .In each test the essential oils were added to fresh suspension after making serial dilution. The essential oil was assayed for antibacterial activity in triplicate. Before conducting experiments, all the conditions were standardized to determine MIC and MBC values *in vitro.*

Filter paper disc diffusion assay

Agar disc diffusion method was used for screening of antimicrobial activity of essential oil of *Pimenta racemosa*. For antimicrobial activity testing essential oil was diluted by adding equal volume of solvent. From this a known volume of essential oil was coated on separate sterile filter paper discs (Whatman No. 1) measuring 6 mm in size. These oil-impregnated discs were made dry under laminar flow cabinet. Bacterial inoculum was spread evenly on to the surface of each agar plate with sterile rubber pad spreader and essential oil coated discs were positioned in the centre of inoculated agar plate. The essential oil was assayed in triplicate. Sterile distilled water was used as negative control, while broad-spectrum antibiotics i.e. Nitrofurantoin, Methicillin and Cefixime were used as positive control for obtaining comparative results. All treated and untreated plates were incubated for 24 h at 37 ºC and size of inhibition zone diameters surrounding filter paper disc was measured. For determination of Minimum Bactericidal Concentration (MBC) growth inhibitory assays were performed. For this purpose, inoculum size was adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml) in sterile agar plates. Both test and control cultures were kept at 37ºC for 24 h. For comparison, both negative and positive controls were set and bacterial colony number was counted. The least concentration at which no visible growth was obtained in agar plates was considered as MBC. For evaluation of inhibition two parallel controls were set and bacterial growth was obtained in presence and absence of various quantities of essential oils.

Results and Discussion

Determination of MIC and MBC values

The MIC value of essential oils are presented in Table-1. The essential oil of the leaves of *Pimenta racemosa* almost has an antimicrobial activity very interesting against *Staphylococcus aureus* ATCC 25923. MIC = (0.30 ± 0.02) mg/mL and E.coli and *Pseudomonas aeruginosa*(2.24 ± 0.13) mg/mL respectively, an average activity against the three others microbial stocks.

Inhibition zone diameter

In the present study, effectiveness of *P.racemosa* essential oil was also confirmed by filter paper disc diffusion assay and growth inhibitions zone diameters was measured. Results are presented in Table-2. The essential oil have shown larger growth inhibition zone diameters in comparison to synthetic antibiotics (Plate-1) against *Staphylococcus aureus*. The essential oil has shown 33 mm inhibition zone diameter against *Staph aureus* followed by *E.coli and Pseudomonas aeruginosa*.

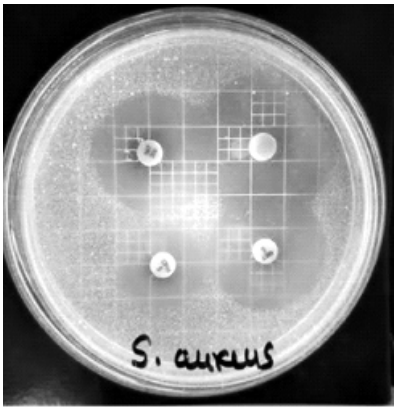

Plate-1 Antimicrobial activity of *Pimenta racemosa* essential oil against *Staph. aureus*

Present study reveals the antimicrobial susceptibility of essential oils against *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa*.. It is proved by low MIC and MBC values obtained in essential oils when used against each bacterial culture. Lowest MIC values (0.30 mg/mL) against *Staphylococcus aureus.*

Further effectiveness of essential oil was determined by agar disc diffusion method and inhibition zone diameters were measured. (Plate-1). Based on growth inhibition zone diameters obtained bacterial strains were divided in to three categories i.e. resistant (>7 mm), intermediate (>12 mm), and susceptible (>18 mm). As in the present study, inhibition zone diameters were obtained more than 18 mm in size, which are significantly much larger than the antibiotic drugs and proves susceptibility of essential oil. In the present study, essential oil have shown strong antimicrobial effects on gram positive than gram-negative bacteria in suspension culture. However, inhibition zone diameters obtained in filter paper disc diffusion assays have shown better effectiveness of essential oil against Gram-positive bacteria. It may be due to volatile action of essential oil and due to absence of lipo-polysaccharide layer in Gram positive bacteria that might function as an effective barrier against any incoming bio-molecule (Inouye *et al*. 2001; Delaquis *et al*. 2002).

Table-1 MIC of Essential Oil of *Pimenta racemosa*

S.No.	Microbial cultures	Minimum Inhibitory Concentration (MIC) (mg/mL)
1.0.	*Escherichia coli* ATCC 25922	2.24 ± 0.13
2.0.	*Staphylococcus aureus* ATCC 25923	0.30 ± 0.02
3.0.	*Pseudomonas aeruginosa*	2.24 ± 0.13

Table-2 Antimicrobial Activity of *Pimenta racemosa* Essential Oil Against Test Organisms

S.No.	Microbial cultures	Diameter of zone of inhibition(mm) *Pimenta racemosa* essential oil	Diameter of zone of inhibition(mm) (Antibiotics)		
			Nitrofurantoin	Methicillin	Cefixime
1.0.	*Escherichia coli* ATCC 25922	28	20	18	20
2.0.	*Staphylococcus aureus* ATCC 25923	33	29	28	19
3.0.	*Pseudomonas aeruginosa*	23	19	16	20

Conclusion

The present study clearly indicates the antibacterial properties of *Pimenta racemosa* essential oil against human pathogens. It has been observed that the essential oil possesses both bacteriostatic and bactericidal activity much stronger than that of synthetic antibiotics when tested *in vitro*.

Hence, this essential oil can be recommended for therapeutic purposes and be used as an alternative medicine after toxicolological analysis and clinical studies.

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Phytochemical Analysis and Evaluation of Antimicrobial Activity of *Berberis aristata* Root Extract

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Abstract-The study was endeavored to observe the phytochemical evaluation and antimicrobial activity of methanolic extract of roots of *Berberis aristata.* Phytochemical analysis of the root extract showed the presence of vital phyto-constiteunts namely alkaloids, carbohydrates, phenolic and tannins while saponins, sterols and triterpenoids were found to be absent. DPPH (1, 1-diphenyl-2-picrylhydrazyl) was used to evaluate the antioxidant activity of the methanolic extract. Soxhlet apparatus was used for extract preparation from the crushed roots *B. aristata* using methanol by hot percolation.The antimicrobial activity of root extract was determined by combined principle of dilution method with that of disk diffusion method at different concentrations (40, 20, 10, 5 and 2.5 mg/ml) to calculate the MIC value. The methanolic extract of root showed antimicrobial activity against drug resistant bacterial strains viz, *Bacillus cereus, Escherichia coli, Pseudomonas fragi, Staphylococcus aureus, and fungal strains of Microsporum audouinii, Microsporum canis and Trichophyton mentgraphytes.*

Keywords: *Berberis aristata*, DPPH (1, 1-diphenyl-2-picrylhydrazyl), Phytochemical screening, Anti-microbial activity, MIC, Methanolic extract

Introduction

Berberis aristata, otherwise known as Indian Berberry or Tree Turmeric, belongs to Berberidaceae family. Around 450-500 deciduous evergreen shrubs are comprised in this genus. *Berberis aristata* in local vernacular is known as chitra and daruhaldhi which is abundantly distributed from the Himalayas to hilly regions of Nepal, Bhutan and Sri Lanka[1-2]. The height of the plant is about 2000-3000m predominantly in Chamba region of Himachal Pradesh and Kumaon region of Uttarakhand. *Berberis aristata* has a high content of an alkaloid i.e. Berberine, a quaternary isoquinoline alkaloid as a major phytoconstituent yielding about 2.23% followed by palamatine. It is mainly found in stem, bark and root parts of the plant. The presence of berberine in this plant is mainly due to the moisture content of soil and potassium. Rasaut/Rashut, a beneficial Ayurveda prepared from this plant is used for curing various diseases such asmeningitis, onychomycosis, ophthalmic, pneumonia, jaundice and skin diseases[3-5]. Ayurveda as a traditional system is used in a wide range of applications for improving and maintaining individual health and well-being. Ayurvedic health care is mainly focused on restoring the uniformity of mental, physical and emotional state of the patients, thereby improving the health status. Thus, it not only cures but averts various diseases and ailments. Herbal medicines are plant-derived components which are in humongous demand for human healthcare in the present developing world[6]. *B. aristata* fruits are consumed by native inhabitants for healing several diseases. It is also an adequate source for dye which is widely used for dyeing fabric and leather[7]. *Berberis aristata* is used to treat all sort of diseases related to ENT, vaginal disorders, indigestion and dysentery[8]. It

is often used in the formulation of polyherbal for treating diarrhea[9, 10] and eye diseases[11]. Phytochemicals namely chlorogenic acid, quercetin, E-caffeic acid, rutin and meratin are present in the flowers of *Berberis aristata*[12]. In Chinese medicine, this plant is used to increase the count of platelets and white blood cells after chemo-cancer therapy and the fruit part is used for the preparation of wines and jellies. The metabolic dysfunction in type-2 diabetes is characterized by hyperglycemia[13, 14]. Considering this, former studies suggested that Berberine can improve glucose tolerance in mice and rat models and are able to reduce body weight[15]. In some literature of previous reports, have shown that Berberine can possibly increase glucose-stimulated insulin secretion[16]. The fruit of the plant can also be used as food additive[17]. Current studies indicate that Berberine can trigger stimulation of glucose metabolism via glycolytic pathway, which then inhibits glucose oxidation in mitochondria[18]. However, in some ongoing research, it has indicated that Berberine can also inhibit Tricarboxylic acid cycle pathways (Krebs cycle) [19-21]. Other investigation of this particular alkaloid has also revealed that it improves the function of insulin[22] and reduces insulin resistance[23].

Material and Methods

Collection of material and preparation of root extract

Berberis aristata plant material (root) was collected from Chamoli, Uttarakhand, India.The roots were thoroughly washed with running tap water and dried in shade at room temperature (24-26°C). The powder component of the plant material was passed through a 40-mesh sieve and stowed in a closed vessel until use.

The air-dried roots of *Berberis aristata* were crushed. Soxhlet apparatus was used for extraction of crushed roots with methanol by hot percolation. The residue was obtain only after the extract evaporated till dryness.

Phytochemical analysis of the root extract

Berberis aristata

B. aristata roots

Number of tests, for phytochemical analysis of root extract, were performed to detect the presence of vital chemical components like alkaloids, carbohydrates, phenols, tannins, proteins, amino acids, sterols and triterpenoids.

Test for alkaloids

1ml of solvent extract was mixed with 2 to 3 drops of dil. HCl and then filtered it. The following color test were done from the filtrate obtained.

i. Mayer's test

a. In 60 ml distilled water, mercuric chloride weighed 1.36 gm was dissolved.

b. In 20 ml distilled water, 5 gms of potassium iodide was dissolved.

Both (a) and (b) were mixed to a volume of 100 ml with distilled water. With Mayer's reagents, cream color precipitate demonstrated the presence of alkaloids.

ii. Wagner's test: 1.27gm of iodine with 2gm of potassium iodide was dissolved in 5ml of distilled water and volume was made up to 100 ml of with distilled water. Reddish brown precipitate appeared with Wagner's reagent showed the presence of alkaloids.

iii. Hager's test: 2-3ml of extract with 20ml of saturated picric acid solution was mixed. Appearance of yellow color was observed showing the presence of alkaloids.

Detection for carbohydrates and glycosides

i. Molisch's test: 10gm of alpha naphthol was weighed and dissolved in 100ml of 95% alcohol. With this solution, extract was tested and 0.2ml of conc. sulphuric was poured along the sides of the test tube. At the junction of two liquids violet or purple colour appeared.

ii. Benedict's test: Few drops of Benedict's reagent were mixed with the test solution and boiled in water bath. Presence of reducing sugars was observed as reddish brown precipitate.

Test for sterols and triterpenoids

Salkowski test: Few drops of conc. Sulphuric acid were added to the extract. The mixture thus obtained was mixed by shaking and then allowed to stand for some time. Red color appearance in the lower layer indicated the presence of steroids and the appearance of yellow colour in the lower layer indicatesd the presence of triterpenoids.

Test for proteins and amino acids

Ninhydrin test: 1gm Ninhydrin (indane l, 2, 3 trione hydrate) was dissolved in n-butanol and the final volume was made up to 100ml. With this solution, the extract was treated and on boiling violet color was observed.

Test for saponins

Foam test: 1ml of extract was diluted in 20ml distilled water and continuously shaken in a graduated cylinder for about 15minutes.The presence of saponins is indicated by the formation of one centimeter layer of foam.

Test for tannins and Phenolic compounds

i. Ferric chloride test: Ferric chloride solution was used to test the extract. If condensed tannins are present green color appears and presence of hydrolysable tannin was indicated by appearance of blue color.

ii. Vanillin hydrochloride test: Dissolved 1gm vanillin in 10ml alcohol and 10ml concentrated HCl solution. Solution prepared was used to treat the extract. Appearance of red or pink color indicated the presence of tannins and phenolic compounds.

Antioxidant and Anti-microbial activity of the extract

Preparation of DPPH

DPPH is an extremely oxidisable compound. It gets easily oxidized in the presence of light, so preparation of DPPH takes place in a dark

room and 0.0019gm was weighed accurately, which was then dissolved in 50ml of methanol.

Preparation of standard Ascorbic acid solution

Ascorbic acid is a strong anti-oxidizing agent. It is taken as standard. 200µg/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg/ml standard solutions of ascorbic acid were prepared.

Preparation of different concentration of *Berberis aristata* roots extract

Different concentration of the test sample *Berberis aristata* roots extract which was examined for antioxidant was prepared. *viz.* 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml.

Preparation of test sample

1ml of different concentration of test sample *Berberis aristata* extract was added in 3ml of DPPH solution in dark.

Preparation of standard

1ml of different concentrations of standard solutions of ascorbic acid was added in 3ml of DPPH solution in dark.

Incubation

For half an hour the prepared solutions of ascorbic acid and test samples were incubated at room temperature.

Measurement of absorbance

After incubation, absorbance was recorded at 517nm with the help of U.V. spectrophotometer,.

Calculation

The % activity of individual concentration of the extract was calculated from the following formula:-

$$\%\text{Activity} = \frac{\text{Abs. of control} - \text{Abs. of individual concentration}}{\text{Abs of Control}} \times 100$$

Abs. = Absorbance.

Antimicrobial activity of root extract

The microbial strains used in this study were provided by department of Microbiology and Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Manduwala, Dehradun.The bacterial strains were maintained on nutrient agar slants at first and incubated at 37°C for about 18-24 hours whereas the fungal strains were maintained Saubouraud Dextrose Agar at 25°C for about 32-48 hours and both the cultures were stored at 4°C as stock for antimicrobial activity.

Results

Table-1 Anti-bacterial activity of Berberisaristata roots extract of various solutions

Extract solutions (mg/ml)	Bacteria (Zone of Inhibition in mm)			
	B. cereus	*E. coli*	*P. fragi*	*S. aureus*
40	19	18	18	15.6
20	15	12.6	13	13
10	13.3	8.3	11.3	10.6
5	10.6	Resistant	Resistant	9
2.5	7	Resistant	Resistant	Resistant

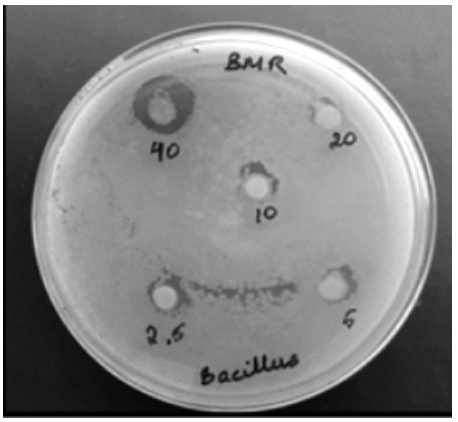

Fig.-1 *B. cereus*

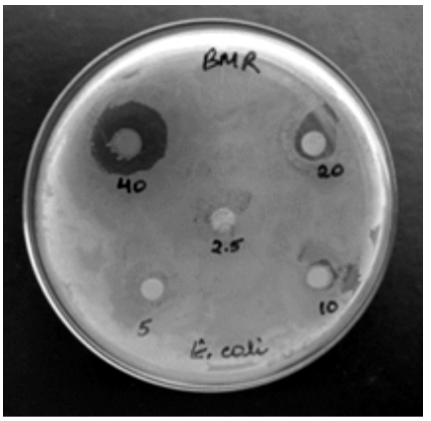

Fig.-2 *E. coli*

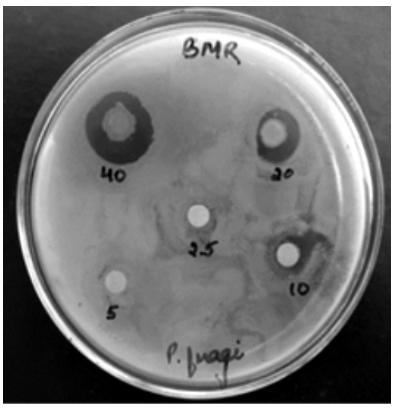

Fig.-3 *P. Fragi*

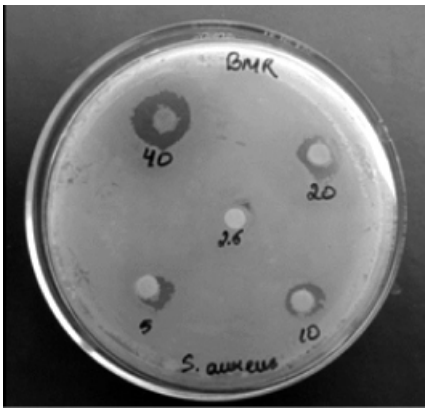

Fig.-4 *S.aureus*

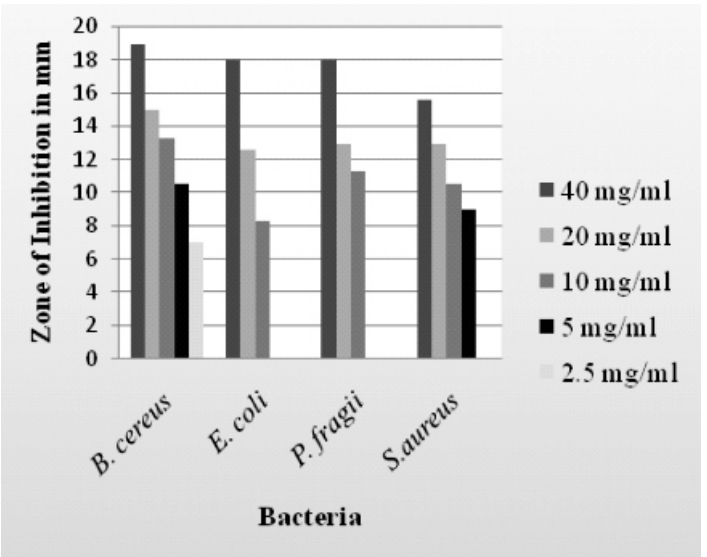

Fig.-5 Graph of antibacterial activity of *Berberisaristata* roots extract

Table-2 Anti-fungal activity of *Berberisaristata* roots extract of various solutions

Extract solutions (mg/ml)	Fungus (Zone of Inhibition in mm)		
	Microsporum audouinii	*Microsporum canis*	*Trichophyton mentagrophytes*
40	18.3	20.3	22
20	10.8	18.6	19.3
10	9.3	17	13.6
5	Resistant	15.3	8.6
2.5	Resistant	15	Resistant

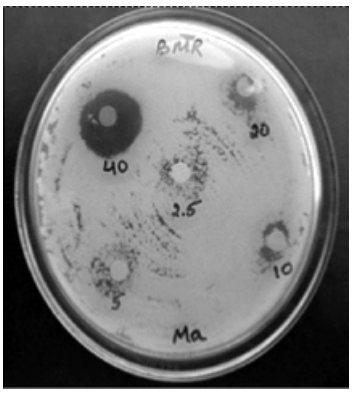

Fig.-6 *Microsporum audouinii*

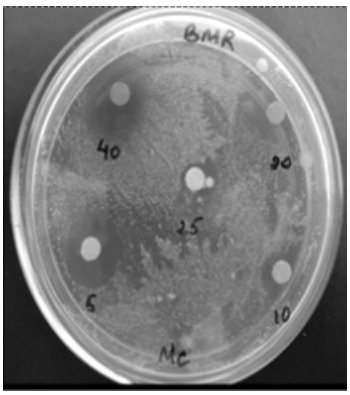

Fig.-7 *Microsporum canis*

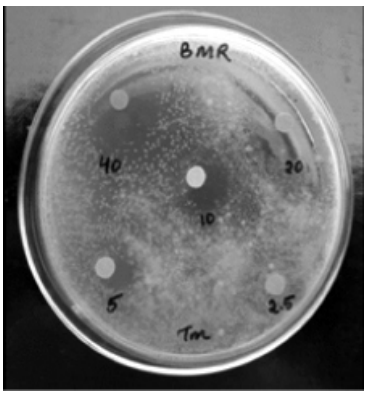

Fig.-8 *Trichophytonmentagrophytes*

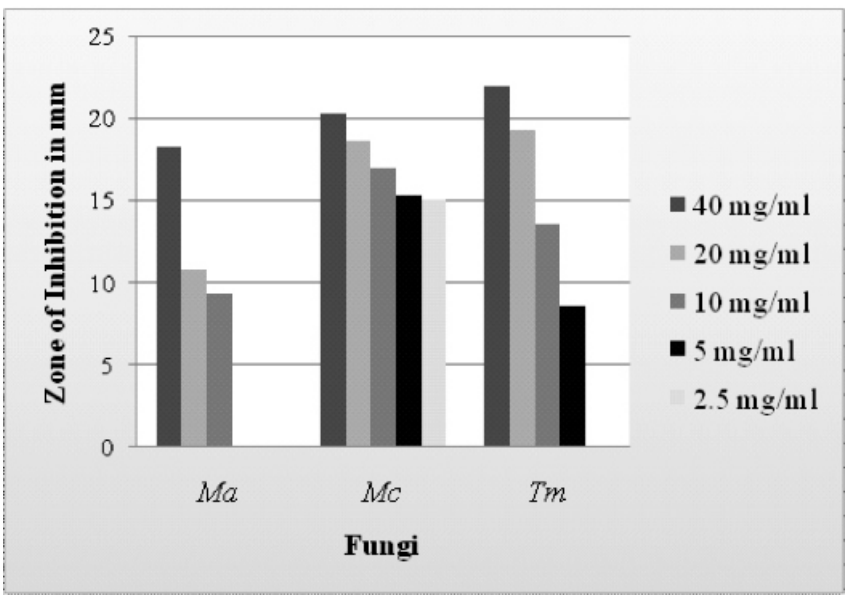

Fig.-9 Graph of Anti-fungal Activity of *Berberis aristata* Roots Extract

Antioxidant Activity

Table-3 Absorbance table of Ascorbic acid at 517nm

S. No.	Concentration (µg/ml)	Ascorbic acid	% antioxidant activity
1	**Control**	0.52684	
2	200	0.022877	95.65
3	400	0.021917	95.83
4	600	0.021908	95.84
5	800	0.020347	96.13
6	1000	0.019937	96.21

Table - 4 Absorbance table of *Berberis aristata* roots extract at 517nm

S. No.	Concentration (µg/ml)	Methanol extract	% antioxidant activity
1	**Control**	0.52684	
2	200	0.33797	35.84
3	400	0.216888	58.83
4	600	0.166365	68.42
5	800	0.110391	79.04
6	1000	0.081023	84.62

Table-5 Qualitative Phytochemical Analysis of *Berberis aristata* roots extract

Test performed	Test for Alkaloids			
Methanol Extract	Mayer's test	Hager's test	Wagner's test	Dragendroff's
	+	+	+	-
	Test for Carbohydrates			
	Fehling's test	Molisch's test	Benedict's test	Barfoed's test
	-	+	+	-
	Test for Phenolic compounds and Tannins			
	Vanillin HCl test	Ferric Chloride test	Lead Acetate test	
	+	+	-	
	Test for Proteins and Amino acids			
	Ninhydrin test	Biuret test		
	-	-		
	Test for Saponins			
	Foam test	-		
	Test for Sterols and Triterpenoids			
	Salkowski test	-		

Results and Discussion

Methanolic extract of roots of *B. aristata* showed antimicrobial activity against the selected bacterial and fungal cultures at different concentrations of 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml with inhibition zone of 7 mm against *Bacillus cereus*, 8.3 mm against *Escherichia coli*, 11.3 mm against *Pseudomonas fragi* and 9 mm against *Staphylococcus aureus* likewise it showed inhibition zone of 9.3 mm against *Microsporum audouinii,* 15 mm against *Microsporum canis* and 8.6 mm against *Trichophyton mentagrophytes* fungi respectively.

This shows the methanolic extract of roots of *Berberis aristata* from Chamoli Garhwal, Himalayan region possess high potential to be used as antimicrobial agent against above microbes. The diseases caused by the bacteria under study are cholecystitis (inflammation of gall bladder), bacteremia, osteomyelitis (inflammation of bone), cholangitis (infection of biliary tract), and urinary tract infection (UTI) which are caused by *Escherichia coli* whereas diseases like fatal non-gastrointestinal-tract infections, eye infections, meningitis, pneumonia, and endophthalmitis are caused by *Bacillus cereus*. The bacteria *Staphylococcus aureus* is responsible for gastrointestinal tract infections, mastitis (inflammation of breast) in dairy cows and cellulitis while *Pseudomonas fragi* causes bacteremia, lung infections and folliculitis. The fungi under study are the root cause of the many diseases such as *Trichophyton mentagrophytes* causes tinea pedis (athlete's foot), toe web or vesicular(blister like) infections and can manifest as ringworm and the fungi *Microsporum canis* causes infections on the body and scalp and also lead to high inflammatory lesions related with hair loss and onychomycosis (nail infection) whereas *Microsporum audouinii* causes tinea capitis(scalp ringworm) and tinea corporis (infection of arms and legs). Antimicrobial study reveals that the plant selected for study, *Berberis aristata* is capable of inhibiting the growth of pathogens even at a very low concentration, showing its high potency for medicinal use.

This study suggests that the roots of *Berberis aristata* can therefore be utilized in perparing various antibacterial, antifungal and antioxidant drugs, supplements, creams, lotions and applicants. *Berberis aristata* is a natural source of antimicrobial and antioxidant agents which makes it economical and easily available to the public, for the benefit of common man. Thus, the present study holds value as a novel and beneficial work which can be further extended to explore the putative active phyto-constiteunts and their potential targets for therapeutic purpose against various diseases to make the use of natural agents instead of artificial sources.

Conclusion

The methanolic root extract of *Berberis aristata* have prominent antimicrobial properties and phytochemical constituents. Since the plant has been reported to have a major plant alkaloid known as berberine, so the amount of alkaloids are expected to be high. Also, the extract exhibits good antioxidant activity comparable to that of ascorbic acid taken as standard. Therapeutic potential of this plant can be further explored as an alternative treatment for diverse types of diseases.

Acknowledgement

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Beneficial Effects of HiOwna on Haematological Parameters of Charles Foster Rats

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Abstract-A new nutraceutical-HiOwna prepared by The Himalaya Drug Company was observed to have significant effect on haematological parameters of Charles Foster rats. It also caused remarkable immunomodulatory effects in the tested animals in our experiment.

Introduction

HiOwna is a nutritional health supplement for adults which promotes overall health and well being . It contains a combination of herbal ingredients, macro and micro-nutrients that bridge essential nutritional gaps. Macronutrients provide energy, promote growth and development and regulate body functions, while micronutrients meet the additional nutritional demands at various physiological stages. HiOwna is beneficial for reviving physical capacity in convalescing and elderly patients , increasing the fatigue and improving immunity. Many plants were observed to possess antioxidant activity (Agbor *etal*., 2012 ; Jayashree *et.al*., 2003; Shivananjappa and Joshi, 2012).

In our studies, we have seen the effect of nutraceutical HiOwna at different doses on haematological parameters-Haemoglobin, Total Red Blood Count (T-RBC), Haematocrit, Platelet Count and Total Leukocyte Count (TLC) of Charles Foster rats.

Material and Methods

Test Animals

Fourty Charles Foster rats of both the sexes were obtained from National Laboratory Animal Center, Central Drug Research Institute, Lucknow. They were allowed to acclimate for seven days prior to experimentation. Animals were kept in a controlled environment at the temperature of 22 + 2 0C and 30 – 50 / relative humidity with a 12 hour light and dark cycle. The rats were fed a standard rodent pellet diet and ad libitum water. These studies were conducted according to the regulations of Institutional Animal Ethics Committee of Central Drug Research Institute, Lucknow.

Experimental Procedure

Test animals were divided into four groups of 10 animals (5 males and 5 females) in each group. The dose groups were as follows:-

Group I Control- Distilled Water- 10ml / Kg Body Weight

Group II – 500 mg / Kg Body Weight HiOwna in Distilled Water

Group III -1,000 mg / Kg Body Weight HiOwna in Distilled Water

Group IV-2,000 mg / Kg Body Weight HiOwna in Distilled Water

All the animals of Groups II, III and IV were given HiOwna daily for 56 days and I was given Distilled water only for the entire period of experimentation. Body weight, food and water intakes were recorded at weekly intervals. Haematological parameters- Haemoglobin, Total Red Blood Cell Count, Haematocrit,Platelet Count and Total Leukocyte Count were done at two

weekly intervals by using MS 9 Fully Automated Haematology Analyzer.

Observations and Results

1. **Body Weight:** There was significant increase in body weight of all the HiOwna fed groups of rats as compared to control and it was dose related.
2. **Food and Water Intakes:** Food and water intakes in all the treated rats were well comparable to control and within physiological limits of normalcy.
3. **Haematological Parameters**

A. Haemoglobin

Haemoglobin values increased in all the rats of HiOwna fed groups as compared to control and it was dose related. Maximum increase was in Group IV and minimum was in Group II.

B. Total Red Blood Cell Count

Haematinic effect of HiOwna was seen in all the rats which was evident by increase in count of erythrocytes.

C. Haematocrit

It was evident that HiOwna caused increases in haematocrit values of all the animals which was dose related.

D. Platelet Coun

Increases in platelet counts were seen in all the treated groups of animals and it was dose related establishing increase in count effect of nutraceutical in our experiment.

E. Total Leukocyte Count

Remarkable increase in total leukocyte count was observed in all the treated groups of rats as compared to control . This has established that our product is very useful and may help in improvement of immunity of the body defense system.

Discussion

The chief constituents of HiOwna are *Eleusine coracana*, *Centella asiatica*, *Emblica officinalis* and *Piper nigrum*. These plant parts and active ingredients isolated from these have shown immunomodulatory activity, antioxidant activity, cytopritective activity, cognitive and memory enhancing activity (Anturlikar *etal*., 2013).

It was observed that HiOwna, a polyherbal health drink supplement was effective in accelerating postoperative recovery . There was improvement in postoperative parameter like Haemoglobin, WBC count and time taken for complete recovery. Also , there was significant gain in body weight (Roy and Rugvedi, 2012). In another experiment, it was concluded that HiOwna Jr given in addition to regular balanced diet helps to maintain adequate natural linear growth, enhanced immunity and favorably modified cognition in children in children (Palani *etal*., 2012).

In our experiment, HiOwna fed rats were healthy and active through the period of experiment and also, no adverse effect was observed in any of the animals. Haematinic effect was very well seen with evidence of haematological parameters. Thus, HiOwna is a very promising nutraceutical and is recommended for human use in anaemic patients.

Conclusion

In our experiments, HiOwna showed very promising haematinic effects in all the rats of treated groups. This was evident by increases in haematological parameters.

Remarkable increase in platelet counts and total leukocyte counts were also recorded.

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Antioxidant Activity of *Carica papaya* Leaf and *Tinospora cordifolia* Stem Extract

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Abstract-Antioxidants play a major role in protecting the body against oxidative stress that is associated with many chronic diseases and disorders including chronic wounds. Plants are the richest source for antioxidants and are effective in the management of oxidative stress caused by free radical damage.The aim of this study is to carry out the antioxidant activity of papaya (*Carica papaya*) and Giloe (*Tinospora cordifolia*) and known in Ayurveda for their use in dengue fever treatment.Methanol extract was tested for its antioxidant activity. Different concentrations of *Carica papaya* leaf extracts (Wild Species) and *Tinospora cordifolia* stem were prepared and allowed to stand for 30 minutes at room temperature. The antioxidant activity was studied at 517 nm by DPPH free radical scavenging activity. Our results showed that the methanol extract of both the plants are able to extract compounds having high antioxidant activity, even when compared to ascorbic acid. The TLC-bioautography using DPPH as a detection reagent, indicated the major components responsible for the strong antioxidant activity.The results suggest that methanolic extract of *Carica papaya* leaf and *T.cordifolia* stem have potent antioxidant activity and could be explored as a novel natural antioxidant.

Keywords: Antioxidant, Bioautography, DPPH, *Carica papaya*,*T.cordifolia*

Introduction

Humans generate knowledge and develop technologies. With them, they synthesize a wide variety of compounds to improve their quality of life. Such compounds (e.g. drugs or insecticides), although solve health problems, cause damage both to the consumers and to the environment. However, humans, in order to solve health and environmental problems, have turned their attention to the use of natural alternatives, such as plant extracts. Plant extracts having the advantage of having a biological origin, are biodegradable and demonstrate a positive impact on human health and the environment (Bravo et al., 2000).

Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. Antioxidants are the substances which inhibit oxidation, which have the ability to remove the potentially damaging oxidizing agents in a living organism. Many phytochemicals present in the plants are able to reduce or prevent the oxidative damage to the human cells which can cause even cancer in humans. Free radicals are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Free radicals promote beneficial oxidation that produces energy and kills bacterial invaders. In excess, however, they produce harmful oxidation that can damage cell membranes and cell contents. Each plant contains hundreds of phytochemicals (plant chemicals) whose presence is dictated by hereditary factors. Only well-designed long-term research can determine whether any of these chemicals, taken in a pill, would be useful for preventing any disease.

Papaya *(Carica papaya)* belongs to the family of Caricaceae, and several species of Caricaceae have been used as medication against a variety

of diseases (Mello et al., 2008). *Carica papaya* is a constant plant and it is presently distributed over the whole tropical area. All parts of the papaya plant can be used as medicine; the fruit flesh, flowers, seeds and the flowers. Many scientific investigations have been conducted to evaluate the biological activities of various part of *C. papaya* including their fruits, shoots, leaves, rinds, seeds, roots or latex. Now a days papaya leaves are widely known as a natural cure for dengue fever. Research has proved that the papaya leaves juice can increase the platelets count of people diagnosed with dengue fever. *Carica papaya* leaf extract increases the platelet count in dengue fever without any side effect and prevents the complication of thrombocytopenia. So, it can be used in dengue fever with thrombocytopenia patients. (Gadhwal, A.K. et al., 2016).

Guduchi *(Tinospora cordifolia)* is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, antiperiodic, antispasmodic, anti-inflammatory, antiarthritic, antiallergic and antidiabetic properties(*Nadkarni, K.M. et al.,* 1976). The roots of this plant are known for its anti-stress, anti-leprotic and antimalarial activities. Earlier studies reported the antiosteoporetic,hepatoprotective, immunomodulatory (*Kapur, P. et al., 2008)*, properties of *Tinospora cordifolia*. Keeping in view the above importance of the plant, present study was undertaken to determine the antioxidant activity of *Carica papaya* and*Tinospora cordifolia* stem.

Material and methods

Plant material

The mature leaves of *Carica papaya* and stem of *Tinospora cordifolia* was collected from the garden of The Himalaya Drug company, Dehradun. The collected material was kept in polythene bags which were subsequently sealed to protect from dust and microbes. The collected plant material was brought to the laboratory and stored in a refrigerator. The stored material was thoroughly washed with tap water followed by sterilized distilled water. After cleaning, the plant material was dried in shade and grinded into powdered form for further analysis.

Extraction

Organic extract was prepared by soxhlet extraction method. A thimble was prepared by using cotton as a filter paper. About 100gm of powdered material was uniformly packed into a thimble and run in soxhlet extractor. *Carica papaya* leaf and *Tinospora cordifolia* stem was exhaustible extracted with methanol (Merck) for the period of about 48 hours or till the solvent in the siphon tube of extractor become colourless. After that, extracts were filtered with the help of filter paper (Whatman No.1) and solvent was evaporated using water wath at 70-80^{0}C to get the syrupy consistency. Then after, the extract was kept in refrigerator at 4ºC to determine antioxidant activity.

Antioxidant assay

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of (Brand-Williams et al., 1995) with modifications. The following concentrations of extracts were prepared 50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL, 250µg/mL, 300µg/mL and 350µg/mL. A stock solution of the sample (50mg/ml) was diluted for 5 concentrations. Each concentration was tested in duplicate. The portion of sample solution (0.5ml) was mixed with 3.0ml of 0.2mM 1, 1-Diphenyl-2-2picrylhydrazyl (DPPH, in 95% distilled ethanol) and allowed to stand at room temperature for 30 minutes under light protection. The absorbance was measured at 517nm which was the scavenging activity of the samples at corresponded intensity of quenching DPPH. Lower the absorbance of the

reaction mixture indicates higher free radical scavenging activity. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation:

Inhibition % = Ac-As/Ac×100

where Ac is the absorbance of the control, as is the absorbance of the sample

TLC/HPTLC fingerprinting

TLC finger printing profile was done for methanol extract to find out the nature of compounds present. The solvent system used was chloroform: methanol (90:10). Test solution was applied on a precoated silica gel 60 F254 TLC plate and run in the previously saturated solvent system. After development, the plates are visualized at UV254nm and UV366nm.

TLC DPPH bio-autography for Antioxidant activity

The filtrate (methanolic extract) were loaded on to the activated Silica gel G (Merck) plate. The antioxidant compounds were separated using solvent mixture of Chloroform: methanol (90:10 v/v). Once the run is completed, plates were air dried for 15 min and the plates were sprayed by 0.2mM DPPH solution in methanol using a spray gun for 5 secs. 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 confirm the antioxidant molecule. The Rf value of the samples were calculated (Rumzhum, N.N. et al., 2012).

Results and Discussion

All the concentrations of the test solution more or less scavenged the free radicals. Both the leaf and stem extracts showed effective scavenging activity against free radicals (Table-1). The methanolic extract of the leaf of *C.papaya* and stem of *T. cordifolia* displayed an excellent activity against the free radicals. The methanolic extract of *Carica papaya* leaf showed the highest scavenging activity (77.99%) at 350µg/ml and lowest (45.11%) at 50µg/ml (Table-1).

The methanolic stem extract of *T.cordifolia* showed the highest scavenging activity (76.02%) at 350µg/ml and lowest (39.44%) at 50µg/ml compared to the ethanolic extract (Table-2).

DPPH accepts hydrogen radical and an electron to become stable diamagnetic molecule and it is a stable free radical at room temperature (Sores, J.R., 1997), with absorption maximum band

Table-1 Antioxidant Activity of Methanolic Extract of *Carica papaya* Leaves.

Concentration (µg/ml)	Plant extract (%) inhibition	Standard Ascorbic aciod (%) inhibition
50	45.11	49.01
100	59.35	69.23
150	65.49	76.99
200	74.23	84.19
250	75.49	88.27
300	76.49	89.54
350	77.99	89.77
IC_{50}	73.14µg/mL	55.23µg/mL

Table-2 Percentage Scavenging Activity of Methanolic Extract of Stem of *Tinosporacordifolia.*

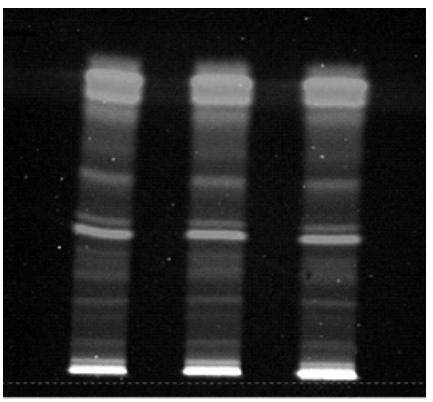
Concentration (µg/ml)	Plant extract (%) inhibition	Standard Ascorbic acid (%) inhibition
50	39.44	49.01
100	56.29	69.23
150	64.92	76.99
200	70.22	84.19
250	75.12	88.27
300	75.42	89.54
350	76.02	89.77
IC_{50}	79.27µg/mL	55.23µg/mL

around 517nm and thus, it is a useful reagent for evaluation of the antioxidant activity of compounds (Sanchez, C., 2002). In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenyl picryl hydrazine, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants. The DPPH anti-oxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of anti-oxidant (Ara, N. 2009).

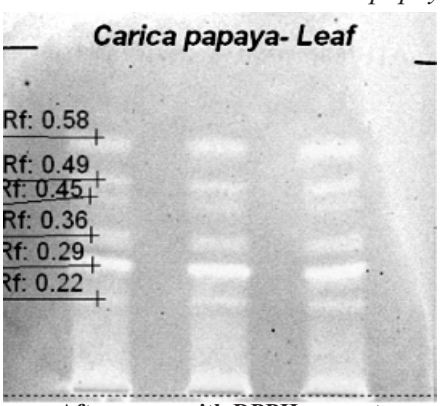
In the present study, it was observed that the methanolic extract of both the plant possessed stronger antioxidant (75-76%). In the DPPH test, antioxidants were typically characterized by their IC_{50} value (Inhibition Concentration of Sample required to scavenge 50% of DPPH radicals).IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. The IC_{50} value for methanolic extract of C*arica papaya* was found to be 73.14µg/mL and the IC_{50} value for methanolic extract of *Tinospora cordifolia* was found to be 79.27µg/mL.

A great number of TLC techniques have been developed and successfully applied for qualitative analysis of antioxidants, and the stable free radical 2,2-diphenyl-1picrylhydrazyl (DPPH) was often used as a derivatization reagent for this purpose (Zhao,J. et al.2010).TLC confirmed the presence of more components extracted in methanol extract using mobile phase (chloroform: methanol= 90:10).Fig.-1 and Fig.-2.

Antioxidants bands for *Carica papaya* leaf

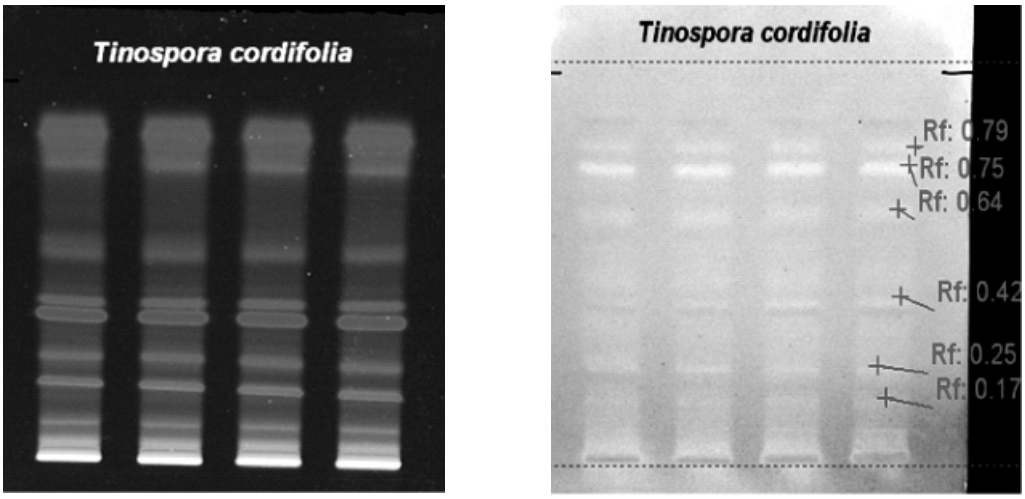


At UV 366nm



After spray with DPPH reagent

Fig.- 1 Antioxidant TLC Bioautography of *Carica papaya* Leaf



At UV 366nm **After spray with DPPH reagent**

Fig.-2 Antioxidant TLC Bioautography of *Tinospora cordifoliastem*

were observed in the TLC plate exposed to DPPH at different Rf values (0.22, 0.29, 0.36,0.45,0.49 and 0.58) Fig-1. Antioxidants bands for *Tinospora cordifolia* stem were observed in the TLC plate exposed to DPPH at different Rf values (0.17, 0.25,0.42,0.64,0.75 and 0.79) Fig.-2. The compounds present in both the plants are antioxidants which act as free radical scavengers.The very strong antioxidant activity of extracts was related to many bioactive compounds as antioxidant within extract may act synergistically. Studies showed a significant improvement in platelet counts. This beneficial effect of aqueous extract of *T. cordifolia* suggests that it probably acts by improving the production of platelets from bone marrow due to its antioxidant and immunomodulatory properties. This beneficial role of *T. cordifolia*. can be attributed to the presence of various phytochemical constituents like alkaloids, sesquiterpenoids and glycosides present in the stem(Chauhan, et al., 2016). *Carica papaya* leaf juice also has beneficial effect in thrombocytopenia associated with dengue (Panda, 2018).

Conclusion

The present study revealed the antioxidant activity of *Carica papaya* leaves and stem of *Tinospora cordifolia*.

Reports on *Carica papaya* and *Tinospora cordifolia* claimed significant improvement in platelet counts. This beneficial effect of aqueous extract of *T. cordifolia* and *Carica papya* suggests that it probably acts by improving the production of platelets from bone marrow due to its antioxidant and immunomodulatory properties.The study and reports further support the use of Platenza, the product of The Himalaya Drug Company in cases of dangue and thrombocytopenia.

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Evaluation of the Antibacterial Activity of Commonly Used Hand Sanitizers on Common Pathogenic Bacteria.

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Abstract-Human Hands are primary source of transmission of microorganisms and infections.The primary hygiene of hand is least expensive and simple means of preventing contagious diseases and hospital acquired infection. Pharmaceutical Industries producing hand hygiene products have given a solution in form of sanitary, antimicrobial, alcohol based hand sanitizers to destroy these microorganisms.

Four different sanitizers viz. A®, B®, C®, D® used for hand hygiene from three different manufacturers were incorporated in this study. The test organisms used were common pathogenic bacteria responsible for day to day infections and hospital acquired infection. The antibacterial activity of hand sanitizers on pathogenic bacteria was carried out using the well agar diffusion method.

Hand sanitizer brand A® was observed as the broad spectrum and strong antimicrobial agent, followed by brand C®. Brand A was the most potent inhibiting all the test pathogens with very wide diameter of zone of inhibitions. Other sanitizer brands B® and C® were also effective against all test bacteria, while the effectiveness of D® were poorest as indicated by minimal activity against the tested microorganisms.

Keywords*:* Disinfectants, Antiseptics, Sanitizers, Hand Hygiene.

Introduction

Hands are primary mode of transmission of microbes and have been considered to be the most important cause of hospital acquired infection (Nystrom, 1994 and Reybrouck 1983). The Center for Disease Control and Prevention (CDC) estimates that approximately 2 million people acquire Hospital Acquired Infections each year and that approximately 90,000 of these patients die as a result of their infections (Zerr et al, 2005). Companies producing hygiene products have offered a solution in form of sanitary, antimicrobial, alcohol based hand sanitizers to destroy microbes responsible for HAI*. Due to its antimicrobial properties, alcohol is used as the main antibacterial component of most waterless antiseptic agents (Boyce & Pittet, 2002). Alcohols have a nonspecific mode of action, consisting mainly of denaturation and coagulation of proteins. Cells are lysed (Isquith and Chesbro, 1963), and the cellular metabolism is disrupted (McDonnell and Russell, 1999). Hand sanitizers are alcohol-containing preparations designed for application to the hands for reducing the number of viable microorganisms. Various preparations of hand sanitizers are available including gel, foam and liquid solutions. Active ingredients of hand sanitizers include isopropanol, ethanol, n-propanol or providone-iodine while the inactive ingredients usually include a thickening agent (such as polyacrylic acid for gels), humectants (such as glycerin for liquid rubs) or propylene glycol and essential oils of plants like lemongrass, tea tree All spice oil, lavender oil, Cinnamon oil and Tulsi oil etc. This investigation have been undertaken to evaluate the inhibitory activity of different herbal and commonly used alcohol based hand sanitizers on some selected common pathogenic bacteria.

Material and Methods

Test organisms

The test organisms used were ATCC strains *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and Salmonella *sp*.

Hand sanitizers

Four different sanitizers used for hand hygiene from three different manufacturers were included in this study. These sanitizers included the following: A®, B®, C® and D®.

Determination of antibacterial activity

The agar well diffusion method (Perez et al; 1994) was modified. Soyabean Casein Digest Agar (SCDA) was used for bacterial cultures. The culture medium was inoculated with the microorganisms suspended in Soyabean Casein Digest broth. A total of 8mm diameter wells were punched into agar and filled with test samples. Standard antibiotic was simultaneously used as positive control. The plates were then incubated at 37°C for 18 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed.

Wells were filled with 0.1 ml of each test sample. Antibacterial activity was determined by measuring diameter of Inhibition Zones (DIZ) in mm.

HAI* Hospital Acquired Infection

Results

According to the zone of inhibition formed resulting from each sanitizer against different test organism, findings in table-1(Plate-1&2) showed that hand sanitizer brand A® was the most strong and broad spectrum antibacterial agent, followed by brand C®. Comparing the antibacterial properties, brand B® and D®, B® were the most potent inhibiting all the bacteria tested with moderate diameter of zone of inhibitions. The sanitizer brands A® and C® were effective against all the test bacteria. While the effectiveness of D® was the poorest as indicated by minimal or no activity against the tested microorganisms. Plate-1 and 2 shows the activity of all the sanitizers against *Staph.aureus* and *E.coli*.

Table-1 Antibacterial Activity of Different Hand Sanitizer/Hand wash

S.No.	Test Brand Samples	Diameter of zone of inhibition(mm)			
		Staph.aureus	*E.coli*	*Pseudomonas aeruginosa*	*Salmonella sp.*
1.0.	A® (HH)	30	25	26	24
2.0.	B® (DA)	18	23	20	18
3.0.	C® (DH)	25	25	26	24
4.0.	D® (ST)	15	10	13	10

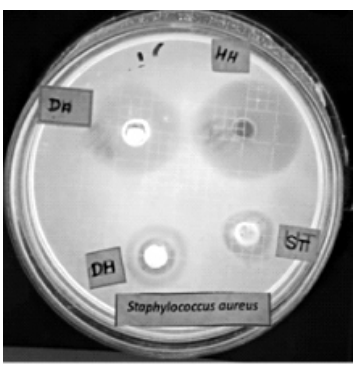

Plate-1

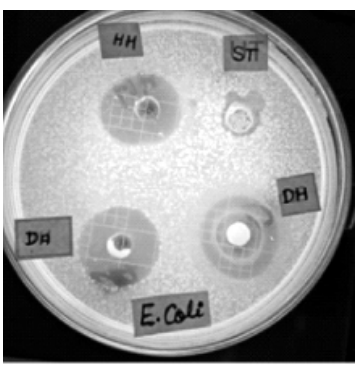

Plate--2

Discussion

Human skin provides nutrients and suitable growth conditions for most pathogens as well as opportunistic bacterial pathogens and these bacteria evidently have the ability to be resistant to most of the cleaning regimen, thus contributing to their persistence in an ecosystem (CDC,2008). Hand hygiene is a simple and least expensive means of preventing hospital acquired infections specially derived from environmental surfaces, (Pittet et al,1999). Alcohol based hand sanitizer is one of the components of hand hygiene and is recommended to reduce infections in healthcare settings. Food and Drug Administration (FDA) recommends a concentration of 60% to 95% ethanol or isopropanol, the concentration range is of greatest germicidal efficacy. The use of hand sanitizers is gaining popularity both among medical and non medical personnel.

Conclusion

The results of this study shows that hand sanitizer reduced the bacterial load on the hands of the subjects to a varying degree. Complete biocidal activity was shown only by brands A® and C® although claimed by all. This is probably because that the reduction in the bacterial load is not just because of alcohol but also other Herbal ingredients incorporated.

The active component of A®, C® has good activity against most vegetative bacteria. The main target is the bacterial cytoplasmic membrane (McDonnell and Russell, 1999; Richards and Cavill, 1979). After bioactive constituents has caused extensive damage to the cytoplasmic inner membrane, precipitation or coagulation of protein and nucleic acids occurs (Russel and Day, 1993). Of the four sanitizer investigated in this study, A® was the most broad spectrum antibacterial agent inhibited of the growth of the tested bacteria followed by C® and B®.

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Phytochemical Characterization of *Butea Monosperma* Flower and Leaf

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Abstract-The traditional systems of medicine together with Homoeopathy, Unani and Ayurvedic medicine continue to play a significant role in the health care system of the human being. Over 55% of all modern clinical drugs are of natural product origin. *Butea Monosperma* belonging to the family, Fabeacae is found in many places of India. The plant is highly used by the rural and tribal people in curing various disorders. The present paper gives information about the chemical composition and pharmacological aspects of the plant. The active substances were isolated by Soxhlet extractor and identified by phytochemical test. The results of analyses of each extract confirm the active substances, tannin, terpenoids, triterpenoids, triterpenes, alkaloids, flavonoids, saponin and glycosides others.

Introduction

There are many natural crude drugs that have the potential to treat many diseases and disorders, one of them is Butea monosperma (Ambasta, 1994). Butea monosperma is commonly known as Flame of forest. It is locally called as palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino and is commonly found throughout India. The flowers are widely used in treatment of hepatic disorders, viral hepatitis, diarrhea,depurative and tonic(Mahato and Sen,1997) . Its Flowers are also good source of flavonoids. (Sindhia and Bairwa, 2010). B. monosperma is used in the treatment of diabetes, leprosy, gout skin disease, eye disease, piles. It has laxative anthelmentic and antistress properties (Kasture, et al., 1999). It is evident that the various parts of the plants are used in Siddhi, Unani, and Ayurvedic medicines for the treatment of various diseases of human beings (Krapper et al., 2005). Its flowers are widely used in the treatment of hepatic disorders and viral hepatitis, diarrhoea and possess anti-inflammatory activity. (Agarwal, 1997)The active principles found in medicinal plants are alkaloids, glucosides, flavonoids, saponins, butrin and other complex compounds. These compounds may be found in root, stem, bark, leaf, fruit, and flower and seed (Nath and Khatri, 2010). There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones (Nostro et al., 2000).The aim of our present research work is to study the prelimnary phytochemical screening of *Butea monosperm*.

Material and methods

Plant material

The leaves and flowers of *Butea monosperma* plant were collected from the village, Rohana Kalan, District of Muzaffarnagar, U.P., India in the month of December – February and it was authenticated by Department of Botany, D.A.V. (PG) College , Muzaffarnagar.

Extraction and isolation

The leaves and flowers (200 gm) of *Butea monosperma* plant were shade dried at room temperature and finely powdered in grinder. The powder were kept in the air tight polythene bags and stored at dry place. The powder was extracted with solvent of water,diethyl ether, acetone and ethanol by using soxhlet. The

extracts were concentrated for further studies at 50 °C in water bath. Test extracts were then dried, crushed and stored in air tight bottle for further study.

Phytochemical analysis: Qualitative phytochemical tests were carried out to detect the presence of particular compounds groups present in different extracts of flowers and leaves using standard procedure.

Detection of carbohydrates: Molish test: 2 ml extract was taken and Molish reagent was added into it. 1 ml concentrated H_2SO_4 was added, it gave raddish violet ring which indicated the presence of carbohydrate.

Detection of Protein: Ninhydrin test: 1 ml extract was taken and 7-8 drops of ninhydrin reagent was added into it, kept in boiling water bath for 1 min. Blue or violet colour indicated the presence of amino acid.

Detection of tannins-$FeCl_3$ test: 200 mg plant material is taken in 10 ml distilled water and filtered. 2ml filtrate was added to 2 ml of $FeCl_3$, blue-black precipitate indicated the presence of tannins.

Detection of flavonoids: NaOH Solution Test:- 2 ml of extracts was added to 2 ml of 10% NaOH Solution. Appearance of yellow to orange colour indicated the presence of flavonoids.

Detection of saponins-Frothing test: 5 ml aqueous extract was taken in 5 ml of distilled water,shaked well for 15 minutes and warm, observed for formation of froth in the upper layer. The presence of froth indicated the presence of saponins.

Detection of glycosides-Keller-Kiliani Test: 2 ml of plant extract was added to 1 ml of glacial acetic acid,few drops of $FeCl_3$ and 3-4 drops of concentrated H_2SO_4 were then added.Raddish brown ring at the junction of liquids indicated the presence of glycosides.

Detection of terpenoids: 2 ml of organic extract dissolve in 2 ml of chloroform, then evapourate to dryness, 2ml of concentrated H_2SO_4 was added and heat for 2 min in boiling water bath. Greyish colour indicated the presence of terpenoids.

Detection of steroids: 2 ml of extract was added to 2ml of chloroform and 1 ml of concentrated H_2SO_4, 1 ml of glacial acetic acid was also added. Green colour indicated the presence of steroids.

Detection of triterpenoids: Liebermann-Burchard's Test: 2 ml extract (200 mg plant material was taken in 10 ml chloroform and filtered) was treated with 2 ml of acetic anhydrate. Few drops of concentrated H_2SO_4 was added. Brown ring appear at the junction and formation of deep red colour, indicated the presence of triterpenoids.

Detection of phytosterol/triterpenes-Salkawski test: 0.5 ml chloroform exctract (200 mg plant material + 2ml chloroform) in a test tube,add 1 ml of concentrated H_2SO_4.Appearance of raddish brown colour indicated the presence of phytosterol.

Detection of alkaloids-Wagner's test: 3 ml aqueous extract was added into 3 ml of 1% HCl and kept on water bath. Meyer's and Wagner's reagent was added which cause turbidity of the resulting smear and indicated presence of alkaloids.

Results and Discussion

Important phytochemically active constituents of *Butea monosperma* (leaves and flower) were qualitatively analyzed by different preliminary screening methods which include different tests such as test for Flavonoids, Triterpenoids, Glycosides, Saponines, Carbohydrates, Alkaloids, Proteins, etc. The preliminary phytochemicals screening of different extracts show presence of tannins, carbohydrates,

Table -1 Phytochemical Activity of *Butea monosperma* Flower in Different extracts

S.NO	Phytochemicals	D.W	Diethylether	Ethanol	Acetone
1	Carbohydrate	+	+	+	+
2	Protein	+	+	+	+
3	Tanin	+	+	+	+
4	Flavonoids	-	+	+	-
5	Saponin	-	+	+	-
6	Glycosides	-	+	+	-
7	Terpenoids	+	+	+	+
8	Steroids	-	+	+	+
9	Triterpenoids	+	+	+	+
10	Triterpenes	+	+	+	+
11	Alkaloids	+	+	+	+

(+ = Positive test, - = Negative test)

Table-2 Phytochemical Activity of *Butea monosperma* Leaves in Different Extracts

S.NO	Phytochemical	D.W	Diethylether	Ethanol	Acetone
1	Carbohydrate	+	+	+	+
2	Protein	+	+	+	+
3	Tanin	+	+	+	+
4	Flavonoids	+	+	+	+
5	Saponin	+	+	+	-
6	Glycosides	-	+	+	+
7	Terpenoids	-	+	+	-
8	Steroids	+	+	+	+
9	Triterpenoids	-	+	+	+
10	Triterpenes	-	+	+	+
11	Alkaloids	-	+	+	-

(+ = Positive test, - = Negative test)

Terpenoids, Glycosides and Alkaloid and Streroids present in all the extracts.

(+ = Positive test, - = Negative test)

Table-1 shows the qualitative phytochemical sceening in *B.monosperma* flower extracts. It indicated that the flower have the presence of carbohydrates, proteins, Tannin, terpenoids, triterpenoids, triterpenes and alkaloids in all the four extracts whereas flavonoids, saponinS and glycosides are absent in aqueous and acetone extracts. Steroids are present in alcoholic, ether and acetone extract and absent in aqueous extract. Gupta *et al.,* (1970) investigated three glucosides, identified as coreopsin, isocoreopsin and sulphurein. The remaining two are new and have been assigned the structures monospermoside and isomonospermoside. Shah *et al*. (1990) isolated and identified free sugars and free amino acids from the petroleum ether extract of the flowers.

(+ = Positive test, - = Negative test)

Table-2 shows the qualitative phytochemical sceening in *B.monosperma* leaves extracts. The study indicateS that the presence of carbohydrates, proteins Tannin, flavonoids and steroids in all the four extracts whereas terpenoids, triterpenoids, triterpenes are absent in aqueous extracts. Alkaloids are present in alcoholic and ether extract and absent in aqueous and acetone extract while saponin is absent in acetone extract. Different solvents have different solubility capacities for different phytochemical constituents (Dhale, 2010).

Conclusion

The present study concluded that the medicinal plants are the source of the secondary metabolites. Leaves and flower of *B. monosperma* have the potential to act as a source of useful drugs because of the presence of various phytochemical contents. These content are useful and improve the health of human being.

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Haematinic and Anti-Anemic Effects of the Ethonalic Extract of *Daucus Carota* Leaf Against Phenylhydrazine-Induced Hemolytic Anemia in Rats

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Abstract-*Daucus Carota* is versatile plant shows anti-cancer, anti-menorrhagic, anti-oxytocic, anti–microbial activity and has extended uses in Ayurveda, Unani and Homeopathy System of Medicine. Aims of the study were to evaluate the hematinic and anti-anemic effects of the ethanol extract of *Daucus Carota* leaves against phenylhydrazine-induced hemolytic anemia in rats. Adult male rats were divided into 5 groups (Gr.I-V) containing 6 rats each. Rats in Gr. I served as control. Gr. II rats were treated with DCLE (250mg/kg) for 14 days. Rats in Gr. III, IV and V were pre-treated with phenyl hydrazine (PHZ,10mg/kg for 7 consecutive days) so as to induce anemia, followed by 0, 250 and 500mg/kg doses of DCLE respectively up to 14 days. To investigate hematinic and anti-anemic profile of plant extract, blood samples were examined for various hematological parameters viz. Hgb, RBCs, WBC, MCV, MCHC, MCH, RDW, Platelet count, etc. Biochemical analysis of marker enzymes for general metabolic, liver and kidney function, and histological examination was done in control and treated-groups of rats. Antioxidant enzymes and level of lipid peroxidation was studied in blood samples from treated and control animals. Results showed that PHZ (10mg/kg) treatment for 7 consecutive days caused a significant increase in MDA activity but, declined SOD and Catalase activity in blood serum. Further, PHZ- treatment caused significant decrease in Hgb (%), RBC, MCV, but increased TLC count in Gr. III-V rats. There was significant increase in Hgb (%), RBC, MCV and TLC count in Gr. IV and V rats treated with DCLE at 250 and 500 mg/kg doses from day 8-14, as compared to PHZ-treated (Gr.III) rats. Rats treated with *DCLE* alone did not alter any haematological/ biochemical/histological aspects and organ weights as compared to controls. In conclusion, the results of the study clearly indicate antioxidant, hematinic and anti-anemic activity of the ethanolic extract of *Daucus Carota* leaf . Findings provide scientific evidence for traditional use of DCLE as natural antioxidant and hematoprotective agent.

Keywords: Antioxidant, Hematinic, Anti-anemic activity, *Daucus Carota,* Leaf extract.

Introduction

Herbal plants and phytomedicines are symbolized for safety of mankind, serving several purposes like health and protection from diseases .In the present scenario the knowledge of traditional medicine has always guided the search for new cures and clues. Therefore, the demand for herbal products is growing exponentially throughout worldwide for the discovery of valuable drugs because of its non-toxicity and safety in spite of modern high drug discovery and screening techniques[1-3]. Nowadays, attention has been focused on the investigation, isolation and fractionation of drugs from plant origin for pharmacological basis of traditionally used plants[4-6].

Daucus carota (*D.carota*) Linn commonly known as “carrot” belong to family Apiaceae and is cultivated almost all over the world as a useful vegetable. The plant has undergone extensive phytochemical studies and a large number of active ingredients have been isolated. These include volatile oils, steroids, triterpenes,

carbohydrates, glycerides, tannins, flavonoids, aminoacid carotene and hydro carotene.[7] D.carota is also used as a noval model to evaluate the effect of light on carotenogenic gene expression isolated animal organ studies. The antioxidant activity of phenolic compounds is mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals or their effects, quenching singlet and triplet oxygen or decomposing peroxides. The flavonoids and terpenoids have multiple biological effects including an antioxidant activity, plays an important role in the defense against free radicals[8]. The oxidative environment of living organism possess a range of free radicals including superoxide radical, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite, which are essential for the production of energy for various biological processes. However, excessive production of free radicals results in the development of carcinogenesis, neurodegenerative diseases, inflammatory diseases, atherosclerosis, aging, immunosuppression, ischemic heart disease, diabetes, hair loss, Alzheimer s disease, cataract and many other problems. The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants[9,10].

The present study was conducted to evaluate the antioxidant potential and anti-anemic effects of the ethanol extract of *Daucus carota leaf* against PHZ-induced hemolytic anemia in rats.

Material and Methods

Chemicals

Ethanol and phenylhydrazine were purchased from Sigma-Aldrich.All other chemicals used in this study were available locally.

Collection of plant material

The fresh plant material of *Daucus carota leaf* was collected locally market nearby Lucknow region, Uttar Pradesh, India. Plant material was identified at Division of botony, CSIR-Central Drug Research Institute, Lucknow.

Preparation of leaf extract

Powdered leaves (2 kg) were extracted with 95% ethanol using a Soxhlet apparatus. The ethanolic extract was filtered and concentrated by distillation process. A green colored residue was obtained (yield 6.79% w/w) and was kept in a desiccators. This ethanolic extract of D. carota leaf extract (DCLE) was used for further experiments[11,12].

Experimental Protocol

Test animals

Sprague–Dawley rats (150-175 gm) of both sexes were obtained from animal house, CSIR-Central Drug Research Institute, Lucknow, India. Animals were allowed to acclimatize to uniform husbandry conditions (22±3 °C, 12h light: 12h dark cycle) for 1 week prior to the experiment. The animals were fed with a standard pellet diet and access to water. Animal studies were conducted according to the regulations of the Institutes Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA[13]

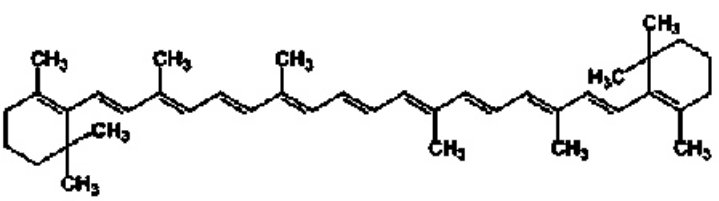

Fig: Structure of β carotene

Phenyl hydrazine (PHZ)-induced haematotoxicity/haematoprotective activity of *Daucu scarota*

Rats (Males and females) were randomly divided into five groups (Gr.) containing five animals each of either sex. Rats in Gr. I (Control) was administered with 1% gum acacia orally (p.o.). The rats in Gr. II were orally administered with ethanol extract of DCL(500mg/kg) for 7days. Rats in Gr. III, IV and V were treated with PHZ (10 mg/kg/day, p.o) for 7 days to induce haematotoxicity. Group III rats severed as PHZ-induced haematotoxicity Control (STD Control). Animals in Gr. IV and V (pre-treated with PHZ for 7 days) were administered orally with *DCL*extract at 250mg/kg and 500mg/kg doses respectively for another 7 consecutive days up to day 14. On day 15, body weights of control and treated animals were recorded. Blood was collected in pre-coated EDTA-vials for hematology and then autopsied by anesthetizing with solvent anesthetic ether. The vital organs (viz. heart, liver, lungs, spleen, kidney and brain) were dissected out, weighed and fixed in 10% Formalin (24h) for histological examination[14,15].

Hematological analysis

Blood samples from control and treated groups were collected on days 0, 7 and 14 of treatment by puncturing tail vein. The hematological parameters were analyzed using MS-9 Fully Automated Hematology Analyzer. The parameters included were hemoglobin (Hgb), total erythrocyte-red blood cells (T-RBCs), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PC) and total leucocytes count (TLC)[16,17].

Biochemical estimations

Blood samples were collected by cardiac puncture and retro-orbital plexus method from all animals into the EDTA sprinkled tubes and were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C until analysis was performed. Serum samples were analyzed for general metabolic function viz. Glucose(GLU), cholesterol(CHO), triglycerides(TG), total proteins(TP) and albumin(ALB) levels. For liver function, serum biochemistry for alanine trasparase (ALT), Aspartate transferase (AST), alkaline phosphatase (ALP) and total billirubin (T-BIL) was done. For kidney function, estimations of blood urea and nitrogen (BUN), creatinin (CRTN), calcium (Ca) and phosphate (P) were carried out using the diagnostic kit (ERBA Diagnostics Mannheim, Germany) in Auto analyzer.

Enzymatic antioxidant assays

On day 15, blood was collected in pre-coated EDTA-vials, centrifuged at 10000xg for 10 min and serum was collected and stored at -20^{0}C in refrigerator till estimation of antioxidant enzyme activity.

Superoxide dismutase (SOD) activity

SOD activity was performed by 19160 SOD determinations Kit (Sigma-Aldrich, USA). Kit contained WST Solution (5ml), Enzyme solution (100µl), Buffer Solution (100ml) and Dilution buffer (50ml). Preparation of working solution was done by diluting 1 ml of WST solution with 19 ml Buffer solution. For enzyme working solution, enzyme solution was centrifuged for 5 sec, mixed thoroughly and diluted 15 µl of enzyme solution with Dilution buffer. SOD was diluted with dilution buffer to prepare SOD STD solution as 200U/ml, 50U/ml, 10 U/ml, 5 U/ml, 1U/ml, 0.05 U/ml, 0.01U/ml,0.001U/ml.

Methods

20µl of sample solution was added to each sample and blank well 2 and 20 µl of double distilled water (DDH2O) was added in each blank well 1 and 3. Then 200µl of WST working

solution was added in each well and mixed. Then added 20 µl of Enzyme working solution to each sample and blank 1 well, and mixed thoroughly. Incubated the Eliza plate at 37^0 C for 20 minutes and read the absorbance at 450nm using a micro plate reader. The SOD activity (inhibition rate %) was calculated using the equation:

SOD activity (inhibition rate %) = {[(Ablank1-Ablank3)-Asample-Ablank 2))]/(Ablank1 – Ablank3)}x 100.

Catalase enzyme activity

Catalase enzyme activity was performed using Catalase Assay Kit (CAT 100) from Sigma-Aldrich, USA. Tissue samples were prepared in 1X Assay buffer solution (500mM potassium phosphate buffer, pH 7.0), then diluted with enzyme dilution buffer(50mM potassium phosphate buffer, pH 7.0 containing 0.1% TritonX-100) in a microcentrfuge tube and added calorimetric assay substrate solution(200mM H_2O_2) and mixed and incubated for 5 min in incubator at 37^{0}C. Then added 900 µl of stop solution and inverted the tube. 10ul aliquot of catalase enzyme reaction was taken in another centrifuge tube and added 1 ml of Color reagent and mixed by inversion and kept for 15 minutes at room temperature for color development and read the absorbance at 520 nm.

Lipid peroxidation (MDA) activity

MDA activity was assayed by using lipid peroxidation assay kit (Catalog no. MAK085, Sigma-Aldirch, USA). Briefly, blood serum (10µl) was gently mixed with 500 µl of 42 mM H2SO4 in a microcentrfuge tube. Then added 125 µl phosphotungustic acid solution, mixed by vortexing and incubated for 5 minutes and then centrifuged at 13000xg for 3 minutes. In a separate tube 2µl of BHT (100x) to 100µl H2O. Resuspended the pellet on ice with water/BHT solution and adjusted the volume for 200 µl with water.

Assay reaction

To form MDA-TBA adduct, added 600 µl of the TBA solution into each vial containing standard and sample. Incubated at 95^{0}C for 60 min. Cooled to room temperature in a ice bath for 10 minutes, pipetted 200 µl from each reaction mixture into 96 well plates for analysis and read the absorbance at 532 nm.

Histology of vital organs

Tissues from liver, kidney and spleen were fixed in Bouins fluid (24 hr) for histology purpose. Further, they were dehydrated in graded series of ethanol, cleared in xylene and infiltrated and embedded in paraffin wax (at 58^{0}C). Transverse tissue sections (5 µm) were stained with routine Haematoxylin-eosin, observed the histological changes under Olympus Trinocular Microscope (Tokyo, Japan) and photo micro graphed[18-24].

Table - 1

Groups	Body wt(g)	Adrenal gland(g)	Brain(g)	Gonads(g)	Heart(g)	Kidney(g)	liver(g)	Lungs(g)	Spleen(g)
control	401.0±20.14	0.030±0.005	2.80±0.42	2.04±0.83	1.20±0.25	0.89±0.19	7.45±1.91	1.92±0.15	0.75±0.21
DCLE(250)	402.0±19.64	0.32±0.005	2.83±0.40	2.48±0.104	1.17±0.22	0.90±0.18	8.02±1.80	2.0±0.19	0.84±0.18
PHZ(10)	388.6±43.40	0.050±0.025	2.99±0.12	2.46±0.33	1.52±0.50	1.09±0.09	11.80±0.52	2.31±0.41	5.44±0.06***
PHZ+DCLE(250)	418.1±15.24	0.04±0.06	1.81±0.34	2.50±0.19	0.90±0.07	0.89±0.09	8.09±1.81	1.87±0.30	1.23±0.33
PHZ+DCLE(500)	406.2±34.20	0.052±0.01	1.80±0.13	2.54±0.29	0.95±0.10	0.87.0.03	8.06±0.64	1.96±0.32	1.19±0.33

Statistical analysis

Analysis for significance of differences between control and treated group of animals was done by Students‘t’ test and one-way ANOVA (one

factor analysis of variance). Values for antioxidant assays represented in a triplicate manner and were expressed as Mean ± SD. Values with $p < 0.05$ were considered as significant.

Results

Body and organ weights

There were no significant differences observed in the body weights of treated male rats as compared to controls. The absolute organ weights viz. adrenal, brain, testis, heart and kidney of treated rats were comparable to controls. However, PHZ (10mg/kg) treatment for 7 days caused significant increase in weights of liver ($p<0.01$), lung ($p<0.01$) and spleen ($p<0.001$) as compared to control rats. Treatment with DCLextract alone did not show any significant difference in body and organ weights. But, oral administration DCL extract at 250 or 500mg/kg doses to PHZ-induced anemic rats, caused significant decrease (recovery) in weights of liver, lung and spleen comparable to that of control rats (Table-1)

Histology of vital organs

Transverse sections of kidney in control rat showed normal distinct glomeruli, Bowman s capsule. PHZ-treated rats showed shrinkage and glomerular and renal damage in kidney of some animals. SI treatment to PHZ-induced anemic rats showed distinct glomerular and tubular structure with improvement as compared to PHZ-treated rats but similar to controls.

In control rats, histology of liver was normal showing hepatocytes, portal tract and leucocytic infiltration. PHZ-treatment caused distortion of hepatocytes, portal tract dilation and inflammatory infiltration. *SI* treatment to PHZ-induced anemic rats showed normal structure similar to in controls. In control rats, spleen showed normal structure. PHZ treatment caused higher erythroblastic islands in spleen with a higher excessive erythrocytic congestion inflammatory infiltration than in liver and kidney in anemic rats. *SI* treatment showed repair of all changes which were comparable to normal control rat spleen[25-28].

PHZ (10mg/kg) treatment caused a significant decrease in SOD and CAT enzyme activity, whereas MDA activity was significantly increased by PHZ treatment as compared to control rats. SI extract alone did not show any significant change in these enzymes activity compared to controls. However, there was a significant increase in SOD and CAT enzyme activity in PHZ-induced anemic rats treated with SI extract at 250 or 500 mg/kg.

Haematology

Adult male rats treated with *SI* (250mg/kg) for 14 consecutive days (Gr. II) did not show any significant change in Hgb, RBC and MCV, Platelets(x103/mm3) and TLC(x103/mm3) concentration in blood as compared to Control rats (Gr.I). Rats in Gr. III treated with PHZ (10mg/kg/day) for 7 consecutive days, caused significant decrease in Hgb (%) ($p<0.01$), RBC (x106/mm3) ($p<0.001$) and MCV (micron3) ($p<0.001$) concentration as compared to control rats. The MCHC (g%) and Platelets(x103/mm3) number did not differ to that of controls, but, increase in number of TLC(x103/mm3) was observed in PHZ-treated rats. When PHZ-treated groups of rats administered *SI* at 250 or 500mg/kg, showed a significant decline in Hgb, RBC and MCV concentration comparable to in controls. The TLC values increased by PHZ treatment were normalized by *SI* extract treatment comparable to controls[29-30].

Biochemical analysis

Oral administration of methanol extract of DCL at 200mg/kg body weight did not show any significant changes in the general metabolic function, liver function or kidney function as evident by analysis of marker enzymes viz. GLU, CHOL, TG, TP, ALB, ALT, AST, ALP, T-BIL, BUN, CRTN, Ca and P as compared to control rats. Treatment of PHZ (10mg/kg body

weight) alone caused significant increase in blood GLU concentration and in marker enzymes of Liver and kidney function e.g. ALT, AST, ALP, T-BIL, and in CRTN and P as compared to control or *SI* (500mg/kg) alone treatment. Administration of DCLE at 250 or 500 mg/kg to PHZ-induced anemic rats caused decline in GLU, AST, ALP, T-BIL, CRTN and P levels as compared to PHZ alone treatment, which were comparable to controls[31-34] .

Discussion

In present study, PHZ-induced anemic rats showed significant increase in MDA activity and decrease in SOD and CAT enzyme activity in blood serum. Oral administration of the methanol extract of *SI* stem bark to PHZ-induced anemic rats caused significant decrease in MDA activity, but increased SOD and CAT enzyme activity equivalent to in *DCL*- treated or normal rats. Previous studies have been shown the antioxidant properties of SI stem bark extract using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and SOD assay system. Further, the antioxidant, antiglycation and inhibitory potential of carotenoid fraction of the leafextract of this plant (DCL) against alpha-glucosidase and alpha-amylase (the enzymes linked to type 2 diabetes) and LDL oxidation have been reported. Pre-treatment of C2C12 cells with SAF prevented the increased formation of MDA, ROS and depletion of GSH induced by H2O2. The ethyl acetate fraction of SAF have also shown the free radical scavenging activity against the DPPH and superoxide radical, along with hydroxyl radical scavenging activity and lipid peroxidation inhibitory potential indicating significant antioxidant and xanthin oxidase (XO; key enzyme linked to inflammation) inhibitory potential. SI leaves extracts (e.g. petroleum ether, chloroform and methanol) administration for 21 days have also been reported to show significant ($P < 0.05$) antioxidant activity at the dose of 500 µg/ml. In concurrence, previously reported studies with herbal extracts e.g. *A. pterocarpoides*, *M. arboreus* and *H. madagascariensis* have been demonstrated to show an increase in the rate of inhibition of DPPH, as a function of time and the extract s concentration which reflect on the kind of scavenging radical kinetic: the fast, intermediate and slow kinetics. The ameliorative effects of SI *leaves* ethanolic extract on hyperglycemia and lipid profile have been demonstrated to be due its contents including phytosterols, phenols, tannin and flavonoids. In plant tissues many phenolic compounds (in addition to tocopherols) are potential antioxidants, flavonoids, tannins and lignin precursors may work as ROS-scavenging compounds. Antioxidants act as a cooperative network, employing a series of redox reactions. Interactions between ascorbic acid and glutathione, and ascorbic acid and phenolic compounds are well known. Therefore, the radical scavenging activity of *SI* extract may to be due to its polyphenolic constituents that play an important role in anti-oxidative effects, act as reducing agents/antioxidants via several mechanisms including the scavenging of free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes.

The body and organ weights (viz. adrenal, brain, testes, heart and kidney) did not show any significant changes in *SI*, PHZ and PHZ+*DCL* extract treated rats as compared to controls. But, there was a significant increase in weights of liver, lung and spleen in PHZ-treated anemic rats as compared to control. Oral administration of *SI* extract (250 or 500mg/kg) caused a significant decrease in these organ weights gain to normalcy. Histopathological studies showed higher erythroblastic islands in spleen with a higher excessive erythrocytic congestion, abundant macrophages than in liver and kidney in PHZ-treated anemic rats. Previous study has been demonstrated that PHZ treatment induces an increase in spleen, kidney and liver weights at 60mg/kg dose and showed severe splenomegaly and marked splenic erythroid hyperplasia on

day 6 of post injection. Erythroblastic islands reported in the spleen, liver and kidney, this being indicative of compensatory erythropoietic activity. In ultrastructural study, sequential transformation of PHZ-induced hemolytic anemia as well as transformation of typical erythrocytes into erythrocytes full of protuberances and transformation of Heinz bodies into damaged erythrocytes by splenic lytic activity have been reported. The treatments with *DCL* extract show recovery of these changes similar to controls. Previous study on *Daucus carota* leaves extract also shown favorable histopathological changes in pancreas, liver and kidney in STZ-induced diabetic mice.[12] The terminal serum biochemistry on day 15 of autopsy, showed significant increase in GLU, ALT, AST, ALP, T-BIL, CRTN and P levels by PHZ-treatment as compared to control or DCLE(250mg/kg) alone treated rats. This increase in by DCLE treatment was decreased to normal level by oral administration of *Daucus carota* leaves extract (500 mg/kg) in PHZ-treated male rats. Previously, Kumar et al. has been shown a significant ($P<0.01$) reduction in blood GLU levels, improved body weight and altered biochemical parameters associated with diabetes in diabetic mice treated with DC leaves extracts (e.g. petroleum ether, chloroform and methanol) for 21 days. Estimation of blood GLU level has been used as marker of hyperglycemia. Hyperglycemia induced-oxidative stress caused by free radical generation and decrease in antioxidant defense system. Dyslipidaemia is associated with elevated total cholesterol, triglycerides and low level of high density lipoprotein (HDL). Treatment with ethanolic extract of DCL has been reported to normalize the altered lipid profile, reduce the elevated glucose level and attenuates the diabetes-induced renal oxidative stress in dose dependent manner.

Administration of DCLextract (250mg/kg) alone for 14 days to normal rats did not alter Hgb, RBC, MCV, Platelets(x103/mm3) and TLC(x103/mm3) concentration as compared to controls. However, PHZ-treatment (10mg/kg/day) for 7 consecutive days, caused significant decrease in Hgb(%)($p<0.01$), RBC(x106/mm3) ($p<0.001$) and MCV(micron3)($p<0.001$) concentration and increased TLC(x103/mm3) infiltration. Treatment of DCLextract at 250 or 500mg/kg body weight to PHZ-induced anaemic rats, showed significant increase in Hgb, RBC and MCV concentration which was comparable to in control rats. The Hct, MCHC and platelets did not show any significant change in PHZ or SI treated either alone or in combination with PHZ when compared with controls. Similarly, previously reported studies have been demonstrated that PHZ decreases the Hgb, RBC and MCV and the rate of haematocrit (HCT) below controls and impairs erythrocyte deformability. It also induces reticulocytosis, increases osmotic resistance, free plasma Hgb, MCH, MCHC and erythropoietin levels, and extramedular haematopoiesis in the spleen and liver. The numbers of erythrocyte-committed progenitors and colony-forming units increases during PHZ-induced acute anaemia. PHZ induce vascular dysfunction and haemodynamic disturbance, but, decreases in mean arterial pressure and hindlimb vascular resistance. Uncompensated respiratory alkalosis, increased arterial CO2 tensions and acidosis were also reported following PHZ administration.This action could be the result from the highest antioxidant potential and the presence of iron as reported earlier. There was also a significant increase in the catalase activity of rat liver and kidney indicating restoration of anaemic condition in the iron deficient group Results of the study with DCLextract administration to PHZ-treated rats also explain the recovery of the decreased haematological parameters (RBCs, Hgb, and MCV) and increased TLC level to normalcy, indicating the action of the plant extract vis-a-vis haemolysis. Further, the toxic effects of PHZ have been shown to increase in ROS, Lipid peroxidation,

and decrease in GSH which to be reversed by N-acetyl cysteine, a known ROS scavenger The sub chronic intoxication of rats with PHZ, results in marked anemia, reticulocytosis, methemoglobinemia and increases hemocatheresis, total iron content, hepatic ferritin and DNA fragmentation, increases levels of 8-oxo-7,8-dihydro-2 -deoxyguanosine (8-oxodGuo), a specific marker of oxidative DNA damage, and hepatocyte y-glutamyl transpeptidase (y-GT, EC 2.3.2.2) activity Acute haemolysis induced by PHZ, exhibited the extramedullary haemopoiesis and increased erythrophagocytosis in the spleen. In consequence, morphological changes include splenomegaly and congestion of haemosiderin deposits Catabolism of haemolysed RBCs is associated with increased expression and activity of haem oxygenase 1 which suggests increased capacity for degradation of Hb products, iron level and DMT1 and TFR1 mRNA expressions in the spleen by PHZ treatment Increased splenic erythrophagocytosis results in a net flux of iron into the circulation from haemolysed RBCs. While the mechanism of iron exchange between macrophages and transferrin is still undefined, ferroportin mRNA increased in the spleen of PHZ-treated mice, consistent with efflux of iron being mediated by this protein. Hepatic non-haem iron levels increases significantly after PHZ treatment and sustained for 7 days after haemolysis[91] which is due to increased absorption driven by haematopoeitic activity in PHZ-treated animals. Liver iron accumulates initially due to haemolysis, later on absorbed iron may be diverted to the liver hepatocytes via serum transferrin Iron deposition in the liver could in addition to the TFR/DMT1 routes, be due to influx of non-transferrin bound iron (NTBI). Furthermore, as PHZ-induced haemolysis is stressful and produces haemderived toxic reactants, the expressions of acute phase proteins hemopexin, haptoglobulin and CD in the hepatocytes and the kidneys are increased.

These organs mop up the haem-derived products from circulation. A flavonoid, silybin dihemisuccinate, an anti-hepatotoxic agent caused protective effects on the hepatic glutathione depletion and lipid peroxidation induced by PHZ. The protective effects of quercetin in a model of PHZ-induced oxidant stress, vascular dysfunction and hemodynamic disturbance, shown to protect the blood glutathione, suppressed plasma malondialdehyde levels and nitric oxide metabolites and superoxide anion production in rats. A significant increase in thiobarbituric acid (TBA)-reactivity in the circulating RBC of PHZ-treated rats increases 3-fold in RBC obtained from the spleen. Since lipid peroxidation accompanies formation of TBA-reactive malonyldialdehyde, phenylhydrazine induces anemia as a consequence of peroxidation of RBC membrane lipids and this effect may be a result of the autoxidation of the drug and the interaction of oxygen radicals with membrane lipids.

Conclusion

In summary, results of the present study provide the evidence that the ethanolic extract of DCL could reduce oxidative stress evidenced by its antioxidant activity. Further, DCL extract also showed protective effects on decreased hematological parameters viz. Hgb, RBC and MCV concentration and increased TLC by PHZ- treated anemic rats. It also normalizes the increased biochemical parameters such as blood GLU level and marker enzymes for Liver function (viz. ALT, AST, ALP, T-BIL) and kidney function (CRTN and P) by PHZ treatment. The increase in organ weights of spleen, liver and kidney and histopathological alterations induced by PHZ-treatment were also restored to normalcy by oral administration of DCL extract. The prevention of PHZ-induced anemia and related changes in vascular dysfunction and dynamics by DCLextract, explore the traditional use of this plant as an

antioxidant, antianaemic and haematoprotectant.

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Isolation, Characterization, Synthetic Modification and Evaluation of Anti-Oxidant Potential of Berberine from Roots of *Berberis aristata*

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Abstract-Roots of plant *Berberis aristata* has been approved to have antioxidant potential which is probably due to presence of alkaloid compound Berberine in the roots, rhizomes, and stem bark with a long history of medicinal use in both Ayurvedic and Chinese medicine. Beside antioxidant activity Berberine extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoan's,several disorders including metabolic, neurological and cardiological problems, In search of new antioxidant compounds in the present study, attempts has been made to isolate *Berberine*, an antioxidant compound from *Berberis aristata* acting as lead compound on which synthetic modifications were carried out to get better antioxidant properties. Berberine was isolated from the roots of *Berberis aristata*.The alkaloidal nature of Berberine was determined with the help of Dragendorff and Wagner's reagents. Its melting point was measured to be 145°C. Co-TLC of the compound with a market sample of Berberine showed similar Rf value. Spectral data is well comparable to spectral data of authentic sample. Berberine was subjected to derivitization to study structure activity relationship firstly by demethylation at position 9 and 10 and then converting to diacetoxy and dibenzoxy derivatives. Synthesized compounds were characterized on the basis of spectral data .All the newly synthesized derivatives along with isolated berberine were evaluated for their antioxidant potential as compared to standard ascorbic acid. From the studies, it could be observed that berberine has very good antioxidant activity (DPPH Radical Scavenging Activity 61% which is comparable to the standard drug Ascorbic acid (DPPH Radical Scavenging Activity, 97.2% @250(µg/ml). 9,10- diacetoxy berberine (3) has comparable effect (60% antioxidant activity) while other derivatives showed decrease in activity as compared to berberine . Increase in dose also caused no betterment in results, however 9,10-dibezoxy derivative(4) at dose of 500(µg/ml had marked effect of 78% DPPH Radical Scavenging property.

Keywords: Berberine, Isoquniloline, Antioxidant, DPPH Radical Scavenging.

Introduction

Plants containing pharmacologically active constituents have been used by man since the dawn of History. Plant drugs offer cure for many diseases which do not find lasting remedies in modern medicine. If used judiciously, drugs of plant origin have better compatibility with human system as the compounds occurring in plants already have biological functions and may have more biologically relevant chemistry to human system, hence lesser side effects. Remarkable diversity in chemical structures and biological activities of the naturally occurring secondary metabolites which make them important as direct use as therapeutic agents, utility as biochemical and molecular probes and utility as prototype lead compounds for the development of new synthetic or semi synthetic drugs. The initial step in the discovery of a new drug is the lead identification**.** In search of lead identification from natural sources which may

act as important entities in themselves or lead to a pathway on which further work could be done. Berberine rich extracts and decoctions from *Berberis aristata (*Anonymous 1985, 2007 Rashmi, et al. 2008) have demonstrated significant antimicrobial activity against a variety of organisms including bacteria,(Meenakshi, et al. 2007) viruses, (Bradley, et al. 1982, Romero, et al. 2005,) fungi (Sharma, et al. 2008), protozoans. Moreover, several clinical and preclinical studies have demonstrated effect of Berberine against several disorders like diabetes (Akhtar et al 2008; Semwal, et al. 2009 ; Ahmad, et al. 2008; Gupta et al. 2010) cancer (Das, et al. 2009; Mazumder, et al. 2010), neprological (Adhikay, 2010) neurological (Gilani, et al. 1999)and cardiological (Rashmi, et al. 2008) problems. It also has heapatoprotectective and antioxidant activity (Brijesh, et al. 2010; Gilani, et al. 1995) profile. keeping this aim in mind present studies attempts were made to isolate Berberine from *Berberis aristata* acting as a lead compound and carry out its synthetic modifications (vogle, et al 2009) (fig.1) to establish structure activity relationship as new antioxidant substances(Barry, 1976).

Material and Methods

Isolation of berberine from roots of *Berberis aristata* and synthetic modification was carried out to study SAR. All the compounds were subjected to spectral analysis. The IR spectra were recorded on Bruker, alpha E ATR FTIR spectrophotometer. NMR and ^{13}C NMR spectra were recorded at 400 MHZ by using $CDCl_3$ as solvents and Mass spectra were scanned on Bruckers micro TOF-QII, ESI Mass spectrophotometer. ^{1}H NMR and ^{13}C NMR shift were reported as parts per million (ppm) downfield from TMS (Me_4Si). Purity of synthesized compounds was determined by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} percolated sheet in chloroform/ methanol mixture and spots were developed using iodine vapours/ultraviolet light as visualizing agents. The melting points were determined by using the capillary method. Solvent removal was accomplished by a rotator evaporator operating at in house vacuum (40-50 Torr). The solvents and reagents were used without further purification.

Collection of Plant Material

Fresh roots of plant *Berberis aristata* was collected adjoining area of Almora district of Uttarakhand in month of Jan-February authenticated from Botany division of forest research Institute, Dehradun. The roots were dried in shade for 12 to 15 days and then kept in oven at 35-40^0 C for 2-3 hrs. The roots were crushed in small pieces. The root was weighed before and after drying in order to estimate the moisture content.

Isolation of Berberine (9,10-dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-7-ium)[1]

Dried roots (250gms) were defatted with pet ether (2l) for 14- 15 hrs followed by extraction with ethanol (2L) for 6 hours. The ethanolic extract was concentrated on hot water bath till syrupy mass obtained. Dissolved syrupy mass in 25ml of hot water and filtered. Added 5ml hot water to the residual syrupy mass, again filtered. To the combined filtrate added slowly 15ml of strong HCL (36.5%w/v) with shaking. Cooled in ice bath and placed in refrigerator overnight to obtain yellow crystals of *Berberine hydrochloride,* 2gms. The salts of Berberine are dissolved in hot water and the solution was made alkaline with few drops of 10% NaOH. Thereafter, a small quantity of acetone is added and solution is diluted with equal amount of water. The precipitate is allowed to settle overnight and washed with ice-cold water. The isolated Berberine was identified on the basic of TLC using silica-G as a stationary phase and chloroform:methanol (95:5) and Ethyl acetate: acetone: formic acid: water (100:11:11:27) as mobile phase and visualized by spraying with

the 5% Sulphuric acid. The Rf value of the Berberine was calculated and compared with the standard value of the same. Berberine(1) was characterized on the basis of spectral studies and comparison with the authentic sample.

E I - M S (*m / z* %) : 3 3 6 (M^+ 2 0) , 322,(35),278(45)281.(50),267(38),207(76),147 (100).

UV(*λmax*.nm): 230,266,348,431.

I R ν_{max}, c m - ^{1} : 2 8 2 0 (C - H) , 1 5 9 7 , (C =C,C=N),1354-1383(C-H)

^{1}H NMR: ($CDCl_3$ 400 MHZ) δ :7.0 (H-4), 3.2 (H-5), 4.9 (H-6), 9.8 (H-8), 8.0 (H-11),8.1 (H-12), 6.1 (H-13), 8.7 (H-14), 4.2 (H-15) and 4.1(H-16) .

^{13}C NMR($CDCl_3$ 100 MHZ) δ: 121(C-1), 132 (1a), 139 (C-2, -3), 122 (C-4), 128 (4a), 24(C-5), 56 (C6), 149 (C-8), 136 (C-8a), 146 (C-9),147 (C-10) 109 (C-11), 123(C-12), 124 (C-12a), 106(C- 153(C-13a) 103 (C-14), 162 (C-15) and 157(C-16).

Synthesis of Derivatives of Berberine

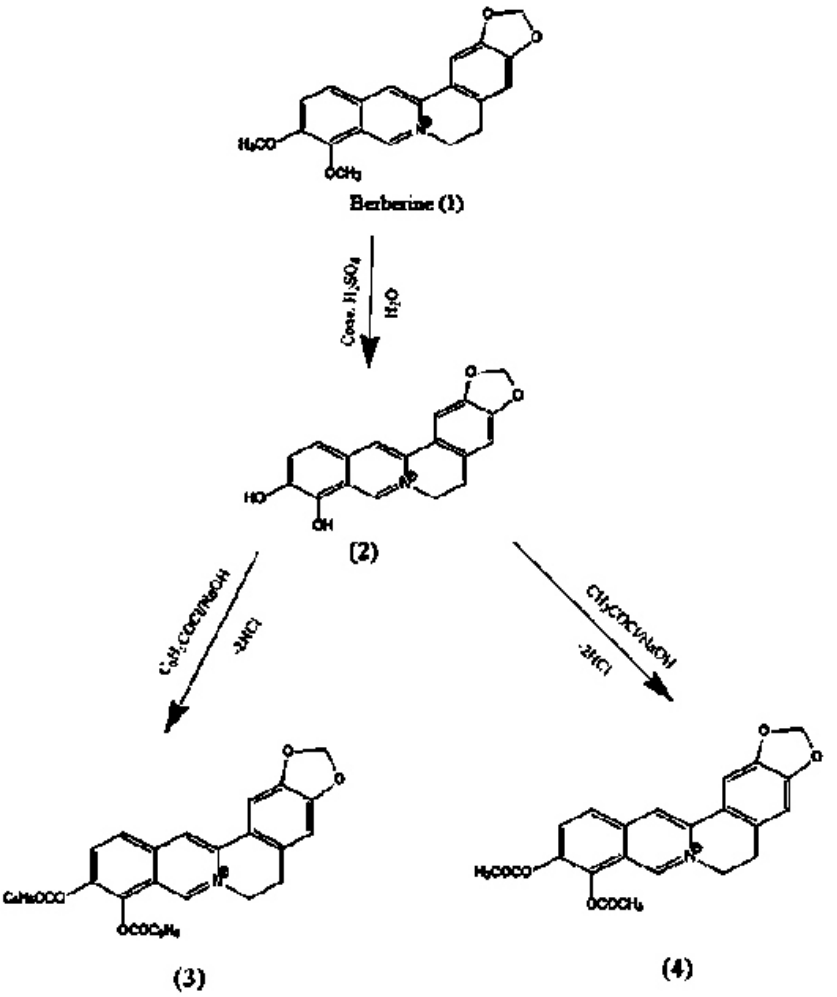

Figure- 1 Dervitization of Berberine

Synthsis of (9,10-dihydroxy-5,6-dihydro[1,3]dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-7-ium) [2]

Berberine (.0.5 mole) was treated with 10 ml of conc. Sulphuric acid and kept for some time . To the mixture was added, few drops of water wherein yellow precipitates of 9,10- dihydroxy berberine were obtained. Filtered and re crystallized with ethanol as bright yellow fine crystals characterised on the basis of spectral data.

EI-MS (*m/z* %):308(M^+42).290(100), 278(26),266(52)

UV(*λmax*.nm): 230,266,335,400.

IR ν_{max},cm-^{1}: 2820(C-H)1597,(C =C,C=N),1354-1383(C-H).

^{1}H NMR: ($CDCl_3$ 400 MHZ) δ: 7.0 (H-4), 3.2 (H-5), 4.9 (H-6), 9.8 (H-8), 8.0 (H-11),8.2 (H-12), 6.1 (H-13), 8.7 (H-14) and 5.49(OH).

^{13}C NMR($CDCl_3$ 100 MHZ) δ::121(C-1), 132 (1a), 139 (C-2, -3), 122 (C-4), 128 (4a), 24(C-5), 56 (C6), 149 (C-8), 136 (C-8a), 146 (C-9),147 (C-10), 109 (C-11), 123 (C-12), 124 (C-12a) 106(C-13), 153 (C-13a) 103 (C-14).

Synthesis of (9,10-benzoxy-5, 6-dihydro[1,3] dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-7-ium) [3]

To (1mole) of [2] in aqueous sodium hydroxide was added (2moles) of benzoyl choloride with continuous vigorous shaking till yellow colored ppts were formed. The reaction mixture was cooled and solid so formed was filtered and given washings with excess cold water so as to remove excess benzoyl choloride. Yellow colored solid so obtained was re crystallized using methanol characterized on the basis of spectral studies.

EI-MS (*m/z* %):370(M^+ 40), 335 (25), 278(43),267(37),207 (62),147 (100).

UV(*λmax*.nm): 235,246352,430.

IR ν_{max},cm-^{1}: 2829(C-H)1600cm^{-1} (C=C,C=N)1354-1383 (C-H).

^{1}H NMR: ($CDCl_3$ 400 MHZ) δ: 7.09 (H-4), 3.19 (H-5), 4.92 (H-6), 9.72 (H-8), 8.36(H-12), 6.1 (H-13), 8.7 (H-14), 7.45-7.98(ArH)),4.24 (H-15))and 4.1(H-16).

^{13}C NMR($CDCl_3$ 100 MHZ) δ: 121(C-1), 132 (1a), 139 (C-2, -3), 122 (C-4), 128 (4a), 24(C-5), 56 (C6), 149 (C-8), 136 (C-8a), 146 (C-9),147 (C-10), 111 (C-11), 123 (C-12), 124 (C-12a), 106(C-13), 153 (C-13a), 103 (C-14), 178(C=O) and 145-159 (2xC_6H_5).

Synthesis of (9,10acetoxy 5,6dihydro [1,3]dioxolo [4,5g] isoquino 3,2a]isoquinolin-7-ium) [4]

A mixture (1mmole), acetic anhydride (1.5mmole) and Zr(HSO4) (.05mmole) in n - hexane (5 ml) was stirred at room temperature. The progress of the reaction was monitored by TLC or GC. After completion of the reaction, solvent was evaporated and water was added (10 ml) wherein bright yellow colored product was obtained re crystallized using chloroform methanol characterized on the basis of spectral studies.

EI-MS (m/z %):382(

M+39), 336(55), 322,(43)278(43),267(37),207 (76), 147 (100)

UV(λmax.nm):255,256367,445.

IR νmax,cm-1:3100,2829 (C-H)1600cm-1 (C =C, C=N)1400-1383 (NO).

1H NMR: (CDCl3 400 MHZ) δ::7.0 (H-4), 3.2 (H-5), 4.9 (H-6), 9.8 (H-8), 8.20(H-11) 8.30(H-12), 6.1 (H-13), 8.7 (H-14) and 2.23(2xCOCH3) .

13C NMR(CDCl3 100 MHZ) δ: 121(C-1), 132 (1a), 139 (C-2, -3), 122 (C-4), 128 (4a), 24(C-5), 56 (C6), 149 (C-8), 136 (C-8a), 146 (C-9),147 (C-10), 114 (C-11), 123 (C-12), 124 (C-12a) 124 (C-12a), 106(C-13), 153 (C-13a), 103 (C-14), 178(CO) and 25,26(2xCH3)

^{13}C NMR($CDCl_3$ 100 MHZ) δ: 122(C-1), 132 (1a), 139 (C-2, -3), 122 (C-4), 128 (4a), 24(C-5), 56 (C6), 149 (C-8), 136 (C-8a), 146 (C-9),147 (C-10), 114 (C-11), 123 (C-12), 124 (C-12a),

Antioxidant Activity

The molecule of 1,1-diphenyl-2-picrylhydrazyl(α,α-dipheny-β-picrylhydrazyl;cteriDPPH:1) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole , so that the molecules do not dimerise, as would be the case with most other free radicals . the delocalization also gives rise to deep violet colour , characterized by an absorption band in ethanol solution centered at about 517 nm. Activity is done under following headings.

A.Preparation of DPPH

DPPH is highly oxidisable compound. It oxidized in light , so DPPH is prepared in dark . Weigh accurately 10 mg DPPH and dissolve in solvent. Generally Methanol and for some cases Ethanol is used as a solvent for DPPH.

B. Preparation of standard Ascorbic acid s7 olution and different concentration of Berberine and its derivative

Ascorbic acid is a strong oxidizing agent. It is taken as standard. Standard solution of ascorbic acid is prepared viz.50, 100, 200,300,400 and 500µg/ml. Different concentration of test sample which is to be examined for antioxidant activity is prepared . viz. 250 , 500, 750, and 1000µg/ml.

C.Preparation of test sample and standard sample

3 ml of different concentration of test sample of prepared Berberine and its derivative and standard (ascorbic acid) were mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 30 minutes . when procedure is done than absorbance is taken with the help of U. V. Spectrophotometer at 517 nm .

Results and Discussion

Drugs of plant origin have better compatibility with human system as the compounds occurring in plants already have biological functions and may have more biologically relevant chemistry to human system, hence lesser side effects. Remarkable diversity in chemical structures and biological activities of the naturally occurring secondary metabolites which make them important in many ways: direct use as therapeutic agents, utility as biochemical and molecular probes and utility as prototype lead compounds for the development of new synthetic or semi synthetic drugs. The initial step in the discovery of a new drug is the lead identification. Berberine, a quaternary protoberberine isoquinoline alkaloid, is a well-known naturally occurring medicine derived from the root and the stem bark of numerous clinically important medicinal plants. The mechanism of action of the highly aromatic nearly planar quaternary structure of berberine is attributed to its ability to intercalate with DNA. The intercalation in combination with the inhibition of protein biosynthes (which is the major mode of action of berberine) could be responsible for the observed cytotoxic effect because both targets are central to all living cells In present study attempts has been made to isolate Berberine from *Berberis aristata* and carry out its synthetic modification to establish structure activity relationship as as new antimicrobial and antioxidant substances. Berberine was isolated from the roots of *Berberis aristata*.The alkaloidal nature of Berberine was determined with the help of Dragendorff and Wagner'sreagents. Its melting point was measured to be 145°C. Co-TLC of the compound with a market sample of Berberine showed similar Rf value. The molar mass of the compound was found to 336 ($C_{20}H_{18}NO_4^+$) from a m/z value 336.15 (base peak) in its mass

spectrum. Other intense peaks at 322and 278 found in the MS were due to the loss of one CH_2 and two OCH_3 fragments respectively from m/z 336.The ^{1}HNMR showed the signals at δ 7.6 (H-1),7.0 (H-4) 9.8 (H-8), 8.0 (H-11),8.1 (H-12), 6.1 (H-13), 8.7 (H-14), for substituted aromatic rings , for aliphatic protons at 3.2 (H-5), 4.9 (H-6), 4.2 (H-15) and 4.1 (H-16) ppm well comparable to spectral data of authentic sample. Berberine so isolated was subjected to derivitization to study structure activity relationship firstly by demethylation at position 9(I) and 10 (II) and then converting to diacetoxy (III) and dibenzoxy derivatives (IV). Synthesized compounds were characterized on the basis of spectral data. All the newly synthesized derivatives incorporated with while other derivatives showed decrease in activity as compared to berberine. Increase in dose also caused no betterment in results however 9,10-dibezoxy derivative(4) at dose of 500(µg/ml had marked effect of 78% DPPH Radical Scavenging activity.

Conclusion

In the present study Berberine was isolated from *Berberis aristata* roots acting as a lead compound and synthetic modifications (figure-1) were attempted to establish structure activity relationship as new antioxidant. The Results revealed that methoxy gps in berberine needs to be retained in the skeleton, further attempts could be made to extend the series with incorporation of other groups for better results.

Table -1 Antioxidant Activity of Test and Standard samples.
Concentration and % Antioxidant activity of Test and Standard samples

Test Samples (% antioxidant activity)					Standard Samples(% antioxidant activity)	
Concentration of Test samples (µg/ml)	I	II	III	IV	Ascorbic acid	Concentration of Standard samples (µg/ml)
250	61.46	48.43	54.70	60.98	96.98	50
					96.61	100
500	41.02	39.74	78.03	18.74	97.29	200
					97.10	300
750	27.6	49.39	72	7.72	97.23	400
1000	20.59	56.47	38.69	3.45	97.17	500

chemotherapeutic pharmacophores along with isolated berberine were evaluated for their in vitro antioxidant activity as compared to standard ascorbic acid summarized in table -1. From the studies, it could be observed that berberine has very good antioxidant activity (DPPH Radical Scavenging Activity 61% which is comparable to the standard drug Ascorbic acid (DPPH Radical Scavenging Activity, 97.2% @250(µg/ml)). 9,10- diacetoxy berberine (3) has comparable effect (60% antioxidant activity)

Conflict of Interests

The authors declare the absence of conflict of interests.

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Determination of Phytochemical Content and Antioxidant Activity in Methanol and Aqueous Extract of *Acmella paniculata*: A Comparative Study

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Abstract-Most of the medicinal plants were with a long history of use in folk medicine against a variety of diseases. Recently, many researchers have taken a great interest on medicinal plants for their phytochemical constituents and biological activities including antimicrobial activity. *A.paniculata* is locally known as "Mariti" is a member of the family, Asteraceae. This species is famous as a folklore remedy for toothache and the throat and gums infection, earning it the English name "toothache plant" It is used in the treatment of rheumatism, fever dysentery, constipation. Leaf and root extracts were investigated for their phytochemical and antioxidant properties using methanolic solvent. Total phenolics, total falvonoids and total flavonols were estimated by three different methods. Antioxident activities were also determined in all extracts .In the finding it was observed that the leaves of *A. Paniculata* contains high concentration of phytochemicals and significant amount of antioxidant activity. Result of the study revealed the methanolic extract of leaves of *A. Paniculata* contains higher amount of phenolics i.e. 799.125 µg Gallic acid extract/mg extract, total flavonoids i.e. 886.0294 µg quercetin eq/mg extract , total flavanol i.e. 980.1471 µg quercetin eq/mg extract, than methanolic root and aqueous leaf and roots. This study also revealed that the methanolic leaves of *A. Paniculata showed* significant total antioxidant activity i.e. 131.3766 µg ascorbic acid eq/mg extract The highest IC50 value was observed in aqueous leaf (757.5758 µg / ml) and root extract (588.2353 µg/ml) while methanolic root (384.6154 µg/ ml) and leaf (406.5041 µg/ml) shows lowest IC_{50} value and in methanolic leaf and methanolic root IC_{50} value of methanolic leaf is lower than methanolic root. That is why the methanolic leaf extract has better DPPH radical scavenging activity than methanolic root. By comparing the IC_{50} value of methanolic and aq. Leaf and roots with IC50 value of ascorbic acid and BHT, it was found that Ascorbic acid had best DPPH radical scavenging activity among all. So, the overall result obtained from the study showed that methanolic extract of leaves of *A. Paniculata* contains higher amount of phytochemical content as compared to roots and leaf extracts of *A. Paniculata.* It also showed significant amount of antioxidant activity.All the experimental results suggested that the methanol extracts of *A. Paniculata* contains constituents having significant phytochemical and antioxidant properties.

Introduction

Knowledge and belief systems which use plants and animal based remedies maintain well being (WHO, 2003). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Plants are being an effective source of both traditional and modern medicines, a genuinely useful for primary healthcare. Plants have been rich source of medicine because they produce wide range array of bioactive molecules Agharkar, S.P. (1991). The World Health Organization (WHO) estimates that about 80%

of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs.

The people of India have a very longstanding tradition in the use of natural medicines and the local practices are still quite common in the treatment of diseases Srinivasan *et al.,* (2001). However, studies on plants are very limited. It is estimated that 2, 65,000 flowering species grace the earth, of these less than 1% have been studied exhaustively for their chemical composition and medicinal values until early 20thcentury, plants are the only known antimicrobials, Aboaba *et al*., (2006). However, since the advent of antibiotics from bacterial and fungal sources in 1950s, the use of plant derivatives antimicrobials is virtually nonexistent. It is reported that an average two to three antibiotics derived from microorganisms are launched every year, Atlas, (1997). After a down turn in the discovery of new microbial agents from microorganisms, it was quickly realized that plants were an excellent source for new antibiotics. There are several reports on the antimicrobial activity of different herbs in different regions of the world.

Acmella paniculata is a genus of plants in the aster family, Asteraceae, described as a genus in 1807. It is native to the Americas and has been introduced to Asia, Africa, the Pacific islands, and Australia. The wild populations naturally grown in a total geographical area of around 90,000 Sq. km and elevation ranging from 200–3000 m from the mean sea level covering tropical and temperate region of Eastern Himalaya.

One familiar species is *Acmella paniculata* which has been widely cultivated for centuries. Its synonyms are *Spilanthes calva, Spilanthes paniculata*. Its common name are Akkalgaro, , Mariti. *Acmella paniculata* is a medicinally important species commonly known as toothache plant among the Bengali community, and Bud and Marsang among the Nyishi and Adi community of Arunachal Himalayan Region of India. The genus comprises of 30 species and nine additional infra specific taxa that are mainly distributed in the tropical and subtropical regions around the world . It is also called Para cress or Eyeball plant, which is native to the tropics of Brazil, and is grown as both medicinal and ornamental species in various parts of the world.It is used for food and medicine, and as an insecticide and an ornamental plant. Its common use as an herbal remedy for toothache and oral infections earned it the nickname toothache plant. These are annual or perennial herbs with branching stems usually reaching 10 to 20 centimeters in length, growing prostrate or erect. The oppositely arranged leaves are smooth-edged or toothed, opposite, ovate, acute, glabrous and usually have rough or soft hairs. The flower heads are usually solitary at the tips of the stem branches, or occasionally borne in inflorescences. There are several too many disc florets with bell-shaped throats and 4 or 5 triangular lobes, usually yellow, or sometimes orange. Its fruits are 0.15-0.2 cm long, oblong, compressed, glabrous. The whole plant, particularly the floral head is very pungent chewings, the head relives toothache or a tincture made from the flower heads and applied in some lint to the teeth and gums, even more effective against toothache. .

Shariful *et.al.,(*2015) reported that the presence of different bioactive component like phenolic and flavonoid play an antioxidant activity through scavenging oxygen radical and inhibit per oxidation.Flavonoids present in the plant are an important part of the diet because of their effect on human nutrition (Frankle, 1988). These phytochemicals can modulate lipid peroxidation in atherogenesis, thrombosis and carcinogenesis. The known properties o flavonoids include free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and anti-inflamatory action (Frankle, 1965).

The bioactive compounds like akylamide,

terpenes, esters and other compounds in *Acmella paniculata* exhibit various biological activities like radical scavenging activity, diuretic (Rajesh *et al*., 2011), immunomodulatory (Savadi *et al*., 2010), antipyretic (Trease and Evans 1972; Rajnarayan *et al*., 2001). The plant is also used in traditional system of medicine for treatment of various diseases and complications including infection of throat and paralysis of tongue. It is a popular remedy for stammering in children and as diuretic (The Wealth of India, 2004).

Material and Methods

The present investigation was performed in department of biochemistry Dolphin (PG) institute of biomedical and natural science, Manduwala, Dehradun, Uttarakhand, India.

Plant material collection

Plant material was freshly collected in the month of March, 2018 from Kandoli (Manduwala, Dehradun) region. Geographical coordinate of the collecting area is 30° 25' 28''North, 77° 52' 57" East at altitude of 25ft or 764m from sea level. After collection, the different plant parts (leaves and roots) were separated very carefully. Plant material was washed carefully and damaged portion of the plant parts were also removed very carefully. Leaves and roots were chopped and air dried in a shady place for 10 days. After that, these plant parts were dried in hot air oven at 40°C for 10-12 hours so that additional moisture was removed from the plant parts. The fully dried plant parts were then grinded into powder with the help of a mechanical grinder.

Preparation of extracts

Fine plant powder (2g) was weighed and placed in 100 ml beaker containing 30ml of methanol. Then the plant sample was placed on a shaker for overnight at room temperature in dark. The solution was then filtered through Whatman no.1 filter paper, the filtrate was collected in 50ml beaker and remnants were again mixed using 10 ml methanol. The filtrate collected was evaporated using a rotator evaporator at a temperature between 56°C to 60°C. Dried extract was diluted with methanol and stock solution of 10mg/ml extract was prepared. All the extract were stored at 4°C. The working solution was made by diluting the stock solution with methanol.

Determination of percentage of yield of extract

The percentage yield of the extracts was based on dry weight and was calculated as follows:

$$\text{Yield of extract \%} = \frac{\text{weight of extracts(gm)}}{\text{weight of dry plant powder (gm)}} \times 100$$

Phytochemical Analysis

Total phenolics content: The total phenolics contents of the extract was estimated according to method folin-Ciocalteu described by Swain and Hills (1959).

Total Flavonoid content: Flavonoids contain in the extract was determined by aluminium chloride colorimetric assay as explained by kim. *et al*., (2003).

Total Antioxidant activity: The antioxidant activity of extract was estimated by phosphomolybedenum method described by Prieto *et al*., (1999).

DPPH Radical Scavenging activity: The free radical scavenging capacity of the extract was determined using DPPH method described by Braca *et al*., (2001).

Results and Discussion

This study was performed in the department of biochemistry, Dolphin (PG) Institute of Biomedical and Natural Science, Manduwala, Dehradun, Uttrakhand. The study was conducted to estimate photochemical content and antioxidant activity in the roots and leaves

of *Acmella paniculata* and the result of the study are as follows.

Percentage Yield of extract

Percentage yield for the extract of leaf and roots of *A. Paniculata w*ere determined based on dry weight as described above and presented in Figure 1. The aqueous leaf had the highest yield 13.5% followed by aqueous root and methanolic leaf and root.

Total Phenolic Content

From the present study, it was observed that total phenolic content present in the methanolic leaf of *A. Paniculata* is **799.12 µg Galic acid extract/mg extract** and in methanolic root, total phenolic content is 764.12. The total phenolic content in the aqueous leaf is 669.12 and total phenolic content in aqueous root is 512.87.This is shown in figure-2.

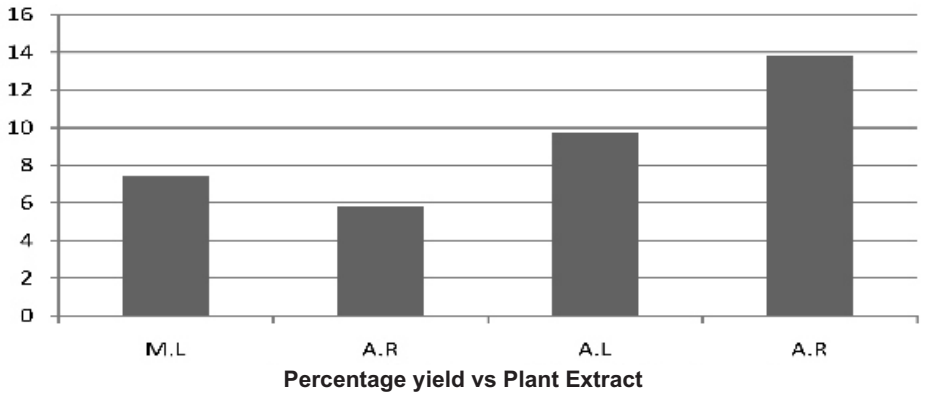


Figure-1 Percentage Yield of Extract

Phytochemical Estimation

Phytochemicals are the chemical compounds that occur naturally in the plant and have protective and disease- preservative properties, but are not essential nutrients, however an analysis of phytochemical is vital to make proper use of any medicinal plant.

Previous study conducted on *S. Acmella* by **Tanwar *et al.*,(2011)** showed that the total phenolic content in leaf extract of *S. Acmella* was 523± 1.6 mg GAE g extract and in root total phenolic content was 32±0.75 mg GAE/g extract. Thus leaf extract show higher phenolic content. By comparing the total phenolic content

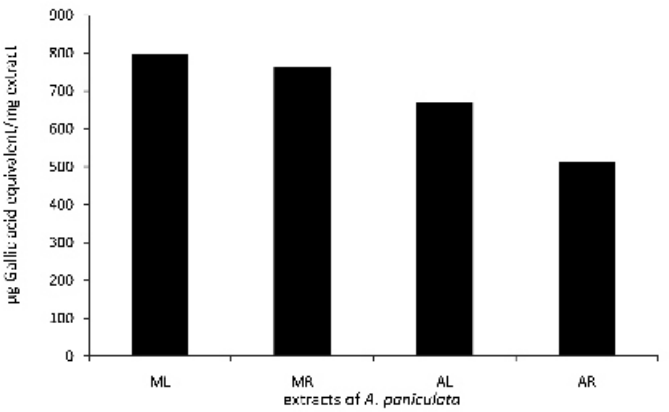


Figure-2 Total Phenolic Content in Methanol and Aqueous Leaf and Root Extracts of *A. Paniculata*

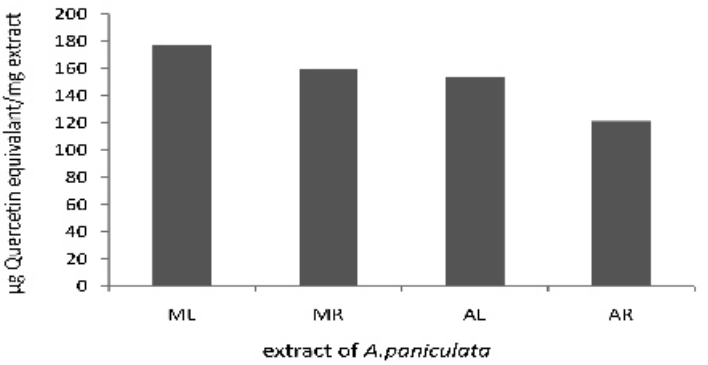
of leaves of *A.paniculata* with the leaves of *S. Paniculata* leaves, this study revealed that leaves contain higher phenolic content than roots.

Total flavonoid content

Aluminium chloride colorimetric method was used for determination of total flavonoid contants of the methanol extract of leaf and roots of *A. Paniculata* with reference to a quercetin standard curve. TFC was expressed as µg of quercetin equivalent per mg of extract (µg quarcetin eq/mg extract). The methanolic extract of leaf of *A. Paniculata* contains eq/mg extract. This is shown in figure 3.The leaves of *A. Paniculata* are rich source of flavanoids than roots as per results. Methanolic leaf have more flavonoid content then aq. Leaf. From the previous literature of Senguttuvan *et al*., (2014) on Hypochaeris redicata it was seen that methanolic extract of leaf of H.radicata showed higher flavonoid content than root extracts. As the plant is of the same family, this literature supports present study finding.

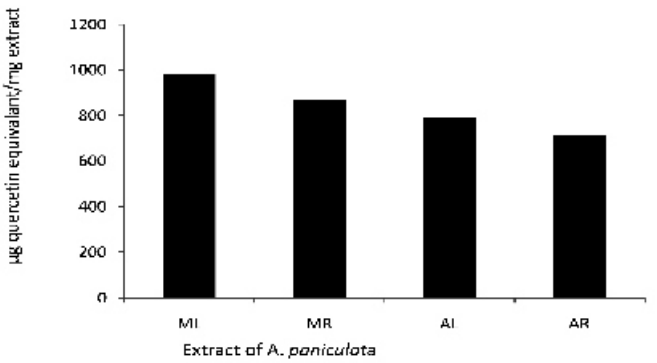
Total Flavonol content

In the present study, total flavonol present in



Figuare-3 Total Flavanoid Content in Methanolic Leaf and Root Extracts and aq. Leaf and Root of A. *Paniculata*

886.029 µg quercetin eq/mg extract and methanolic root of *A. Paniculata* contains 796.32 µg quercetin eq/mg extract. The aqueous extract of leaf of *A. Paniculata* contains 766.91 µg quercitin eq/mg extract and aqueous root contains 666.61 µg quercetin methanolic leaf is 980.14 µg quercetin eq/mg extract and in methanolic root of *A. Paniculata* contains 869.85 µg quecetin eq/ mg extract. The total flavonol content in aq. Leaf is788.97µg quercetin eq/mg extract.



Figuare-4 Total Flavonol Content in Methanolic and aq. Root and Leaves Extracts of *Acmella paniculata*

The estimation of total flavonol present in methanoilic extract of leaves and roots of *Acmella paniculata* was done by method given by Yermkov, *et. al*., (1987). In this study, it was estimated that the leaf extract contains 275±8.91mg quercitin eq/g extract and root extract contains 35.22± 1.01 mg/g extract. This is shown in figure-4.The extract of leaf contains the higher amount of flavonols followed by the root extract.

Antioxident Activity

The antioxidant properties of many plants mainly contributed by phenolic compounds present in them (Brown and Rice-Evans, 1998 and Krings and Berger, 2001). Phenols and flavonoids are active antioxidant compounds showing many other medicinal properties.

Total Antioxidant activity

In the present study total antioxidant activity of methanolic leaf is 131.37 µg ascorbic acid eq/mg extract and methanolic root of *A. Paniculata* contains 117.15 µg ascorbic acid eq/mg extract. The total antioxidant activity in aq. leaf is 104.46 µg ascorbic acid eq/mg extract and in aq. Root total antioxidant activity is 79.08 µg ascorbic acid eq/mg extract. This is shown in figure- 5.

The total antioxidant activity of the extract was determined by phosphomolybdenum method described by **Prieto *et al*., (1999).** The leaves of *A. Paniculata* shows 74.01± 0.73 mg ascorbic acid eq/g extract and the root extract shows 34.67005± 1.46556 mg ascorbic acid eq/g extract total antioxidant activity that is leaves of plants have higher antioxidant activity than the roots.

The previous study performed by **Kamanashis *et al.*, (2014)** on medicinal herb also showed higher antioxidant activity in leaf extract than root extracts, thus it justified the present work.

DPPH radical scavenging activity

DPPH radical scavenging assay is the most common method used to evaluate antioxidant activity because it is simple and rapid. The mechanism of radical scavenging activity employed in the assay via the hydrogen atom denoted by phenolic compound with antioxidant activity which binds to the purple coloured DPPH thus reducing DPPH which turns yellow in colour (Gulsin *et al*., 2007). IC_{50} stands for the compound concentration where half maximal inhibition is observed. DPPH radical scavenging activity is inversely proportional to IC_{50}

The methanolic leaf extract had IC_{50} value were 384.61 µg/ ml and methanolic root had value 406.50 µg/ml, the aq. Leaf extract had value 757.57 µg / ml and aq. Root have 588.23 µg/ml.

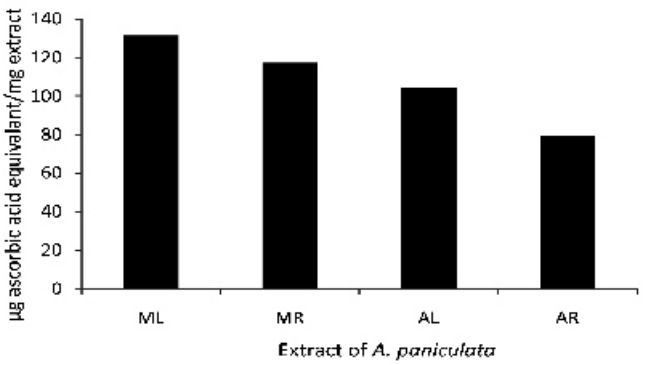


Figure-5 Total Antioxidant Activity in Extracts of *A. Paniculata*

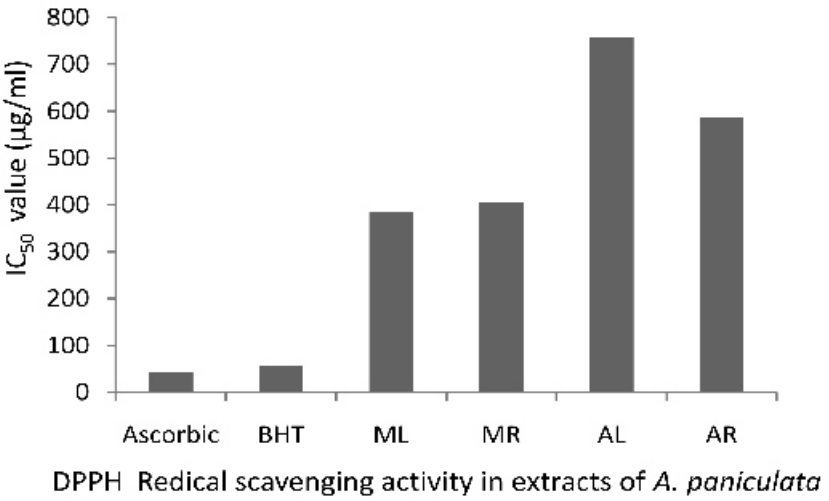


Figure-6 DPPH Radical Scavenging Activity in Extract of *A. Paniculata*

The highest IC_{50} value was observed in aqueous leaf extract while methanolic leaf shows lowest IC_{50} value. That is why the methanolic leaf and root have better DPPH radical scavenging activity than aqueous leaf and roots. In the methanolic leaf and root, best DPPH radical scavenging activity is better in methanolic leaf extract. By comparing the IC_{50} value of methanolic and aq. leaf and roots with IC_{50} value of ascorbic acid and BHT, it was found that Ascorbic acid had best DPPH radical scavenging activity among all.This is shown in figure 6. Previous study of Tamwar *et al*., (2011) also showed the highest scavenging activity in roots, thus supported the findings of present work. The study of Senguttuvan, *et al*., (2014) also reported higher DPPH radical scavenging activity of roots than leaves.

Conclusion

Acmella paniculata (Asteteracaea) locally known as plant is an annual or short lived perennia l herb found in all about all parts of the world. *Acmella paniculata* is an important medicnal plant with rich source of therapeutic and medicinal constituents of family asteraceae. *Acmella paniculata* contains different phytochemicals like alkaloids glycocides, phenolics and saponins, steroids, esters, alcohol, hydrocarbons, terpenes and gums. The terpenoids present in A. Paniculata are copaene, cadinene, caryophyllene and logifolin. *A.paniculata* has been used as a tradicnal medicine for toothache, rheumatism and fever. The flower heads are chewed to relive the toothache and the other mouth related troubles. Leaves are used externally for treatment of skin diseases.

In Phytochemical contents the estimation of total phenolic, total flavooids were performed. The total phenolic content of the extract was estimated according to the method Folin-Ciocalteu method. Total flavonoid content in the extract was determined by the aluminium chloride colorimetric assay and total flavonol content in extract was estimated by method given by Yermakov. Antioxident activity was detrmined by three methods viz total antioxidant activity, DPPH radical scavenging activity and reducing power. Total antioxidant activity of the extract was determined by phosphomoly bedenum method. DPPH radical scavenging capacity of the extract was determined by the method of Oyaizu.

From the present study, it was observed that total phenolic content present in the methanolic leaves and aqueous leaf of *A. Paniculata* are 799.125 µg Galic acid extract/mg extract and 669.12 µg GAE/ mg extract. And in methanolic and aqueous roots of *A. Paniculata* are 512.87 µg GAE/ mg extract and 764.125µg GAE/ mg extract. Total flavonoid content in methanolic and aq. Leves are 886.0294 µg quercetin eq/mg extract and 766.91 µg quercitin eq/mg extract ,and in total methanolic and aq. Roots extract are 666.61 µg quercetin eq/mg and 796.32 µg quercetin eq/mg extract. The total flavonol content in methanolic and aqueous leaves and roots are 980.14 µg quercetin eq/mg extract ,869.85 µg quecetin eq/ mg extract ,788.97µg quercetin eq/mg extract and 715.44 µg quecetin eq/ mg extract. Additionally study reveled that the methanolic leaves of *A. Paniculata* showed 131.3766 µg ascorbic acid eq/mg extract and methanolic root showed 117.15 µg ascorbic acid eq/mg extract and aqueous leaf showed 104.46 µg ascorbic acid eq/mg extract and in aq. Root showed 79.08629 µg ascorbic acid eq/mg extract total antioxidant activity. The methanolic leaf extract had IC50 value were 384.61 µg/ ml and methanolic root had value 406.5041 µg/ml the aq. Leaf extract had IC50 value 757.57 µg / ml and aq. Root have 588.23 µg/ml. The highest IC50 value was observed in aqueous leaf and root extract while methanolic root and leaf shows lowest IC50 value and in methanolic leaf and methanolic root the IC50 value of methanolic leaf is lower than methanolic root. That is why the methanolic leaf extract is better DPPH radical scavenging activity then methanolic root. By comparing the IC50 value of methanolic and aq. Leaf and roots with IC50 value of ascorbic acid and BHT, it was found that Ascorbic acid had best DPPH radical scavenging activity among all. So, the overall result obtained from the study showed that methanolic extract of leaves of A. Paniculata contains higher amount of phytochemical content as compared to roots and leaf extracts of *A. Paniculata* also showed significant amount of antioxidant activity then roots.

Based on overall results of study, it was seen that the leaves of *A.paniculata* showed significant amount of antioxidant activity and contain higher phytochemical concentration. So on the basis of these finding it can be concluded that both leaves and roots of *A. Paniculata* are rich source of phytochemical content as well antioxidant activities. According to previous literature as well as these findings, this Plant serves as good source of various threauptically important phytochemicals and those therapeutic chemicals have good antioxidant activity. So, in future more studies can be performed on these aspects in an advance level.

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Antiatherogenic and Antioxidant Impact of α-Tocopherol and Tocotrienols (Tocomin) on Copper Mediated Oxidative Modification of Low Density Lipoprotein in normal subjects

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Abstract-The oxidation of LDL cholesterol has been implicated in the development of atherosclerosis because macrophages a type of immune system cell absorb oxidized LDL in the arterial wall and can form foam cells an important step in lesion formation. Epidemiological and some clinical studies have shown a protective role for vitamin E against Cardiovascular diseases**.** The purpose of this study was to determine the anti-oxidant impact of two forms of Vitamin E, α-Tocopherol and Tocotrienol on Copper mediated *in vitro* oxidation of LDL and its subfractions. Both α-. Tocopherol and Tocotrienol showed a significant decrease in Conjugate diene , Lipid hyderoperoxides and TBARS formation. However Tocotrienol proved to be more potent than α-Tocopherol. A decrease of 18.61% and 2.41% was observed in CD formation during LDL oxidation on the addition of Tocotrienol and α-Tocopherol respectively. Similarly LHPO formation also reduced by 53.39% and 36.16% and TBARS formation by 32.97% and 21.49% on the addition of Tocotrienol and α-Tocopherol respectively. In case of oxidation of and more atherogenic and a strong and independent predictor of CVD, sd-LDL, this decrease was found to be 22.85%, 49.21% and 32.25% when Tocotrienol was added and 11.37%, 33.89% and 15.48% when α-Tocopherol was added in CD , LHPO and TBARS formation respectively. Similarly when lb-LDL was subjected to oxidation, Tocotrienol and α-Tocopherol again blocked the rise in above mentioned parameters. However the Lag phase successfully increased in presence of both Tocotrienol and α-Tocopherol by 77.4% and 70.58% in case of LDL oxidation; 50% and 16.67% in case of sd-LDL oxidation and 93.34% and 40% respectively in case of lb-LDL oxidation. Hence Tocotrienols and α-Tocopherols proved to be effective against LDL oxidation with the former one being more potent.

Key words: α-Tocopherol, Tocotrienol, LDL Oxidation, CVD

Introduction

According to WHO, Cardiovascular diseases or CVDs are the number one causes of death globally: more people die annually from CVDs than from any other cause. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke. The number of people, who die from CVDs, mainly from heart disease and stroke, will increase to reach 23.3 million by 2030. Most cardiovascular diseases can be prevented by addressing risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, high blood pressure, diabetes and raised lipids.

Increased LDL oxidation is associated with coronary artery disease. The predictive value of circulating oxidized LDL is additive to the Global Risk Assessment Score for cardiovascular risk prediction based on age, gender, total and HDL cholesterol, diabetes,

hypertension, and smoking. Circulating oxidized LDL does not originate from extensive metal ion-induced oxidation in the blood but from mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase. Oxidized LDL induces atherosclerosis by stimulating monocyte infiltration and smooth muscle cell migration and proliferation. It contributes to atherothrombosis by inducing endothelial cell apoptosis, and thus plaque erosion, by impairing the anticoagulant balance in endothelium, stimulating tissue factor production by smooth muscle cells and inducing apoptosis in macrophages.

One of the various forms of Vitamin E, α-Tocopherol, is an important lipid-soluble antioxidant. It performs its functions as antioxidant in the glutathione peroxidase pathway (Wefers and Sics, 1988) and protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Traber and Atkinson, 2007). This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidized α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Wang and Quinn, 1999). Other than antioxidant protection of LDL α-Tocopherol may even have important physiologic effects that are not directly related to the protection of LDL against oxidation in vivo. For example, α-tocopherol has been shown to influence leukocyte adhesion to endothelial cells (Faruqi *et al*., 1994) , monocyte transmigration (Navab *et al*., 1991) and oxidant-mediated cytotoxicity (Hennig *et al*., 1987) Moreover, α-tocopherol is known to inhibit protein kinase C in vascular smooth muscle cells (Boscoboinik *et al*., 1991) and protein kinase C activation has been implicated in vascular disease due to diabetes (Tesfamariam *et al*., 1991)and ox-LDL (Ohgushi *et al*., 1993). Thus, these alternative effects of α-tocopherol have the potential to influence processes that are known to impair endothelium-dependent arterial relaxation. Compared with Tocopherols, Tocotrienols are sparsely studied. Less than 1% of PubMed papers on vitamin E relate to Tocotrienols (Sen *et al*., 2006) The current research direction is starting to give more prominence to the tocotrienols, the lesser known but more potent antioxidants in the vitamin E family. Some studies have suggested that Tocotrienols have specialized roles in cholesterol reduction (Das *et al*., 2008) by inhibiting the activity of HMG-CoA reductase; δ-tocotrienol blocks processing of sterol regulatory element binding proteins (SREBPs). Oral consumption of Tocotrienols is also thought to protect against stroke-associated brain damage *in vivo* (Khanna *et al*., 2005). The purpose of this study was to examine the antioxidant impact of α-Tocopherol and Tocotrienol on *in vitro* Copper mediated oxidative modification of LDL.

Material and Methods

Chemicals

Softgel capsules (TOCOVID™ Supra Bio™), with its new biooptimum absorption system, provides 200% to 300% higher absorption of its natural combination of phytonutrients in our body, each contains 50% Tocomin®, in which d-β-tocotrienol (15.38 mg), d-β-tocotrienol (28.20 mg), d-β-tocotrienol (6.42 mg) and d-β-tocopherol (15.32 mg or 29.90 IU) were supplied as a gift from CAROTECH BHD, Chemor, Malaysia. All other chemicals and reagents used in this study were of analytical grade.

Estimation

Plasma triglyceride (Trinder, 1969), Plasma cholesterol, LDL and HDL and its subfractions (Annino and Giese, 1976), Plasma VLDL-C (Friedwald *et al*., 1972), HDL and its fractions-HDL_2, HDL_3 (Patsch *et al*., 1989), isolation of sd-LDL and lb-LDL (Hirano *et al*., 2004), *ex vivo* and *in vitro* Cu^{++} -mediated LDL, sd-LDL and lb-LDL oxidation (Esterbauer *et al*.,

Table-1 Average age, Body weight, Body mass index and lipid parameters of the study subjects.

PARAMETERS	VALUES
Age (years)	23.83±0.686*
Body weight (Kg)	64.70±1.14
BMI (Kg/m^2)	24.61±3.32
Triglycerides (mg/dl)	104.77±1.01
Total cholesterol (mg/dl)	106.19±1.60
VLDL -C (mg/dl)	23.41±0.368
LDL-C (mg/dl)	82.98±0.278
sd-LDL (mg/dl)	26.22±0.590
lb-LDL (mg/dl)	55.17±0.801
HDL-C (mg/dl)	45.49±4.17
HDL_2-C (mg/dl)	14.17±0.162
HDL_3-C (mg/dl)	30.08±0.057

*Values are mean ±SD from individual subjects

1989,1992), basal and Cu^{++} induced generation of LHPO (Nourooz-Zadeh *et al*., 1996) and MDA formation (Niehaus and Samuelsson, 1968).

Statistical evaluation

Results were expressed as mean ± SD. Statistical analysis of data was done by employing two-tailed Student t-test as described by Bennet and Franklin (Bennet and Franklin, 1967).

Experimental design

Fresh blood samples of 12 normal subjects were collected from Pathology Lab. of Sardar Bhagwan Singh Post Graduate Institute, Balawala, Dehradun . Plasma obtained from them was pooled and LDL and its sub-fractions were isolated. The average age, body weight and body mass index of the study subjects were 23.83±0.686 years, 64.70±1.14 kgs and 24.61±3.32 kg/m^2 respectively.

Results

Table-2 Effect of Tocomin and α-Tocopherol on *ex vivo* and copper-mediated in *vitro* oxidation of LDL isolated from normal subjects plasma.

LDL treatment with*	LDL oxidation						
	Conjugated dienes++			Lipid hydroperoxides#		TBAR S††	
	Basal	Maximal	Lag phase**	Basal	Maximal	Basal	Maximal
None (Control)	407.93*	1025.95 (+151.50%)+	85	2.51±0.382	527.50±0.252 (+20915.93%)§	9.96±0.493	33.26±0.881 (+233.93%)+
Tocomin	382.38* (-6.26%)+	835.00 (-18.61%)†	185 (+77.64%)	1.48±0.125[c] (-41.83%)§	245.86±0.183[a] (-53.39%)τ	5.09±0.105[a] (-48.89%)+	22.26±0.086[a] (-32.97%)†
α-Tocopherol	400.23* (-1.88%)+	1001.19 (-2.41%)†	145 (+70.58%)	1.85±0.123[b] (-26.29%)§	366.71±0.315[a] (-36.16%)τ	7.57±0.063[d] (-23.93%)+	26.11±0.163[a] (-21.49%)†

++The conjugated diene values are expressed as nmole malondialdehyde (MDA) equivalents/mg protein. Basal conjugated dienes represent the status of oxidized LDL *in vivo*. Maximal *in vitro* oxidation of LDL was achieved after 12 h incubation with $CuSO_4$. **The lag phase is defined as the interval between the intercept of the tangent of the slope of the curve with the time expressed in minutes. #The lipid hydroperoxide values are expressed as nmole hydroperoxides formed/mg protein. Basal lipid hydroperoxides represent the status of oxidized LDL *in vivo*. Maximal *in vitro* oxidation of LDL was achieved after 12 h incubation with $CuSO_4$. ††The TBARS are expressed as nmole MDA/mg protein. Basal TBARS represent the status of oxidized LDL, *in vivo*. Maximal values were obtained after 12 h of LDL oxidation. *Values are obtained from LDL, isolated from pooled normal subjects plasma (n=12), LDL incubated alone, with 10μM Tocomin, or 2.3 μM α-Tocopherol for 12 h at 37 ^{0}C. +Percent change with respect to basal value in control. †Percent decrease with respect to maximal value in control. ¶Percent increase with respect to lag phase value in control §Percent change with respect to basal value in control. τPercent decrease with respect to maximal value in control .Significantly different from control at [a]$p<0.001$, [b]$p<0.05$, [c]$p<0.02$, [d]$p<0.01$.

Effect of Tocomin and α-Tocopherol on *ex vivo* and copper mediated *in vitro* oxidation of LDL

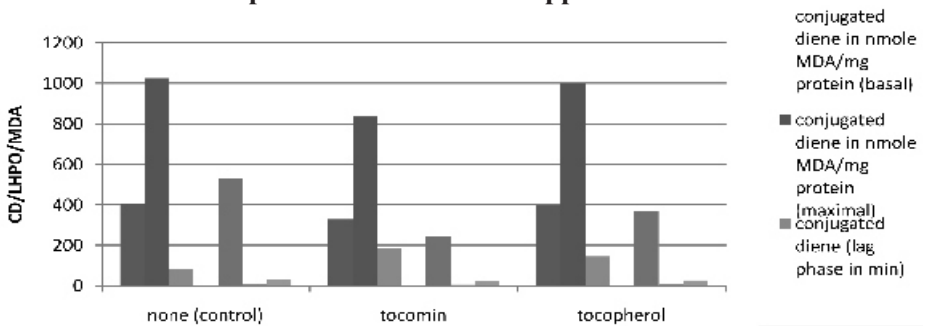

Figure-1

Table-3 Effect of tocomin and α-tocopherol on *ex vivo* and copper-mediated *in vitro* oxidation of sd -LDL isolated from normal subjects plasma

Sd-LDL treatment with*	Sd-LDL oxidation						
	Conjugated dienes++			Lipid hydroperoxides#		TBARS††	
	Basal	Maximal	Lag phase**	Basal	Maximal	Basal	Maximal
None (Control)	337.22*	1098.73 (+225.81%)+	12	2.56±0.253	477.84±0.185 (+18565.62%)§	8.70±0.121	25.58±0.226 (+194.02%)+
Tocomin	295.00* (-12.52 %)+	847.67 (-22.85%)†	18 (+50.0%)	1.69±0.252[c] (-33.98%)§	242.68±0.722[a] (-49.21%)τ	4.22±0.223[a] (-51.49%)+	17.33±0.352[a] (-32.25%)†
α-Tocopherol	322.93* (-4.23%)+	973.80 (-11.37%)†	14 (+16.67%)	2.02±0.181[b] (-21.09%)§	315.90±0.127[a] (-33.89%)τ	6.07±0.173[a] (-30.22%)+	21.62±0.195[a] (-15.48%)†

++The conjugated diene values are expressed as nmole malondialdehyde (MDA) equivalents/ mg protein. Basal conjugated dienes represent the status of oxidized sd-LDL *in vivo*. Maximal *in vitro* oxidation of sd-LDL was achieved after 12 h incubation with $CuSO_4$. **The lag phase is defined as the interval between the intercept of the tangent of the slope of the curve with the time expressed in minutes. #The lipid hydroperoxide values are expressed as nmole hydroperoxides formed/mg protein. Basal lipid hydroperoxides represent the status of oxidized sd-LDL *in vivo*. Maximal *in vitro* oxidation of sd-LDL was achieved after 12 h incubation with $CuSO_4$. ††The TBARS are expressed as nmole MDA/mg protein. Basal TBARS represent the status of oxidized sd-LDL, *in vivo*. Maximal values were obtained after 12 h of sd-LDL oxidation. *Values are obtained from sd-LDL, isolated from pooled normal subjects plasma (n=12), sd-LDL incubated alone, with 10μM Tocomin, or 2.3 μM α-Tocopherol for 12 h at 37 ^{0}C. +Percent change with respect to basal value in control. †Percent decrease with respect to maximal value in control. ¶Percent increase with respect to lag phase value in control §Percent change with respect to basal value in control. τPercent decrease with respect to maximal value in control .Significantly different from control at [a]p<0.001, [b]p<0.05, [c]p<0.02, [d]p<0.01.

Effect of Tocomin and α-Tocopherol on *ex vivo* and copper mediated *in vitro* oxidation of lb-LDL

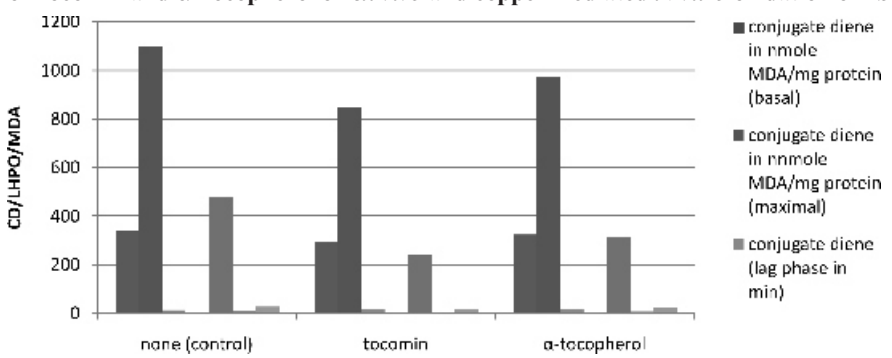

Figure-2

Table-4 Effect of tocomin and tocopherol on *ex vivo* and copper-mediated *in vitro* oxidation of lb-LDL isolated from LDL of normal subjects plasma

Lb-LDL treatment with*	Lb-LDL oxidation						
	Conjugated dienes++			Lipid hydroperoxides#		TBARS††	
	Basal	Maximal	Lag phase**	Basal	Maximal	Basal	Maximal
None (Control)	328.57	1077.22 (+227.85%)+	75	1.73±0.123	495.50±0.256 (+28541%)§	8.30±0.196	30.01±0.152 (+261.56%)+
Tocomin	296.58 (-9.73%)+	758.49 (-29.58%)†	145 (+93.34%)	0.992±0.122[d] (-42.65%)§	237.97±0.315[a] (-51.97%)τ	4.01±0.173[a] (-51.68%)+	22.02±0.219[a] (-26.62%)†
α-Tocopherol	320.71 (-2.39%)+	915.47 (-15.01%)†	105 (+40.0%)	1.36±0.121[b] (-21.38%)§	301.18±0.183[a] (-39.21%)τ	6.40±0.095[a] (-22.89%)+	25.49±0.221[a] (-15.06%)†

[++]The conjugated diene values are expressed as nmole malondialdehyde (MDA) equivalents/mg protein. Basal conjugated dienes represent the status of oxidized lb-LDL *in vivo*. Maximal *in vitro* oxidation of lb-LDL was achieved after 12 h incubation with $CuSO_4$.[**]The lag phase is defined as the interval between the intercept of the tangent of the slope of the curve with the time expressed in minutes.[#]The lipid hydroperoxide values are expressed as nmole hydroperoxides formed/mg protein. Basal lipid hydroperoxides represent the status of oxidized lb-LDL *in vivo*. Maximal *in vitro* oxidation of lb-LDL was achieved after 12 h incubation with $CuSO_4$.[††]The TBARS are expressed as nmole MDA/mg protein. Basal TBARS represent the status of oxidized lb-LDL, *in vivo*. Maximal values were obtained after 12 h of lb-LDL oxidation.[*]Values are obtained from lb-LDL, isolated from pooled normal subjects plasma (n=12), lb-LDL incubated alone, with 10μM Tocomin, or 2.3 μM α-Tocopherol for 12 h at 37 ^{0}C.[+]Percent change with respect to basal value in control.[†]Percent decrease with respect to maximal value in control.[¶]Percent increase with respect to lag phase value in control[$]Percent change with respect to basal value in control.[τ]Percent decrease with respect to maximal value in control .Significantly different from control at [a]$p<0.001$, [b]$p<0.05$, [c]$p<0.02$, [d]$p<0.01$

Effect of Tocomin and α-Tocopherol on *ex vivo* and copper mediated *in vitro* oxidation of lb-LDL

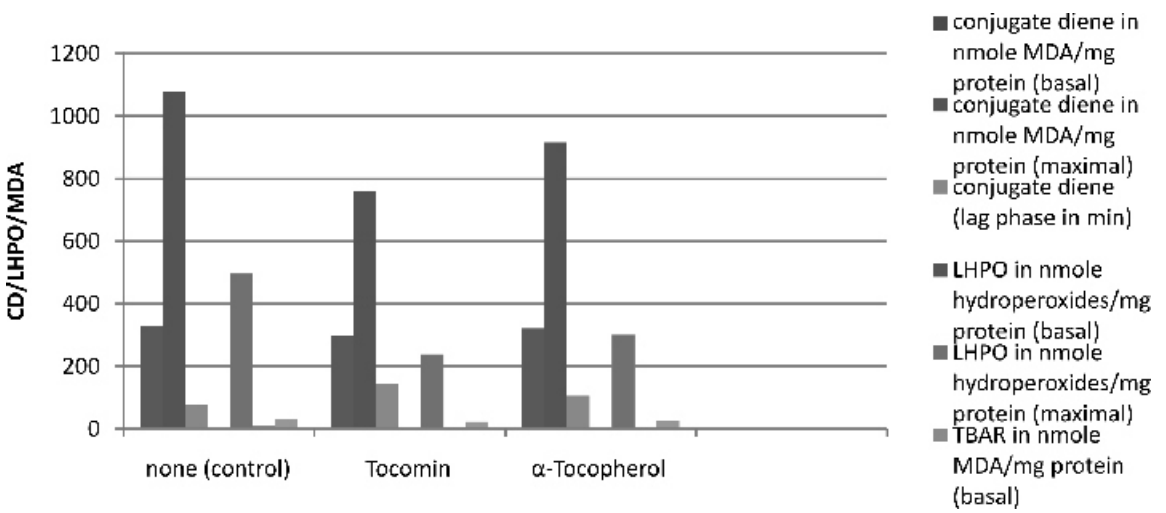

Figure-3

Results and Discussion

Because of their lipophilic character, Tocopherols are located in the membranes or with storage lipids where they are immediately available to interact with lipid hydroperoxides. They react rapidly in a non-enzymic manner unlike many other cellular antioxidants, which are dependent on enzymes, to scavenge lipid peroxyl radicals, i.e. the chain-carrying species that propagate lipid peroxidation. In model systems *in vitro*, all the Tocopherols ($\alpha > \gamma > \beta > \delta$) and Tocotrienols are good antioxidants, with Tocotrienols being the most potent. The present results demonstrated the same (Christae, 2013).

As seen in Table no.-2, when LDL was subjected to Cu^{++} mediated *in vitro* oxidation in presence of Tocotrienol (Tocomin) the basal conjugate diene formation was significantly decreased by 6.26% and maximal conjugate diene formation which was achieved after 12 hours of incubation showed a significant decrease of 18.61% as compared to the control. The lag phase as expected increased by 77.64%. In case of LHPO formation a decrease of 41.83% was observed in basal values and 53.39% in maximal values. The basal and maximal amount of TBARS also decreased in presence of Tocomin by 48.89% and 32.97% respectively. However when α-Tocopherol was used in place of Tocomin, these changes were 1.88%, 2.41%, 70.58%, 26.29%, 36.16%, 23.93% and 21.49% respectively.

In case of Cu^{++} mediated *in vitro* oxidation of the more atherogenic and a strong and an independent predictor of CVD i.e sd-LDL, same kind of results were obtained. As evident from Table no.-3 in presence of Tocotrienol the basal conjugate diene formation decreased by 12.52% whereas the maximal conjugate diene formation decreased by 22.85% as compared to control. The lag phase increased by 50% and in case of LHPO formation the decrease was 33.98% and 21.09% in basal and maximal values respectively. TBARS formation also showed a significant decrease in basal and maximal values being 51.49% and 30.22% respectively. Addition of α-Tocopherol also showed favourable results when compared to control. However it proved to be less efficient in comparison to Tocotrienols with the above mentioned changes being 4.23%, 11.37%, 16.67%, 21.09%, 33.89%, 30.22% and 15.48% respectively.

When lb-LDL was subjected to Cu^{++} mediated in vitro oxidation , in presence of Tocomin again significant changes were observed. The basal conjugate diene formation was decreased by 9.73% whereas maximal conjugate diene values were decreased by 29.58% as compared to control. The lag phase however as expected to be, increased largely i.e by 93.34%. Similar changes were observed in LHPO formation. Tocomin decreased the basal values by 42.65% and maximal values by 51.97%. The formation of TBARS also decreased in presence of Tocomin, the decrease being 51.68% in basal value and 26.62% in maximal values. In presence of α-Tocopherol these changes were 2.39%, 15.01%, 40%, 21.38%, 39.21%, 22.89% and 15.06% respectively.

Conclusion

The present results have shown that three trans double bonds containing Tocotrienols posses more potent capacity a Tocopherols to protect LDL, sd-LDL and lb-LDL from Cu^{++} catalysed oxidation in normal subjects. These results are consistent with earlier reports showing more potent anti-oxidative impact of Tocotrienols than Tocopherols (Christie, 2013). Both α-Tocopherol and Tocotrienol showed a significant decrease in Cu^{++} mediated in vitro oxidation of LDL, be it conjugate diene formation, LHPO formation or TBARS formation. Therefore, Tocopherols and Tocotrienols can be used as dietary supplements to avoid and cure cardiovascular diseases. These are cost effective as well as free from side effects.

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A Comparative Estimation of Flavonoids and Tannins in Some Barks of Indian Medicinal Trees

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Abstract-Total flavonoids and tannin content was estimated quantitatively by using UV-Vis Spectrophotometry method by aqueous extraction in the barks of *Oroxylum indicum* (L.) Kurz (Arlu), *Terminalia arjuna Wight & Arn. (Arjuna), Azadirachta indica* A. Juss (Neem), *Tecomella undulata* (G.Don) Seem (Roheda). Flavonoids may exist as free aglycone, but usually they are bound to sugar as glycosides and any one aglycone may occur in single plant in several combinations. For the measurement of total flavonoid content and tannin content original plant extract was hydrolysed and aglycone was measured before and after hydrolysis to calculate the presence of free aglycone content. Flavonoids content of the extracts in terms of rutin equivalent and Tannin content of the extract in terms of tannic acid content was measured. The flavonoid content of the Arlu, Arjun, Neem and Roheda barks was present 1.07%, 1.38%, 1.72% and 1.13% and the tannin content of the Arlu, Arjun, Neem and Roheda barks was present 6.24%, 4.87%, 6.57% & 5.02 % respectively.

Key words: Flavonoids, Taninns, *Oroxylum indicum* (L.) Kurz (Arlu), *Terminalia arjuna Wight & Arn. (Arjuna), Azadirachta indica* A. Juss (Neem), *Tecomella undulata* (G.Don) Seem (Roheda)

Introduction

Plant phenolics are secondary metabolites with diverse chemical nature and potential including phenolic acids, flavonoids, tannins, coumarins, lignans, xanthones and stilbenes (Liu, 2004; Harborne, 1980). Flavonoids and tannin detected from the bark of Arlu, Neem, Roheda and Arjun. Flavonoids play important role in imparting bright colours to flowers, fruits and berries that make the biosphere beautiful (Brouillard and Dangles, 1993). In addition to their biological, nutraceutical and clinical effects (Maimoona et al., 2011), flavonoids including proanthocyanidins are implicated in various plant defense mechanisms (Stafford, 1988). Flavonoids may act as phytoalexins which are produced in response to the attack of microorganisms (Laks and Pruner, 1989; Synder and Nicholson, 1990; Dixon, 1986). These bioactive defense compounds are also responsible for plant responses to environmental hazards, such as temperature fluctuations (Alonso et al., 2007), air pollution (Gietych and Karolewski, 1993) and UV radiation (Tegelberg et al., 2004). The increasing numbers of automobiles, industries and thermal power plants have continuous additive effect on atmospheric pollutants. Plants which are rightly spoken as the lungs of nature, act as the sinks for various pollutants by absorbing, accumulating and integrating them. Pollution can be detected at low levels and at an early stage by computing the chemical compounds as the physiological changes are faster than the morphological and anatomical parameters (Pasqualini et al., 2003). Moreover, a significant increase in total flavonoid and tannins content has been related with the increased vehicular pollution (Qayoom et al., 2009). Flavonoids and tannins have been proven to be UVB protectants in many studies in different plants (Kenneth et al., 2008; Ryan et al., 1998), including conifers (Fischbach et al.,

1999). In addition, plant phenolics not only hinder the pest attack at larval stage (Clifford et al., 1997), but also provide defense against mammalian herbivory (Harborne, 1991) acting as antifeedant. The proposed research is aimed at estimation of flavonoids and tannins in some Indian medicinal tree bark like Arlu, Neem, Roheda and Arjun. The research may have considerable advantage to agricultural and horticultural industry and authorities involved in environmental conservation.

Material and Methods

Based on the significance of total flavonoids and total tannins, experimental procedures were adapted to analyze their content obtained from bark of Arlu, Neem, Roheda and Arjun using agues extract. Flavonoids and tannins were calculated as Rutin hydrate and tanic acid equivalent respectively.

Plant material

Plant material was collected from The Himalaya Drug Company, Faridabad. The above material were dried under shade, powdered and passed through 40 mesh sieve and stored in closed container for further use.

Instruments

UV-Vis spectrophotometer (Shimadzu, UV-1800)

Reagents and solutions

The solvents used were all of analytical grade, obtained from Merck Germany. Other reagents and standards Rutin hydrate and Tanic acid, were purchased from Sigma Aldrich available commercially.

Method

Estimation of flavonoids by spectrophotometry

Sample preparation: Extracted the finely powdered test substance in methanol (1 mg in 50 ml) on a water bath at 80 ºC ± 5ºC for 30 minutes and transfer into 100 ml volumetric flask. Make up the volume with methanol and filter.

Standard preparation: Weighed 100 mg of standard rutin in 100 ml standard volumetric flask and made up the volume with methanol. Pipetted out 5 ml of the above solution in 100 ml standard volumetric flask and made up the volume with methanol.

Procedure (Standard): Take 1 ml of the standard solution in 10 ml volumetric flask. Add 1 ml of aluminum chloride reagent and add ethanol to make the volume to 10 ml. measure the optical density of the solution against the reagent blank (1 ml of aluminum chloride make up to 10 ml with ethanol) at 410 nm exactly after 15 min after he addition of regent.

Sample: Took 1 ml each of test solution in 10 ml volumetric flask and follow the same procedure as that of standard.

Blank solution: Prepared standard and sample blank solutions by diluting 1 ml of standard or sample to 10 ml with ethanol and read the OD at 410 nm after 15 min.

Calculation: Subtracted the corresponding blank values from the sample absorbance value (S-SB;T-TB). Calculate the flavonoids content with respect to Rutin and express as % w/w of flavonoid.

$$\frac{\text{Absorbance of sample}}{\text{Absorbance Of standard}} \times \frac{\text{weight of std (mg)}}{100} \times \frac{5}{100} \times \frac{\text{vol. of std taken for reaction}}{\text{Vol. of sample taken for reaction}} \times \frac{\text{Total vol. of sample}}{\text{Weight of sample (mg)}} \times \text{\% purity of standard}$$

Estimation of Tannins by Spectrophotometry

Sample preparation: Weighed accurately about 0.1 gm finely powdered sample in 100 ml purified water at 100 ºC using water bath for 1 hr, cool and decanted the dissolved extract in to 500 ml volumetric flask. Wash the residue with purified water and make the volume up mark with water. Filtered the extract through Whatman filter paper no. 1 filter paper. Discarded first 50 ml of filtrate and use next filtrate for analysis.

Standard preparation: Weighed accurately100 mg of tannic acid in 100 ml volumetric flask and make up to volume with water (stock solution). Pipetted out 1 ml from the above stock solution and made up to 100 ml with water (standard solution).

Procedure

Standard: Took 1 ml of standard solution in 10 ml volumetric flask; added 1 ml potassium ferri cyanide, and 1 ml of ferric chloride. Mixed well and make the volume up to 10 ml with purified water. Exactly after 30 min added the reagents and read the OD at 720 nm against Reagent blank.

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

Test solution: Took 0.2 ml of test solution and followed the same procedure as that of standard and measure the test absorbance (T) against reagent blank.

Test Blank: Took 0.2 ml of test solution and made up to 10 ml with purified water and measured the absorbance (TB) against water.

Results and Discussion

All the aqueous extract of the bark of Arlu, Neem, Roheda and Arjun contains flavonoids and tannins. Flavonoids exist in plants in different combinations, as glycosides so dependent on the sugar moiety, appear in aqueous fraction. However, the flavonoid content of the Arlu, Arjun, Neem and Roheda barks is present in 1.07%, 1.38%, 1.72% and 1.13% and the tannin content is present in 6.24%, 4.87%, 6.57% & 5.02 % respectively. The total flavonoid content maximum in agues fraction of the above barks as Neem, Arjun, Roheda and arlu while the total tannin content is Neem, roheda, Arjun and Arlu., all others are at their show the remarkable content in agues medium. Usually, most of the flavones and flavonols get extracted in dichloromethane and ethylacetate fractions due to their polar nature. The presence of flavonoids and simple phenolics like phenolic acid in different bark has been reported variously (Ye-sil-Celiktas et al., 2009; Senthilmohan et al., 2003; Rohdewald et al., 2002), but as mentioned earlier, plant phenolics are quite variable, including stilbenes, coumarins, tannins, lignans and xanthones as well. Stilbenes, the plant phenolics with somewhat nonpolar nature, have been reported in barks of different plants (Ito et al., 2005; Muhtadi et al., 2006; Wieslaw et al., 2001; Cui et al., 2008). Coumarins, like umbelliferone and its derivative hernarin, have been reported from different plants (Iqbal et al., 2009). The comparison of the results obtained from the methods discussed previously is made on gram bases, that is, the flavonoid content in the extracted solutions is calculated as mg rutin equivalent per 100 g of the dried plant material,

$$\frac{\text{Absorbance of sample(T-TB)}}{\text{Absorbance Of standard}} \times \frac{\text{weight of std (mg)}}{100} \times \frac{1}{100} \times \frac{\text{vol. of std. taken for reaction}}{\text{Vol. of sample taken for reaction}} \times \frac{\text{Total vol. of sample ml}}{\text{Weight of sample (mg)}} \times \text{\% purity of standard}$$

Table-1 Estimation of Favonoids and Tannins by Spectrophotometer

Sl. No.	Name	Botanical name	Part Use	Flavonoid content (%)	Taninn Content (%)
1	Arlu	***Oroxylumindicum (L.)Kurz***	Bark	1.07	6.24
2	Arjun	***Terminaliaarjuna Wight &Arn.***	Bark	1.38	4.87
3	Neem	***Azadirachtaindica A. Juss***	Bark	1.72	6.57
4	Roheda	***Tecomellaundulata (G.Don) Seem***	Bark	1.13	5.02

and the tannin content in the extracted solutions is calculated as mg tannic acid equivalent per 100 g of the dried plant material as presented in (Table 1).

Conclusion

The above different barks are rich in flavonoid and tannin content. These barks contain various types of tannins and flavonoids. The functions of flavonoids and tannins in plant is thought to be that of providing resistance against fungi and insects to them. So, Neem bark is the best in the Pharma industry and is rich in tannins. Neem also plays a role as antimicrobial activity and anti-pollutant, keeping the air fresh and this is probably attributable to their flavonoid and tannin contents which act as sinks for different pollutants. Therefore, to control pollution in urban and industrial areas, plantation of Neem, Arjun, arlu and Roheda etc. trees should be promoted if possible. Nonetheless, utilization of this raw material as herbicides might be possible.

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A Physicochemical Based Comparative Study of Binding Agents: Sodium Carboxymethyl-Cellulose, Gum Acacia and Starch

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Abstract-To improve quality attributes and shelf-life of many food formulations, Sodium Carboxymethyl cellulose, Gum acacia and Starch are widely used. The main uses are as thickening, binding and gelling agents. As thickening agents, they find uses in soups, gravies, salad dressings, sauces and toppings while their binding ability is different. Hence, in this study aimed to evaluate the physicochemical and binding properties of Sodium Carboxymethyl cellulose, Gum acacia and Starch to correlate their thickening and binding ability.

Keywords: Gum Acacia, Sodium Carboxymethyl Cellulose, Starch

Introduction

Hydrocolloids are frequently used in several foods for thickening. The process of thickening involves the nonspecific entanglement of conformationally disordered polymer chains; it is essentially a polymer-solvent interaction (Philips et al., 1986). Thickening occurs above a critical concentration known as overlap concentration. Below this, the polymer dispersions exhibit Newtonian behavior but show a non-Newtonian behavior above this concentration (Philips and Williams, 2000). Hydrocolloids that have been used as thickening agents in various food systems include starch, modified starch, xanthan, galactomannans like guar gum and locust bean gum (LBG), gum Arabic or acacia gum, gum karaya, gum tragacanth and carboxymethyl cellulose (CMC). The thickening effect produced by the hydrocolloids depends on the type of hydrocolloid used, its concentration, the food system in which it is used and also the pH of the food system and temperature. Therefore, in the present study it is aimed to evaluate the pharmacognostic analyses to correlate it with the thickening and binding similarities between Maize starch, Gum Acasia or Gum Arabic and Carboxymethyl cellulose (CMC).

Material and Methods

Chemicals

For the current study, starch, Gum Acasia and carboxymethyl cellulose (CMC) were obtained from the Himalaya drug company, Faridabad and all chemicals and reagents were of analytical grade in purity.

Physicochemical parameters

The various physicochemical parameters such as total ash, acid insoluble ash, sulphated ash, pH value and moisture content were determined by the method reported by (Sailor, G.U. et al., 2010*)* with slight modification.

Ash value

Total Ash: About 1-2 g of powdered material was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The powder was spread as a fine even layer on the bottom of the crucible. The crucible was heated to make it dry red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight.

Sulphated Ash

To ash content obtained added 1 ml con. Sulphuric acid. The crucible was again heated at 850 ºC to make it dry red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight.

Acid Insoluble Ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transfered into a silica crucible, ignited and weighed. The procedure was repeated to get constant weight.

Loss on drying

About 1-2 g of powdered material was accurately weighed and taken in a LOD bottle, which was previously dried at 105°C and weighed. The powder was spread as a fine even layer on the bottom of the bottle. The bottle was dried at 105°C in a hot air oven. The LOD bottle was cooled and weighed. The procedure was repeat to get constant weight.

pH: The Ph value conventionally represents the acidity or alkalinity of an aqueous solution. In pharmacopeia, standard and limits of pH have been provided for those pharmacopeia substance in which pH as a measure of the hydrogen ion activity is important from the standpoint of stability or physiological suitability. 1% w/v of each aqueous solution was used for the pH estimation and the result is shown in Table-1,2 and 3

Results

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a binding agent. The total ash content is sometimes useful to determine the ratio of water-soluble to water-insoluble ash as this gives a useful indication of the quality of binding agent. The ash constituents comprise potassium, sodium, calcium and magnesium, which are present in larger amount as well as slighter quantities of aluminum, iron, copper, manganese or zinc, arsenic, iodine, fluorine and other elements present in traces. The quality of binding agents mainly depends on the concentration and type of minerals they contain, including their taste, appearance, texture, and stability because high mineral contents are sometimes used to retard the growth of certain microorganisms. It is often important to know the mineral content of binding agents because this affects the physicochemical properties of foods or drug during processing. Thus, determination of the ash and mineral content of food is important for some reasons such as Nutritional labeling, Quality, Microbiological stability, Nutrition, Processing. Moisture content is one of the most commonly measured properties of food materials. Therefore, it is important because the susceptibility of microorganisms to grow in foods depends on their water content. In the present study, it was observed that the ash content value is higher in CMC than Gum Acacia and Starch as shown in table-1, 2 and 3.

Table-1. Gum Acacia

Sl.No.	Parameter	Observation
1	Loss on drying	6.69
2	Ash Content	7.15
3	Sulphated Ash	2.21
4	Acid insoluble ash	0.45
5	pH	6.66

Table -2. CMC

Sl.No.	Parameter	Observation
1	Loss on drying	6.96
2	Ash Content	38.15
3	Sulphated Ash	28.26
4	Acid insoluble ash	30.12
5	pH	6.98

Table-3.Starch

Sl.No.	Parameter	Observation
1	Loss on drying	6.84
2	Ash Content	1.89
3	Sulphated Ash	0.82
4	Acid insoluble ash	0.014
5	pH	6.45

Discussion

Starch is the most commonly used hydrocolloid thickener, the reason being it is relatively cheap, abundant and possibly it does not impart any noticeable taste if used at a low concentration of 2 to 5%. Further, as starch is a common ingredient of many foods we encounter, addition of starch does not offer any foreign taste which may be true for different gums. It is mainly the hydrocolloid providing a base texture in soups and sauces. Thickening of sweet and sour sauces with various polysaccharide combinations like potato starch-xanthan gum and oat starch-xanthan gum has been studied. The evaluation of the thickener performance is considered on the basis of its effect upon sensory properties and rheology. Oat starch-xanthan gum combination has better thickening property compared to potato starch-xanthan gum combination as evaluated from their energy of thixotropy. Both potato starch and xanthan are anionic polysaccharides and hence are thermodynamically incompatible for efficient intermolecular interaction required for thickening (Gibinski et al., 2006). Carboxymethyl cellulose (CMC) is soluble in either cold or hot water. The concentration, molecular weight and degree of substitution (ds) are important factors for flow behaviour of CMC in aqueous dispersions. Commercial products usually have ds values from 0.7 to 1.5. Dispersions of the gum show shear thinning properties, but products of lower ds are thixotropic and viscosity decreases with increase in temperature (Kulicke, et al., 1996; Alexander, 1999b). Other cellulosics include methyl cellulose (MC) and hydroxypropylmethyl cellulose (HPMC). Unlike CMC, they form weak gels on heating when the temperature rises above 52 °C for MC and 63–80 °C for HPMC. These are mostly used for binding and shape retention in reformed vegetable products like onion rings, potato croquettes and shaped soya protein (Murray, 2000). Another hydrocolloid used as a thickener is gum targacanth. This gum swells rapidly, in either cold or hot water to form highly viscous dispersions, up to 4000 mPas at 1% solids, depending on the grade. The dispersions are shear thinning and possess good yield value (Alexander, 1999a). Gum arabic (Acacia gum) is widely used in the food industry mainly to impart desirable qualities because of its influence over viscosity, body and texture. Mainly, it is used as an emulsifier in beverage emulsions (Buffo, et al., 2001). It is a low viscosity gum and has been found to produce low viscosity at 30% concentration compared to 1% xanthan or CMC at low shear rates (Williams and Philips, 2000). Mothe and Rao, (1999) in their study on the rheological behaviour of aqueous dispersions of gum Arabic have found that dispersions of the gum (4–50%, w/v) exhibit shear-thinning characteristics at low shear rates (<10 s”1) and Newtonian plateaus at shear rate above 100 s”1. The infinite shear rate viscosity (ηα) however, is found to increase with increasing concentration of gum.

Conclusion

The physicochemical property of binding agent such as Sodium Carboxymethyl cellulose, Gum acacia and Starch suggested that the binding property mainly depends on the ash value of the agent, as the ash content of CMC is higher in comparison to Gum Acacia and Starch.

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About Flowers (Shown on the cover page)

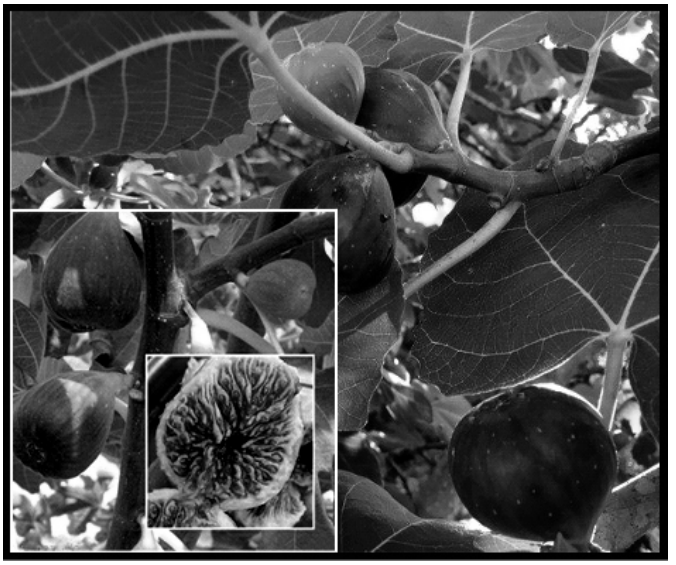
Herbal Neutraceuticals

Syzygium cumini

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	*Syzygium L.*
Species	:	*Syzygium cumini*

Medicinal Properties

1. Jamun the highly nutritious, refreshing and succulent fruit flooding the summer markets has innumerable health benefits.
2. This juicy fruits holds a great significance in holistic treatments like Ayurveda, Unani and Chinese medicine as it attenuates Kapha and Pitta.
3. Jamun is found with a special mention in Ramayana and is prized as the "Fruits of Gods" as Lord Rama.
4. The fruit is diuretic, anti-scorbutic and carminative in properties and is a rich source of polyphenolic compounds. Ayurveda strongly recommends this berry for treating various conditions related to heart, arthritis, asthma, stomach pain, bowl spasm, flatulence and dysentery. The diuretic effects of jamun flushes out of the kidneys, while the high fibre content aids in digestion and prevent nausea and vomiting.



Ficus carica

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Urticales
Family	:	Moraceae
Genus	:	*Ficus L.*
Species	:	*Ficus carica]*

Medicinal Properties

1. Figs are amongst the oldest fruits consumed by humans and have endless benefits. Figs are known for its amazing health benefits, which are high on nutrition and taste best.

2. Anjeer helps in treating various ailments and is loaded with essential vitamins, minerals and antioxidants.

3. Figs are commercially grown in Middle Eastern countries and several Asian countries. Anjeer is globally is known for its medicinal values and is simply a power house of nutrients which help human body to attain good health.

4. Improving overall health often balances skin and helps with circulation of blood.

Cucumis sativus

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Violales
Family	:	Cucurbitaceae
Genus	:	*Cucumis* L.
Species	:	*Cucumis sativus*

Medicinal Properties

1. High in water and low in calories, fat cholesterol and sodium.
2. They have been linked to a lower risk of obesity,diabetes, heart diseases, promoting a healthy complexion, increased energy and a health body weight.
3. As a member of the cucurbitaceae family of the plants they contain Vitamins A, B, C, D and E and high levels of nutrients known as cucurbitacins,which may help prevent cancer by stopping cancer cells from proliferating.
4. Cucurbitacins stimulate insulin release and regulate the metabolism of a key hormone in the processing of blood sugar hepatic glycogen.

Daucus carota

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Apiales
Family	:	Apiaceae
Genus	:	*Daucus L.*
Species	:	*Daucuscarota*

Medicinal Properties

1. Carrots are often thought of as the ultimate health foods.

2. Carrots contain Vitamin A, antioxidants and other nutrients. Evidence suggests that eating more antioxidants rich fruits and vegetables such as carrot can help reduce the risks of cancer and cardiovascular diseases.

3. Carrots contain beta-carotene. Past studies have concluded from trusted source that beta-carotene Supplementation may reduce risk of lung cancer.

4. Carrots contain vitamin C, an antioxidant which helps boost the immune system and prevent diseases.

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January 20-21, 2020
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Theme: Novel Methodologies in Pharmaceutical and Drug Delivery Procedures
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February 12-13, 2020
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Theme: Unveiling prodigious discoveries and exploratory insights in Medicinal Chemistry & Drug Discovery
Website: **https://medicinalchemistrymeetings.pharmaceuticalconferences.com/**

3rd International Conference and Exhibition on Pharmaceutical Nanotechnology and Nanomedicine
February 24-25, 2020
Rome, Italy
Theme: Nanomedicine & Nanotechnology: A prominent epitomes in Pharmaceutical Sciences
Website: **https://nanomedicine.pharmaceuticalconferences.com/usa/**

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Osaka, Japan
Theme: Challenges and New ideas in Pharmaceutical and Medicinal Sciences
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Theme: Designing the future of Biopharma
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Bangkok, Thailand
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Website: **https://pharmaexpo.pharmaceuticalconferences.com/cancellation-policy.php**

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April 24-25, 2020
Pune, India
Website: **http://academicsera.com/Conference2020/India/2/ICPAF/**

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Prague, Czech Republic
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Website: **https://pharmaceuticalchemistry.annualcongress.com/**

3rd International Conference on Herbal & Traditional Medicine
April 27-28, 2020
Dubai, UAE
Theme: Herbal & Traditional Medicine: Innovations and strategies in Alternative Medicine
Website: **https://herbal-traditional.conferenceseries.com/**

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May 04-05, 2020
Kuala Lumpur, Malaysia
Theme: Leading Edge Innovations in Life Sciences
Website: **https://www.proteomicsconference.com/asia-pacific/**

30th Annual European Pharma Congress
May 18-19, 2020
Berlin, Germany
Theme: Innovations for the Next Generation Pharmacy
Website: **https://europe.pharmaceuticalconferences.com/**

857th International Conference on Medical, Biological and Pharmaceutical Sciences ICMBPS
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June 23-24, 2020
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4th International Symposium on Phytochemicals in Medicine and Food
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Northwest University, 229 North Taibai Road
Xi'an, China
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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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