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# Universities' Journal of Phytochemistry and Ayurvedic Heights


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*Cinnamomum zeylanicum*


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## Editorial

Today 's world is in distress due to the COVID-19 pandemic it is possibly one of the most severe pandemics we all are witnessing in this century. The spread of the novel coronavirus is now a major public health threat for whole world. Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered **coronavirus** (SARS COV-2)**.**

Most of the people infected with the COVID-19 virus have experienced mild to moderate respiratory illness and some recovered without requiring special treatment. Older people, and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer patients are more likely to develop serious illness. At the moment, there are no specific vaccines or treatments for COVID-19.

Since the emergence of the novel coronavirus (COVID-19), **biopharmaceutical companies with advanced virology labs and facilities have stepped up their efforts and are engaged in research efforts to develop vaccines and treatments** in order to control the corona virus. Currently a number of existing and new treatments are in various **research phases and clinical trials** to test their efficiency and safety for treating COVID-19. **Glenmark pharmaceuticals Ltd** would begin a clinical trial in India to test a combination of two antiviral drugs,**Favipiravir** and **Umifenovir** as potential coronavirus disease(COVID-19) treatment. According to Indian Council of Medical Research (ICMR) Hydroxychloroquine is effective in reducing intensity and duration of COVID-19.

**New Drug Development** including preclinical(toxicological studies)on animals and clinical studies Phase I to IV in human. The development of new drug is by and large a random process that might result in serendipitous discovery, many pharmaceutical companies are **now focusing on the development of plant derived drugs**.

Indian indigenous system is a **5000 years old system of medicines** with a backup of *in vivo* study support of safety and remarkable clinical results were administrated by the qualified practitioners of AYUSH.

Approximately **25% of drug prescribed worldwide come from plants** but more *in vitro* studies and toxicity studies of current and past formulations are required to promote this indigenous system of medicines against viral diseases.

## Ayurveda's immunity boosting measures for self care during COVID 19 crisis will

**plays an pivotal role in maintaining optimum healthcare.**

We all know that **prevention is better than cure**. While there is **no medicine for COVID-19** as of now, it will be good to **take preventive measures which boost our immunity in these times.**

Bitters like *Andrographis* (**highly medicinal, immune-boosting herb)** is considered to be one of the most powerful herbs intended to improve the immune system and enhance antimicrobial properties.

*Andrographis* is described in several different forms, such as "Indian Echinacea," "False Willow" ," "Bitter King" "Bhunimba," "Bidara," or Green Chereta.

Ministry of AYUSH recommends the following self-care guidelines for preventive health measures and boosting immunity with special reference to respiratory health. These are supported by Ayurvedic literature and scientific publications.

Spices like Haldi (Turmeric)-Golden milk(Half tea spoon Haldi (turmeric) powder in one cup of milk).In the daily food Jeera (Cumin), Dhaniya (Coriander) and Lahsun (Garlic) are recommended in cooking.

This issue of UJPAH is focused on current scenario and published research paper on immunoboosters herbs to boost up the immunity and supports the body in the fight with covid-19.

Keeping in view of the above problems facing by the ailing humanity I must say that the best way is the nature's way. Promotion of indigenous system of medicines through research using most advanced equipments by young scientists is a now indispensible.

Iam gratefull to Honourable Minister of HRD Dr. Ramesh Pokhriyal Nishank for very kind consenting to preside over the Webinar and release this journal. He is a wonderful person his ideas will definitely give encouragement to the participants for research with available resources on Indian Herbs. I am fortunate to be in touch with him since long and very thankful to him to be a part of this ceremony of opening of Universities' Journal of Phytochemistry and Ayurvedic Heights.

My best wishes to all those scientists, Research scholars, students and teachers who contributed for bringing out this issue in this crisis time 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 with hope that research published in it may become fulfill to fight COVID-19 pandemics. Thanks to all.

**Dr. S. Farooq**
Chief Editor

# Contents

# *Azadirachta Indica*: Indian Neem, A Natural Killer of Microorganisms

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**Abstract**-Medicinal Plants are always of immense vital component for pharmaceutical word. *Azadirachta indica*, commonly known as neem, has attracted worldwide prominence in recent years owing to its wide range of medicinal properties. Neem is a medicinal plant which is always at top from ancient world to modern world. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem- a tree for solving global problems'. Various parts of neem are sources for mankind to treat many severe diseases. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. Keeping these points in view the present study is aimed to study antimicrobial activity of plant extracts prepared from leaves of neem plant against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli.* There is a significant antimicrobial activity observed by neem leave extract against these microbial strains.

**Keywords:** *Azadirachta indica,* Antibacterial activity, Immunomodulatory activity.

## Introduction

Plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations obtained from commonly used herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. Thousands of natural products obtained from plant sources have been used by mankind for alleviating diseases and/or their symptoms.

The evergreen tree neem has been used as a traditional medicine for many centuries in

India. Various preparations of neem obtained from its different parts have been found to exert antibacterial, antimalarial, contraceptive and antiulcer activities (Biswas, et al, 2002).

Neem is perhaps the most commonly used traditional medicinal plant in India. Almost all parts of the plant are endowed with medicinal property. During the past few decades, apart from the studies in the chemistry of neem compounds, considerable progress has been made in evaluating biological activity of phytochemical compounds for medicinal applications. In the modern era, Neem is a component of Ayurvedic medicine, which has been practised in India since post-vedic period. The Neem plant possesses antibacterial, antifungal, antiviral and antiseptic properties (Subapriya, 2005).

First of all the three bioactive compounds namely nimbin, nimbinin, nimbidin were derived from Neem tree in 1942 (Siddiqui et al., 1986). These compounds have anthelmintic, antifungal, antibacterial and antiviral activities. Nimbin is a sulphur free crystalline product with melting point 205$^0$C. Nimbidin have potential antibacterial ingredient and highest yielding bitter compound. These compounds are stable and found in substantial quantities in the Neem plant.

Neem leaves have antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premises. Administration of alcoholic extract of neem disrupts the estrous cycle in sprague dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent (Gbotolorun et al., 2008). The aqueous extract of neem have powerful chemotherapeutic and antiviral agent (Hassan et al., 2010). Aqueous leaf extract of neem has good therapeutic potential as an anti-hyperglycemic agent (Sonia and Srinivasan, 1999). More than 135 compounds have been isolated from different parts of neem and the chemical properties have been studied and structural diversity of these compounds has been elucidated (Khan and Wassilar, 1987).

In view of the medicinal value of Neem tree, the present work was intended to study the antibacterial activity of aqueous and methanolic extracts of the leaves of *Azadirachta indica*.

## Material and Methods

### Collection of Leaves

Leaves of *Azadirachta indica* (neem) were collected from Yashwant Singh Parmar, University of Horticulture and Forestry, Nauni, Solan, India.

### Bacterial culture

The bacterial strains i.e., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis, Staphylococcus aureus* and

*Escherichia coli* for evaluating antibacterial activity were obtained from the Department of Microbiology, SILB, Solan. These strains were then sub-cultured and maintained in Molecular and Immuno-parasitology Laboratory over Nutrient Agar medium.

**Preparation of Methanolic and Aqueous extracts of Neem leaves**

For preparing methanolic extract, the dried and crushed neem leaves were used. Twenty grams of this powder was weighed and loaded to the Soxhlet apparatus. 200 ml of methanol was used as solvent for preparing the alcoholic extract. Extraction was carried out for 16 hours. After extraction the solvent containing the crude extract of neem was dried in a china dish under air current. The dried extract was scrapped from the china dish after 4-5 days with the help of surgical blade. This final product was stored at 4°C until use. For preparing aqueous extract of neem leaves, the same procedure as that of methanolic extract was involved. Only the solvent used here was 200 ml distilled water for 20 grams of neem leaves (Syarifah *et al*., 2014).

**Antibacterial activity**

Antibacterial activity of the methanolic and aqueous extracts was studied by agar-well diffusion method on Mueller Hinton Agar (MHA) medium using five different species of bacteria namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli* as described by NCCLS methods. Methanolic extract was dissolved in Dimethyl Sulphoxide (DMSO) and concentrations of 10 mg/ml i.e., 500 µg crude extract per well were used for the study. 50 µl of aqueous extract was poured in one of the wells without dissolving in DMSO. Ciprofloxacin was used as a standard antibiotic and was applied in the fourth well with a concentration of (1 mg/ml i.e., 50 µg per well). DMSO alone was also applied in one of the four wells to check whether it has any activity or not. These Petri plates were then incubated at 37 °C for two days. After two days zone of inhibitions around the wells were measured using an antibiotic zone scale.

## Results and Discussion

The results of antibacterial assay have shown that *E.coli* has the maximum zone of inhibition for both methanolic and aqueous extracts, whereas *Bacillus subtilis* has shown minimum zone of inhibition for both methanolic as well as aqueous extracts (Table-1, Fig. 1-5) Ciprofloxacin was used as a control.

**Table -1 Antibacterial activity of aqueous and methanolic extracts of leaves of *Azadirachta indica***

| S. No. | Bacterial species | Gram+ or Gram- Bacteria | Zone of inhibition (mm) | | |
|---|---|---|---|---|---|
| | | | Methanolic | Aqueous | Ciprofloxacin |
| 1 | *Staphylococcus aureus* | G+ | 32 | 22 | 38 |
| 2 | *Bacillus subtilis* | G+ | 29 | 17 | 35 |
| 3 | *Escherichia coli* | G- | 34 | 19 | 36 |
| 4 | *Pseudomonas aeruginosa* | G- | - | - | 39 |
| 5 | *Klebsiella pneumoniae* | G- | - | - | 33 |

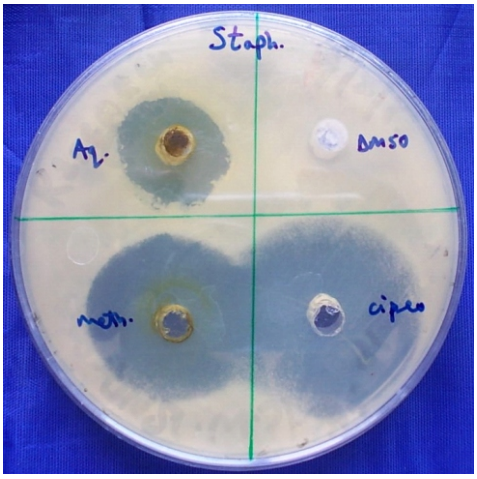


Fig.-1 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Staphylococcus aureus.*

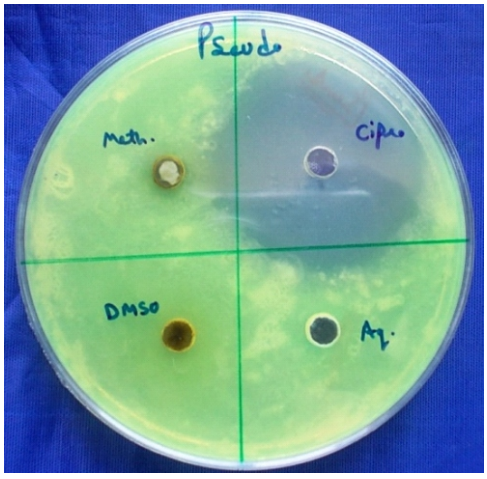


Fig.-2 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Pseudomonas aeruginosa.*

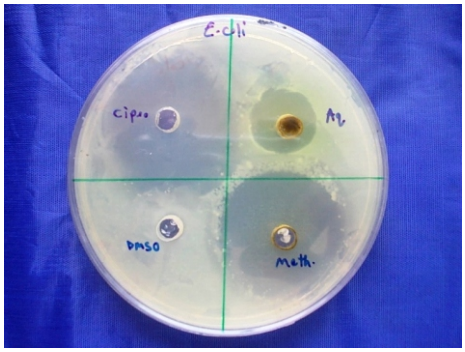


Fig.-3 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Escherichia coli*

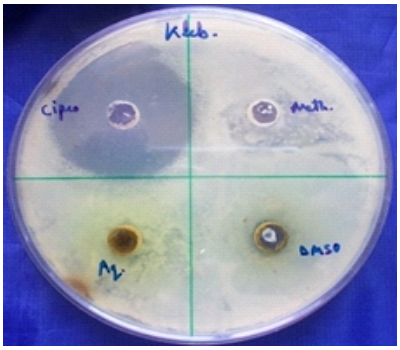


Fig.-4 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Klebsiella pneumoniae*

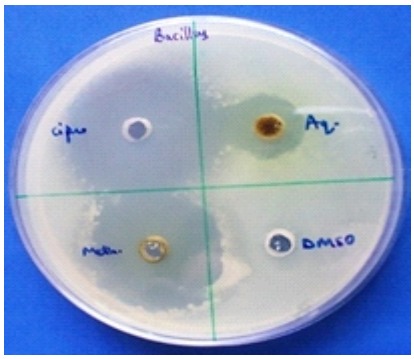


Fig.-5 Antibacterial activity of methanolic and aqueous extracts of neem leaves against *Bacillus subtilis*

*Azadirachta indica* (Neem) is perhaps the most commonly used traditional medicinal plant in India. Neem is one of the major components in Ayurvedic medicine, which has been practised in India since many centuries due to its antibacterial, antifungal, antiviral and antiseptic properties**.** It is also reported to have antiplaque and many other pharmacological properties. Microorganisms are concealed enemies to the mankind. They are small but cause a very profound damage in human body as well as on other living organisms which may be fatal. The agents which have the capacity to kill the microbes or arrest the multiplication are called the antimicrobial agents or drugs. Keeping these points in view**,** we planned to study the antimicrobial activity of Neem leaves. The extracts of plant leaves were prepared in aqueous and methanol solution. The bacteria selected for the study were *E. coli, P. aeruginosa K. pneumoniae, S. aureus and B. Subtilis*. These are the most commonly found micropathogens causing number of infections in human in day to day life.

Methanolic and aqueous extracts prepared form leaves were then subjected for antimicrobial activity by using five bacterial species on Muller Hinton Agar plates by agar well diffusion method. Our findings suggest that methanolic extract of neem is highly efficient in inhibiting *E. coli* followed by *S. aureus* and *B. subtilis.* Jahan *et al*., *(2007)* has also reported that the neem leaves are active against these three bacteria with similar zones of inhibition. Neem extract has not shown any activity against *P. aeruginosa* and *K. pneumoniae.* A study carried out by Maragathavalli et al., (2012) have shown considerable activities of neem extract against *P. aeruginosa* and *K. pneumoniae* which is contrary to the results obtained in the present study. This might be due to the different bacterial strains used in the antibacterial assay. Similarly in another study antibacterial activity of neem extract was reported against *Mycobacterium*

*tuberculosis* which is a potential human pathogen (Chakraborty *et al*., 2001). *Alam sher*, (2015) has reported anti-dermatophytic activity of the ethanolic and aqueous extracts of the neem leaves. Neem extracts have also been reported to have inhibitory action against food borne pathogens which include *Aeromonas hydrophila, Alcaligenes faecalis, Pseudomonas putida* etc. (Mahfuzul *et al*., 2007). The present work and the literature reports suggest that the neem tree has broad spectrum of inhibition of microbial pathogens and this may be further evaluated along with the standard antibiotics to determine the synergistic effect of neem extract against the pathogenic microflora to serve the mankind.

## Conclusion

Neem is used as a traditional medicine and as a source of many therapeutic agents in India. It grows well in the tropical country like India. In the present study, antimicrobial activity of neem leaves was studied against different pathogenic bacteria. Methanolic extract of Neem possesses a good antibacterial activity and is comparable to the standard antibiotic.

We found potent antimicrobial activity of neem leaves extract against different bacteria. It has been observed that the leaf extract have shown strong antimicrobial activity against *E. coli,* whereas weak antimicrobial activity was observed against *B. subtilis* as zone of inhibition was smallest. No zone of inhibition in the case of *P. aeruginosa* and *K. pneumoniae* was observed revealing the inefficiency of neem extracts in inhibiting these bacteria. We observed high efficacy of methanolic extracts of neem leaves and moderate activity of aqueous extract against three out of five bacterial species involved in the study.

To conclude, the study that *Azadirachta indica* leaf extract has potent antibacterial activity against different pathogenic bacteria. Further studies are required in order to understand its mechanism of action which may limit the use or replace the antibiotics which are now no longer safe and efficient to full extent.

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# Study on Phytoconstituents and Antimicrobial Potential of *Sapindus mukorossi* Fruit Extract

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**Abstract**-*Sapindus mukorossi* is well known for its folk medicinal values. The fruit is valued for the saponins (10.1%) present in the pericarp which constitutes up to 56.5% of the drupe known for inhibiting tumour cell growth. Recently many of the pharmacological actions of this plant have been explored which include the antimicrobial molluscicidal and insecticidal actions. The fruits are of considerable importance for their medicinal value for treating several diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis etc. The powdered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea. Keeping in view the medicinal importance of the fruit, it was thought worthwhile to carry out systematic study on phytochemical investigation and antimicrobial potential of fruit extract of *Sapindus mukorossi.* Fruit were dried, powdered and extracted with different solvent systems with increasing polarity. The extracts were screened for the antibacterial and antifungal activity. Predominance of antibacterial activity was observed at dose level of 50µg/ml of petroleum ether extract. Maximum zone of inhibition (19mm) &(18mm) was observed against *Salmonella typhii* and *Shigella dysenteria* respectively as compared to standard drug Ampicillin 20µg/ml. Since antibacterial activity was observed in 50µg/ml concentration of petroleum ether extract, it was chosen for exploring its antifungal potential also. Maximum zone of inhibition was observed against Aspergillus sulfurous as compared to standard drug ketoconazole 20µg/ml. From the above study, it could be concluded that *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml) have good antimicrobial potential and can be explored further to isolate active principles from the same.

**Key words:** Epilepsy, Phytochemical Screening, Zone of Inhibition, Antimicrobial potential

## Introduction

The traditional knowledge and use of medicinal plants for curing of disease has been widely established across the globe. 80% of the world's population in developing countries uses traditional medicine as per WHO guidelines. Furthermore, the traditional knowledge with its holistic and systematic approach supported by experimental base can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines. *Sapindus mukorossi* also known as soap-nut tree belongs to the family sapindaceae widely grown in upper reaches of Indo-Gangetic plains, Shivaliks and sub-Himalayans tracks at altitudes from 200m to 1500m. [1]The fruit is valued for the saponins (10.1%) present in the pericarp which constitutes up to 56.5% of the drupe known for inhibiting tumor cell growth.

The fruits are of considerable importance for their medicinal value for treating several diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis. The powdered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea.[1] The fruit are used in the baths to relieve joint pain and the roots are used in the treatment of gout and rheumatism, since ancient times *Sapindus mukorossi* has been used a detergent for shawls and silks. Recently many of the pharmacological actions of this plant have been explored which includes the antimicrobial molluscicidal and insecticidal actions.The fruit of *Sapindus mukorossi* was utilized by Indian Jewellers for restoring the brightness of tarnished ornaments made of gold, silver and other precious metals[2-4]. Various types of triterpene, saponins of oleanane, dammarane and tirucullane type were isolated from the galls, fruits[5-9] and roots of *Sapindus mukorossi*. Oleanane type triterpenoid saponins named Sapindoside A and B were reported from the fruits of [9-11]*Sapindus mukorossi*. Sapindoside C-11, Sapindoside D- 12, which is a hexaoside of hederagenin, and Sapindoside, a nonaoside of Hederagenin was isolated and identified by Chirva et, al from the methanolic extract of the fruits of *Sapindus mukorossi*[12]. Keeping in view medicinal importance of the fruit, it was thought worthwhile to carry out phytochemical investigation and antimicrobial potential of fruit extract of *Sapindus mukorossi.*

## Material and Methods

All the solvents and reagents used in this study were of analytical grade and were always freshly prepared before use (Merck India Ltd. & Ranbaxy Fine Chemicals Ltd.). Fehling reagent, Mayer's reagent, Dragendroff reagent, Hager reagent, Wagner reagent, Molish reagent, Millon reagent, Ninhydrin reagent, NaOH pellets, $FeCl_3$ were purchased from Ranbaxy Fine Chemicals Ltd, New Delhi. Picric acid and Hydrochloric acid was purchased from S.D. Fine Chemicals Ltd. Mumbai. α-naphthol, $H_2SO4$ was purchased from Merck India Ltd. Mumbai. Mueller Hinton Hi Veg Agar, Peptone, Beep extract and DPPH were obtained from Himedia.

**Plant Material**

The fruits were procured from garden of SBS University and authenticated by Deptt. of Pharmacognosy, SBS University and kept as voucher specimen in herbarium of the department. Fruit was dried, powdered and stored in airtight bottles.

**Extraction and Fractionation**

The powdered material (50g) was successfully extracted with solvents of increasing polarity like petroleum ether, chloroform and ethanol using soxhlet apparatus. The extracts so obtained were concentrated and dried concentrates were stored in airtight container and kept in refrigerator until use.

**Qualitative phytochemical analysis**

The various solvent extracts of *Sapindus mukorossi* fruit were subjected to qualitative chemical tests for the identification of various phytoconstituents viz, alkaloids, carbohydrates, glycosides, proteins, tannins, sterols, saponins, amino acids etc. These phytoconstituents are solely responsible for biological activity of these plants.

**Antibacterial Activity**

Antibacterial bioassay13were evaluated against gram positive bacterial strains, *Staphylococcus aureus* (ATCC 11633S. and gram negative bacterial strains, *Salmonella typhi* (MTCC 733) & *Shigella dysenteriae* (ATCC3313) by disc diffusion method. Standard inoculums (1ml/100 ml of medium) with suspension (105cfu/ml) were introduced on to the surface of sterile agar plates, and a sterile bent glass spreader was used for even

distribution of the inoculum. The discs measuring 6 mm in diameter and 2 mm thickness were prepared from Whatman (grade no. 1) filter paper and sterilized by dry heat for 1 hr. Three discs of test samples were placed on three portion together with one disc with reference drug, Ampicillin and disc impregnated with solvent (DMF) as negative control. The sterile discs previously soaked in a known concentration of the *Sapindus mukorossi* fruit extracts samples (petroleum ether, choloroform and ethanol extracts in 25µg/ml, 50µg/ml, 75µg/ml in dimethyl formamide were placed in nutrient agar medium. Ampicillin (20µg/disc) was used as positive control for bacteria. Plates were inverted and incubated for 24 h at 37±20$^0$C. Diameters of zone of inhibition (mm) were determined and average diameter of test samples were calculated in triplicate sets. Zone of inhibition of test samples were compared with that produced by standard.

**Antifungal Activity**

Fungal infections are most common among the human population and several therapeutic agents are also available in the market but most of them are effective as topical applications. Rare drugs are available for deep mucosal infections. We chose petroleum ether fruit extract of *Sapindus mukorossi* to inspect the antifungal activity, as it has shown good antibacterial effect. Strains chosen were *Aspergillus tarrius, Aspergillus sullitrius, Aspergillus niger, Aspergillus fumigatus, Trichophytom and Aspergillus flavus*. Medium employed for antifungal activity was potato dextrose agar (PDA) for isolation of fungal culture and sabraud's agar medium for antifungal assay by plate diffusion method[14]. Readily available PDA powder was used having the following composition and pH: Dextrose 2%, Poatato peel off-200gm/lit., water, Agar-1.5%, pH-5.

## Results and Discussion

**Qualitative chemical examination of extracts of the fruit of *Sapindus mukorossi***

The different extracts of fruit of *Sapindus mukorossi* were chemically analyzed for the presence of various chemical constituents. Petroleum ether extract shows presence of steroids. Chloroform shows presence of carbohydrates, phenols and saponins while ethanolic was found to be rich in alkaloids saponins, phenols, amino acids and carbohydrates summarized in Table-I. Antibacterial bioassay of three extracts namely petroleum ether, chloroform and ethanol (25,50 75 µg/ml) each were evaluated against gram positive bacterial strains, ***Staphylococcus aureus* (ATCC 11633)** and gram-negative bacterial strains, *Salmonella* ***typhi (MTCC 733) & Shigella dysenteriae* (ATCC3313)** by disc diffusion method. From antimicrobial activity (Table- II, fig. I) It was observed that increase in concentration did not have much impact on zone of inhibition. Good and moderate effect was exhibited by petroleum ether and ethanolic extract of the fruit at dose level of 50µg/ml respectively while chloroform extract did not show any prominent results. Maximum zone of inhibition (19mm &18mm) was observed against *Salmonella typhii* and *Shigella dysenteria* respectively in 50µg/ml concentration of petroleum extract as compared to standard drug Ampicillin 20µg/ml. Since antibacterial activity was observed in 50µg/ml concentration of petroleum ether extract, it was chosen for exploring its antifungal potential also against *Aspergillus tarrius, Aspergillus sullitrius, Aspergillus niger, Aspergillus fumigatus, Trichophytom and Aspergillus flavus*. Maximum zone of inhibition (24.5 mm) was observed against *Aspergillus sulfurous* as compared to standard drug ketoconazole 20µg/ml (Table -III, fig. II).

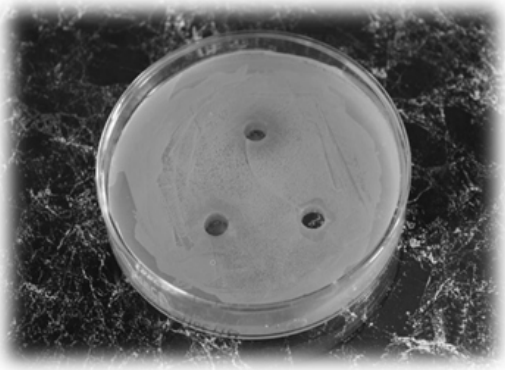
**Table-I Results of Phytoconstituents present in various of *Sapindus mukorossi* fruit extracts**

| S. No | Plant constituent/Test/ reagent used | Petroleum ether Extract | Chloroform Extract | Ethanolic Extract |
|---|---|---|---|---|
| 1 | Alkaloids | | | |
| | Mayer's test | + | + | + |
| | Wagner's test | + | - | + |
| | Dragendroff's test | + | - | + |
| 2 | Carbohydrates | | | |
| | Molish' test | + | - | + |
| 3 | Saponins | | | |
| | Foam test | + | + | + |
| 4 | Amino acids | | | |
| | Million's test | + | + | + |
| | Biuret test | + | + | + |
| | Ninhydrin test | - | - | + |
| 5 | Sterol | | | |
| | Libermann- burchard test | + | + | + |
| 6 | Phenolic compounds/ tannins | | | |
| | Ferric chloride test | + | + | + |
| | Lead acetate test | + | + | + |
| | Alkaline test | + | + | + |

**Table-II Antibacterial effect of *Sapindus mukorossi* fruit extracts**

| | Zone of inhibition | | | Zone of inhibition | | | Zone of inhibition | | | Zone of inhibition |
|---|---|---|---|---|---|---|---|---|---|---|
| | Petroleum ether Extract (con in %) | | | Chloroform extract (con in %) | | | Ethanolic extract (con in %) | | | AMP(20µg/ml) |
| | 25 | 50 | 75 | 25 | 50 | 75 | 25 | 50 | 75 | |
| *Salmonella typhii* | 1 | 19 | 15 | 2 | 5 | 5 | 1 | 14 | 14 | 27 |
| *Staphylococcus aureus* | 0 | 9 | 15 | 3 | 5 | 6 | 3 | 16 | 16 | 25 |
| *Shigella dysenteria* | 2 | 18 | 17 | 2 | 5 | 7 | 0 | 10 | 12 | 26 |
| DMF | - | - | - | - | - | - | - | - | - | |

- AMP =Ampicillin
- DMF = Dimethyl formamide
- SA = *Staphylococcus aureus* (ATCC 11633)
- ST = *Salmonella typhi* (MTCC 733)
- SD = *Shigella dysenteriae* (ATCC3313)



*Salmonella typhii*

*Shigella dysenteria*

**Fig. I Plates showing Antibacterial effect of *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml)**

## Table-III Antifungal activity of *Sapindus mukorossi* petroleum ether fruit extracts (50µg/ml)

| Sr.NO. | TEST ORGANISM | ZONE OF INHIBITION |
|---|---|---|
| 1 | Aspergillus tarrius | 14mm |
| 2 | Aspergillus sulfurius | 24.5mm |
| 3 | Aspergillus niger | 21mm |
| 4 | Aspergillus flavus | 14.5mm |
| 5 | Aspergillus fumigatus | 20mm |
| 6 | Trichophyton | 21mm |
| 7 | ketoconazole20µg/m | 12mm |

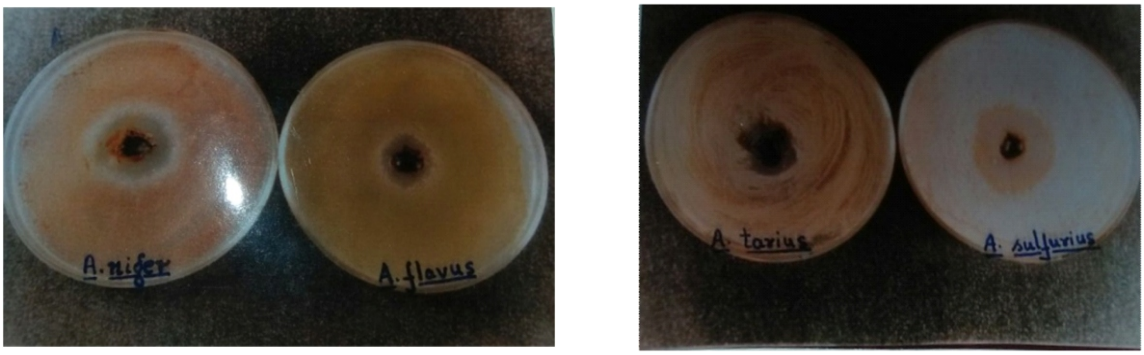



**Fig.II Plates showing Antifungal effect of *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml)**

## Conclusion

From the present study, it was observed that petroleum ether fruit extract of *Sapindus mukorossi* rich in steroids showed maximum zone of inhibition against *Salmonella typhi* and *Shigella dysenteria* respectively at dose, *50*µg/ml as compared to standard drug, *Ampiciline* 20µg/ml. Since antibacterial activity was observed in 50µg/ml concentration of petroleum ether extract, it was chosen for exploring its antifungal potential also. Maximum zone of inhibition was observed against Aspergillus sulfurous as compared to standard drug ketoconazole, 20µg/ml. Thus, from the above study, it could be concluded that *Sapindus mukorossi* petroleum ether fruit extracts(50µg/ml) has good antimicrobial potential and can be explored further to isolate active principle from the same.

### Conflict of Interests

The authors declare the absence of conflict of interests.

### Acknowledgement

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# Phytochemical and Antimicrobial Evaluation of *Terminalia Chebula* Fruit Extracts

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**Abstract-**The present study includes the phytochemical and antimicrobial evaluation of the fruit of *Terminalia chebula* (harad) plant. Phytochemical screening of the fruit extract (shade dried) indicated the presence of flavonoids, terpenoids, tannins, alkaloids, saponin, carbohydrates, protein and glycosides. The antibacterial activity of methanol, ethanol, diethylether, acetone, chloroform and aqueous extracts of fruit of *Terminalia chebula* was evaluated against the human pathogenic bacteria like *Bacillus subtilis, Staphylococcus aureus, E coli, Pseudomonas fluorescens* and fungi like *Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum* by disc diffusion method. Among the extracts analyzed ethanol and acetone extracts showed promising results. The acetone fruit extract showed maximum inhibition against Gram positive bacterial strains *(Staphylococcus aureus,* 12.5mm; *Bacillus subtilis,* 12mm). Phytochemical tests carried out showed that the antimicrobial activity of plant *Terminalia chebula* fruit may be due to the presence of phytochemical compounds present in it.

**Keywords:** *Terminalia chebula,* Phytochemical , Antimicrobial

## Introduction

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances called phytochemicals include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols **(Edeoga *et al*, 2005).** Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens **(Maria, 2017).** These phytochemicals have been used as poisons and others as traditional medicine **(Kannan, 2009).** *Terminalia chebula* (Harad) is one of the imperative herbs rich in medicinal properties used for digestive problems. Harad is a magical herb and its fruit is used to treat acidity, heat burn, heart disease, constipation, ulcers, piles, inflammation, dysentery and diarrhea. Harad is considered as a remarkable medicine for its healing properties. It is also used to remove toxins from body. It is good for lungs, bronchitis and sinus. Harad directly acts on the gastro-intestinal tract and reduces the passage of harmful toxins to the liver and kidneys **(*Tensingh* and *Astalakshmi,* 2014)**. It is one of the constituents of the popular Ayurvedic formula Triphala (which contains equal proportions of Harad (*Terminalia chebula*), Bahera (*Terminalia bellerica*) and Amla (*Emblica officinalis*). This herb is an effective

remedy for chronic ulcer, diarrhoea, dysentery and piles. It is also an effective purgative and helps in removing toxins and fats from the body. Harad contains chebulic acid, which catalyses the production of insulin generated by the pancreatic gland. It gives relief from the irritation and one is able to eat and chew with much less discomfort.(***Jigna Prakash*,2009)**

## Material and Methods

The fruits of *T. Chebula* were collected from the local market in Muzaffarnagar, Uttar Pradesh. The taxonomic identity of the plant was confirmed by Dr. Sanjeev Kumar, plant taxonomist, Head of Botany Department, D.A.V. (PG), College, Muzaffarnagar, U.P., India.

**Preparation of plant extract**

Extracts were prepared according to the method described by **Ahmad and Beg, 1998** with minor modification. The samples were carefully washed under running tap water followed by sterile distilled water and air dried at room temperature (35-40°C) for 4-5 days, homogenized to a fine powder using a sterilized mixer grinder and stored in air tight bottles. Six different 50% solvents, namely ethanol, methanol, acetone, diethyl ether, chloroform and aqueous were used for extraction. Homogenized fruits, 10 g each (10%) were separately soaked in conical flasks each containing 100 ml of acetone, ethanol, methanol, acetone, diethyl ether, chloroform (50%) and sterile distilled water in conical flasks and allowed to stand for 30 min in a water bath (at 100°C) with occasional shaking, followed by keeping all the flasks on rotary shaker at 200 rpm for 24 h. At the end of extraction period, it was centrifuged and supernatant was filtered through Whatman No.1 paper. This extraction was repeated three times. Filtrates were pooled and evaporated to air dry and stored at 4°C in labelled steriled bottles until further use.

**Phytochemical Screening**

Extract of methanol, ethanol, acetone, chloroform, aqueous and diethyl were used for preliminary phytochemical screening using standard procedures. Phytochemical analysis for major phyto constituents of the plant extracts were undertaken using standard qualitative methods as described by various authors (**Vogel, 1958; Kapoor *et al*, 1969**; **Fadeyi *et al,* 1989; Odebiyi and Sofowora, 1990**). The plant extracts were screened for the presence of biologically active compounds like glycosides, alkaloids, flavonoids, saponin, tannin, terpenoids, carbohydrates and proteins **(Harborne, 1973).**

**Test for Tannins**: 10 ml of bromine water was added to the 0.5gm aqueous extract. Decolouration of bromine water showed the presence of tannins.

**Test for Saponins**: 5.0 ml of distilled water was mixed with plants extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

**Tests for Flavonoids**: ***Shinoda Test***-Pieces of magnesium ribbon and HCl concentrated were mixed with aqueous crude plant extract after few minutes. The pink color showed the presence of flavonoid.

**Alkaline Reagent Test-** 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

**Tests for Glycosides**: ***Liebermann's Test***- 2.0 ml of acetic acid and 2 ml of chloroform

were added with 10 ml plant crude extract. The mixture was then cooled and we added $H_2SO_4$ concentrated. Green colour showed the entity of a glycone, steroidal part of glycosides.

***Keller-Kiliani Test:*** A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml plant extract and 1 ml $H_2SO_4$ concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

***Salkowski's Test:*** 2 ml of concentrated $H_2SO_4$ was mix with plant crude extract. A reddish brown colour formed which indicated the presence of steroidal a glycone part of the glycoside.

**Test for Terpenoids:** 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of $H_2SO_4$ concentrated. A grey colour formed which showed the entity of terpenoids.

**Test for Alkaloids: Dragendroff's Test-** Plant extracts were dissolved in chloroform. Chloroform was evaporated and the residue was acidified by adding few drops of Dragendroff's reagent (Potassium bismuth iodide). Appearance of orange red precipitate indicated presence of alkaloids.

**Test for carbohydrates: Molisch's Test-** 5 ml extract was mixed with 5 ml Molisch's reagent, and then 10 ml conc. $H_2SO_4$ was added along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicated the presence of carbohydrates.

**Test for proteins: NinhydrinTest-** Few drops of ninhydrin solution was added to the extract. Appearance of blue colour indicated presence of amino acid or proteins.

## Antimicrobial Screening

### Preparation of Culture Medium

**Nutrient agar medium**: 5gm of NaCl, 20gm agar, 10gm beef extract and 5gm peptone were added in 1000ml of distilled water which was boiled in a round bottom flask by continuous stirring. The mouth of the flask was wrapped with cotton plug and aluminium foil and tied tightly. The medium was sterilized at a pressure of 15 lbs and 121$^0$C for 15 minutes in an autoclaved and used for maintenance of bacterial culture and testing. **(Cappouccino and Sherman, 1998).**

**Potato Dextrose Agar medium** : 200gm of sliced peeled potatoes were boiled in 1 liter of water for 30 minutes. This mixture was filtered through cheesecloth, saving effluent, which is potato infusion. 20gm of dextrose, 20gm agar and water to effluent was added in it. The medium was sterilized at a pressure of 15 lbs and 121$^0$C for 15 minutes in an autoclaved and used for maintenance of fungal culture and testing.(**Cappouccino and Sherman, 1998).**

**Microorganisms used:**The bacterial strains and fungal strains were isolated from spoiled food and then identified the bacteria like *Bacillus subtilis, Staphylococcus aureus (Gram positive), E coli, Pseudomonas fluorescens (Gram negative)* and *fungi* like *Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum.*The bacterial strains were maintained in nutrient agar medium at 37$^0$C whereas fungal strain were maintained in potato dextrose agar medium at 28$^0$C. The stock culture slants were maintained at 4$^0$C.

**Disc diffusion method**

The antimicrobial test was carried out against gram positive and gram negative bacterial strains and some fungal strain. The antimicrobial activity of fruit extracts were tested against bacterial and fungal strains by disc diffusion method (**Cappouccino and Sherman, 1998; Mahato *et al,* 2005; Berghe and Vlietinck, 1991**). 200µl concentration extract

loaded disc were placed on the surface of the agar medium by pressing with sterile forceps in an aseptic condition. The inoculated and treated plates were incubated at $37^0$C and $28^o$C for bacterial and fungal strains respectively for 24 hours. After the incubation, the diameter of zone (zone of inhibition) was measured. The results were recorded in millimeters (mm).

## Results and Discussion

### Phytochemical evaluation

In the present investigation it is indicated that the dried fruit of *T. Chebula plant* contained different types of phytochemicals such as alkaloids, flavonoids, saponin, terpenoids, tannin, glycoside carbohydrates and proteins in all the extracts. There have been reports that the presence of different phytochemicals with biological activity have valuable therapeutic index. It has been observed that the biologically active phytochemicals were present in all the extracts of *T. chebula*. (Table-1).The investigation showed that acetone and ethanol extract show the maximum qualitative results as compared to all other extracts. There has been report of the presence of different phytochemicals with biological activity that have valuable therapeutic index **(*Senthilkumar* and *Reetha*, 2009)**

**Table-1 Phytochemical evaluation of different extracts of fruit of *Terminalia chebula***

| S. No. | Phytochemicals | Extracts | | | | | |
|---|---|---|---|---|---|---|---|
| | | aqueous | acetone | Methanol | ethanol | Di ethyl ether | chloroform |
| 1 | Saponin | + | - | - | - | - | - |
| 2 | Tannin | + | + + + | + + | + + + | + + | + + |
| 3 | Flavonoid | + | + + + | + | + + | + | + |
| 4 | Terpenoid | + | + + + | + + | + + + | + + | + + |
| 5 | Glycosides | + | + + | + + | + + + | + + | + |
| 6 | Alkaloid | + | + + + | + + | + + + | + | + + |
| 7 | Carbohydrates | + + | + + + | + + | + + + | + + + | + + |
| 8 | Protein | + + | + | + | + | + | + |

(+ = Presence of compound and - = Absence of compound)

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (***Sofowora*,1993**). **Ali *SS et al*, 2008** reported that natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols *etc.* The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation **(*Benavente-garcia,* 1997**). The phytochemical analysis of the *P. guajava* extract revealed the presence of tannins while that of *M. indica* showed the presence of alkaloids, saponins and tannin. Tannins have been found to form irreversible complexes with proline-rich proteins **(Hagerman and Butler, 1981)** resulting in the inhibition of the cell protein synthesis. Tannins are polyphenols that are obtained from various parts of different plants **(*Gajendiran and mahadevan*, 1990).**

## Antimicrobial evaluation

In the present study, the antibacterial activity of the fruit of *Terminalia chebula* was tested by the disc diffusion method against bacterial species *like Bacillus subtilis, Staphylococcus aureus, E coli, Pseudomonas fluorescens and fungi like Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum.* The results of the present investigation indicated that the ethanol extracts of the dried fruit show maximum zone of inhibition against S*taphylococcus aureus* as well as *Bacillus cereus* (Gram-positive species) . *It also gives promising results against* Gram-negative bacteria which is used in this investigation (Table -2). The dried fruit showed higher zone of inhibition (12.5 mm in *Staphylococcus aureus* and 12 mm in *Bacillus sutilis)*. The aqueous extracts showed a decreased zone of inhibition when compared to all the extracts.

**Table-2 Shows the antimicrobial evaluation (Inhibition zone in mm) of *Terminalia chebula* dried fruit in various extracts.**

| Strains | | Extracts | | | | | |
|---|---|---|---|---|---|---|---|
| | | Aqueous | Acetone | Methanol | Ethanol | Di ethyl ether | Chloroform |
| Gram+ve bacteria | *Bacillus subtilis* | 5 | 12 | 10 | 11 | 10 | 9 |
| | *Staphylococcus aureus* | 4 | 12.5 | 8.5 | 10 | 7 | 8 |
| Gram-ve bacteria | *E.coli* | 3 | 11.5 | 8 | 12.5 | 9 | 11 |
| | *Pseudomonas fluorescens* | 2.5 | 11.5 | 11 | 12 | 9 | 10 |
| Fungi | *Aspesgillus niger* | 5,5 | 10 | 9 | 9 | 4 | 7.5 |
| | *Aspergillus fumigatus* | 4.5 | 9 | 9.5 | 8.5 | 7.5 | 8 |
| | *Penicillium chrysogenum* | 5 | 10.5 | 11 | 10 | 8.5 | 9.5 |

The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant fruit extract. This finding conforms to the report of ***Anyanwu* and *Dawet*, 2005.** Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection**.** They have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall **(*Marjorie*, 1999).** In addition to use in leather processing industries, tannins have shown potential of antiviral antibacterial (***Akiyama et al*, 2001),** and antiparasitic effects **(*Bhagavathi et al*, 1999)**. In the past few years tannins have also been studied for their effects against cancer through different mechanisms. Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy **(*Thirupathy et al*, 2004).** Medicinal plants have been considered a boon to human society to cure a number of ailments **(Murray, 1995)**. Several works have documented the pharmacological screening of plant extracts which have been exploited as the source of innumerable therapeutic agents **(*Natrajan et al*, 2003; *Yoshikazu et al*, 2001; *Herrera et al*, 1996).**

## Conclusion

Different types of secondary metabolites are reported from *Terminalia chebula* that have effective functions on many diseases. Especially the Terpenoids, Tannins, Flavonoids and Alkaloids are present in more quantity which makes this *plant* with high pharmacological properties. In the present study, it was found that *Terminalia chebula* acetone extract has an excellent antimicrobial activity. The pathogenic bacteria and fungi were inhibited in presence of the fruit extracts of *Terminalia chebula.* Therefore, the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant for controlling pathogenic bacterial and fungal strains. Hence, it is considered as most important medicinal plant used in medicines of Ayurveda, Siddha, Unani and Homeopathy.

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# Inhibitory activity of Immunobooster Tea against pathogens

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**ABSTRACT-**Indian medicinal plants manifest miraculous effects in curing a vast range of diseases and disorders among humans and can be better called as "elixirs of life." Currently, there is much growing interest in the use of these medicinal plants as immunomodulators of the complex immune system. Through a number of vast researches conducted in the area, it is being explored that many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties. Keeping in mind, the tremendous potential of the medicinal plants and their derived drugs, a study is designed to evaluate the inhibitory antibacterial activity of a immunobooster tea formulation against different bacterial strains using methanol, Hexane and water as solvents.

**Keywords**:Immunity; Immunobooster tea; Immunomodulators, Antimicrobial activity

## INTRODUCTION

Traditional medicine research has given high priority to immune boosting medicinal plants due to the prevalence of diseases like HIV/AIDS and other viral diseases which affect the immune system (Devasagayam and Sainis,2002). However, very little information on plants with immune boosting potentials have been documented (Gupta,1994).

Through a number of researches conducted in the area have explored that many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties(Wadood et al,2013).

The term immunity defines body's natural defense system against a vast array of diseases and disorders. Remarkably sophisticated and advanced among vertebrates, the complex immune system is capable to generate a limitless variety of cells and molecules to arrest enormous spectrum of infections and undesirable substances. Immunomodulators refer to those substances capable of inducing, amplifying, and inhibiting any component or phase of the immune system. Immunostimulators and immunosuppressant are two types of immunomodulators are known for use. In fact, immunopharmacology is a newer branch of pharmacology concerned with immunomodulators(Patil,2012). Administration of immunostimulators as in the case of AIDS and use of immunosuppressor in cases of an exaggerated response of an immune system is appreciating to reconstitute the normal immune system and increase the longevity of life. Immunomodulator intake along with antigen, the process is meant to boost the immune system, and the modulator is known as immune adjuvant(Dutt,2013).

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 semi synthetic derivatives of natural products and used in the traditional systems of medicine. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new drugs introduced in the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983

and 1994 (Cragg et al., 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new immunomodulator compounds with also have the potential to act against bacteria and viruses.

According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India.

Ayurvedic system of medicine has its long history of therapeutic potential. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted world wide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995;Nascimento et al., 1990).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). Therefore,researchers are increasingly turning their attention to folkmedicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Bylka et al., 2004; Shimpi and Bendre, 2005; Kilani,2006).

The current research in the area to develop plant-derived natural products as potent and safer leads to act as immunomodulators, is gaining much interest. Generation of herbal medicine as multiple-component agent expected to modulate the complex immune process in such a way so as to prevent the infection rather than treatment and cure of the disease. With all these aspects keeping in mind, the present work focuses on an antimicrobial activity of a immunobooster tea formulation.

## Material and Methods

### *Collection of plant material*

The immunobooster tea formulation studied consists of a combination of 15 indigenous medicinal herbs were collected from in and around Dehradun (Uttarakhand state, India).The Herbs were identified based on the taxonomical characteristics by Dr.Mayaram Uniyal, Department of Pharmacognosy, The Himalaya Drug Company Dehradun, India. The dried herbs were powdered and used for extraction.

### Test microorganisms

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

### *Preparation of aqueous extract*

The finely powdered plant materials (100 grams) were boiled in 500 ml distilled water till one-fourth of the extract initially taken was left behind after evaporation. The solution was first filtered through double layered muslin cloth and centrifuged at 5000 g for 30 min and the supernatant was filtered through whatman No.1 filter paper under strict aseptic conditions and then the filtrate was collected in fresh sterilized bottles and stored at 4°C until further use.

### *Preparation of solvent extract*

100 grams each of the powdered material was extracted with 500ml of methanol &Hexane separately for 24hrs. The extract were filtered with sterile whatman filter paper No.1 into a

clean conical flasks. The solvent along with the sample was transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvent. The evaporated solvent so obtained was weighed and preserved at 4$_o$C in airtight bottles until further use.

***Determination of antimicrobial activity through Agar-well diffusion assay***:

Suspension of 24 h cultures of *Escherichia coli, Staphylococcus aureus, Salmonella enterica ,Pseudomonas aeruginosa and Candida albicans* was made in sterile normal saline. Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5mm diameter was used to make wells on the medium. 100 µL of the various extract concentration were dropped into each, appropriate well(Atata et al;2003& Bonjar,2004).Methanol solvent used for extraction apart from water & Hexane was tested for each organism. The inoculated plates were kept in refrigerator for 2 hours to allow the extracts to diffuse into the agar. The agar plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 30 µg of standard antibiotic streptomycin was used as positive control and respective solvents as negative controls.

## Results and Discussion

The efficacy of Hexane,methanol and aqueous extracts of immunobooster tea formulation against

pathogenic bacteria showed varied level of inhibition (Table -1). It was revealed from the result that the formulation showed different degrees of inhibition against different microorganisms. The maximum zone of inhibition was observed in the case of *Staphylococcus aureus* (28mm) due to action of methanol extracts followed by *Salmonella enterica*(26mm) followed by *Pseudomonas aeruginosa*(25mm).

The results revealed variability in the bactericidal concentration of each extract for given bacteria. It was clear from the present result that methanol extract exhibited pronounced activity against all the tested four bacteria and *Candida albican*. The highest antibacterial activity as seen with methanol extract might be due to the presence of alkaloids and tannins(Okemo,1996). Broad spectrum activity of methanol extract tended to show that the active ingredients were better extracted with methanol. Earlier studies had also shown the greater antibacterial activity of methanol extracts than other solvent extracts(Aqil et al.2003 & Kannan et al;2009)With least antibacterial activity as seen with other solvent extracts, might be due to loss of some active compounds during extraction process of the sample and lack of solubility of active constituents in the solvent(Sampathkumar,2008).

In spite of this permeability difference between Gram positive and Gram negative bacteria, the methanol extract had a broader spectrum of inhibitory activity. This showed the involvement of more than one active principle of biological significance.This study does not only show the scientific basis for some of the therapeutic uses of these herbs formulation in traditional medicine, but also confirms the fact that ethnobotanical approach should be considered when investigating antimicrobial properties of plants(Iwu,1993 & Adesanya,2005).

## Conclusion

On the basis of the results obtained, it can be concluded that methanol can be used for extracting antimicrobial compounds from herbs

and formulations. The present study shows that immunobooster tea extracts possessed the antimicrobial activity against some organisms associated with infections and are highly resistant against antibiotics. Therefore, it suggests that this formulation can be a source of oral drugs to be used in the treatment of opportunistic infections and may be a source for future drug formation.

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# Phytochemical Analysis and Evaluation of Anti-inflammatory Activity of *Bignonia venusta (*Ker Gawl.) Miers Flower Extracts

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**Abstract-**The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extra vasations, cell migration, tissue breakdown and repair which are aimed at host defence and usually activated in disease condition. Currently much interest has been shown in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response is amplifying in the disease process. In the present study, the selection of *Bignonia venusta* plant for evaluation was based on its traditional usages. Preparation of different extracts from non polar and polar solvent were prepared to study the phytochemical analysis in different extracts and anti inflammatory activities of different extracts. Methanol extract was found to be the richest extract for phytoconstituents and from the comparison with the standard drug aspirin, it was observed that the concentration of 2000 µg/ml of methanol extract showed maximum activity (58.0%) at 560 nm while the other extract from petroleum ether, in comparison with standard drug aspirin shows no activity.

**Keywords:** Phytochemical Analysis, Anti-inflammatory, *Bignonia venusta*

## Introduction

Many species belonging to the Bignoniaceae family, such as *Bignonia venustata,* also known as *Pyrostegia venusta* (Ker Gawl). Miers are known to be of medicinal value[2]. In folk medicine, the aerial parts of *B. venusta* are mainly used as an infusion or decoction.

Traditionally, many diseases like dysentery, immoderate menstrual flow, common diseases of the respiratory system, and for the treatment of genital infections, *Pyrostegia venusta (*Ker Gawl.) Miers is used as a medicine. Diseases like diarrhea, vitiligo and jaundice are controlled by general tonic[3, 9]. Tonics made from the stem of *P. venusta* are useful for treating diarrhea, where as flower preparation has been showed to attenuate vomiting[13]. The decoction of aerial parts of *P. venusta* is used for the treatment of cough and flu by local Brazalians. It was shown by Immuno-Modulatory study of the methanol extract of flowers of *P. venusta* that it stimulates the immune system. It supports increase in anti inflammatory and

suppress pro- inflammatory cytokines. *P. Venusta* is a natural source of phytochemicals like terpenoids, alkaloids, tannins, steroids, and saponins which has been been correlated with potential degrees of anti-inflammatory and analgesic activity[1]. Compounds like β-sitosterol, n-hentriacontane, acacetin-7-O-β-glucopyranoside and meso-inositol having anti-inflammatory activities [8]. LPS is responsible for sickness behavior due to production of pro-inflammatory cytokines which provokes a number of neuropsychological symptoms [5]. Many studies have showed that acacetin inhibits the induction of nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) in macrophages that are activated with LPS by inhibiting the transcriptional activation [6]. The objective of this study was to evaluate the phytochemical constituents and anti-inflammatory effects of the methanolic and petroleum ether extract of *B. venusta* flower.

**Material and Methods**

Experimental work was carried out under following headings on the flowers of *Bignonia venusta* for their anti-inflammatory activity.

- Collection and identification of flowers of *Bignonia venusta*
- Extraction of flowers of *Bignonia venusta* in non-polar and polar solvents.
- Phytochemical analysis and thin layer chromatography of different extracts.
- Anti-inflammatory activity of different extracts.

**Qualitative Phytochemical Tests**

The different extracts made from the flowers of *Bignonia venusta* were tested for the various components as follows.

**Test for alkaloids**

Small portion of solvent free extract was stirred with few drops of dilute HCl and filtered. The filtrate was then tested for the following color tests.

**Mayer's Test**: (a) 1.36 grams of mercuric chloride was dissolved in 60ml distilled water .(b) 5gm of potassium iodide was dissolved in 20ml of distilled water. (a) and (b) were mixed and the volume was adjusted to 100ml with distilled water. Appearance of cream color precipitate with Mayer's regents showed the presence of alkaloids.

**Wagner's Test**: 1.27 grams of iodine and 2 grams of potassium iodide was dissolved in 5ml of water and made up to the volume to 100ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloid.

**Hager's Test**: Took 20ml of saturated solution of picric acid added few drops of it to 2-3ml of extract. A yellow color was absorbed.

**Detection for carbohydrates and glycosides**

**Molisch's Test**: 10 grams of alphe naphthol was dissolved in 100ml of 95% alcohol. Extract was treated with this solution and 0.2ml of concentrated sulphuric acid was slowly added through the side of the test tube, purple or violet color appeared at the junction. This indicated the presence of carbohydrates and glycosides.

**Benedicts Test**: The test solution was treated with few drops of Benedict's solution (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed, showing reducing sugars were present.

**Fehling's Test**: 6.932 grams of copper sulphate was dissolved in distilled water and make volume upto 100ml (solution a). 34.6 grams of potassium sodium tartarate and 10 grams of sodium hydroxide was dissolved in distilled water and make volume upto 100 ml (solution b). two solutions were mixed in equal volume prior to use and few drops of sample were added and boiled, a brick red precipitate of cuprous oxide were formed, if reducing sugar were present.

**Barfoed's test:** 16.5 grams of copper acetate was dissolved in 24 ml of water and 2.5 ml of glacial acetic acid was added to it. Reddish brown precipitate were formed on boiling if reducing sugar were present.

**Test for Sterols and Triterpenoids**

**Salkowski Test:** Extract was treated with few deops of concentrated suphuric acid, shake well and allow to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow coloured lower layer indicated the presence of triterpenoids.

**Sulphur Powder Test:** Small amount of sulphur powder was added to the test solution, it sinks at the bottom, showing presence of sulphur powder.

**Test for Proteins and Amino acids**

**Biuret Test:** To 3 ml test solution 4 % w/v NaOH and few drops of 1% w/v copper sulphate solution were added. A blue color was absorbed.

**Ninhydrin Test:** 1 gram of ninhydrin (indane 1,2,3 trione hydrate) was dissolved in n-butanol and made the volume 200 ml. Extract treated with this solution gave violet color on boiling.

**Test for saponins**

**Foam Test:** 1ml of extract was diluted with distilled water to 20 ml and shook in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicated the presence of saponins.

**Test for Tannins and Phenolic compounds**

**Ferric Chloride test**: Extract was treated with ferric chloride solution, blue color was appeared if hydrlysable tannin was present and green color was appeared if condensed tannins were present.

**Vanillin Hydrochloride Test**: 1 gram vanillin was dissolved in 10 ml alcohol and 10 ml concentrated hydrochloride solution. Extract was treated with this solution gave pink or red color due to the presence of tannins and phenolic compounds.

**Anti-inflammatory Activity of extract**

There are several methods to determine anti-inflammatory activity. In the present study, we had studied the anti-inflammatory activity by Hypotonic induced hemolysis method.

**Preparation of NIH solution**:

| | | |
|---|---|---|
| Trisodium citrate | - | 5.5 gm |
| Citric acid | - | 2.0 gm |
| Dextrose | - | 6.125 gm |
| Distilled water | - | 250 ml |

Used 0.8 ml of NIH solution to preserve 5 ml of blood [14]

**Effect of Hemolysis** [11]

**Erythrocyte suspension**

Whole blood was collected from goat under ether anaesthesia. Heparin was used to prevent clotting. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (Ph 7.4).which was contained in 100ml of distilled water. The isotonic buffer solution was composed of 154mm NaCl in 10mm sodium phosphate buffer (pH 7.4).

**Hypotonic solution-induced hemolysis**:

Stock erythrocyte suspension was mixed with hypotonic solution containing the *bignonia venusta* flower extract at different concentrations, while the control was kept drug free. They were incubated for 10 min at room temperature and centrifuged at 3000g for 10 min. All the experiments were performed in triplicates and the absorbance (O.D) of the supernatant was measured at 560nm.

Acetyl salicylic acid (200mg/ml) was used as a reference standard.

**Calculation**

The percentage inhibition or acceleration of hemolysis in tests was calculated according to the equation:

% acceleration or inhibition of hemolysis = 100*(OD1-OD2/OD1)

Where, OD 1 = optical density of hypotonic saline solution + blood (control) and OD 2 = Optical density of test sample in hypotonic saline solution + blood.

## Results and Discussion

**Phytochemical Analysis**

The extract of flowers of *Bignonia venusta* undergoes various qualitative chemical tests. They showed their presence and absence in the both solvent system which is summarized in table-1. From the table, we can find out that methanol extract was the richest extract for phytoconstituents except proteins and amino acids. It contains all tested phytoconstituents viz. Alkaloids, carbohydrates, saponins and phenolic compounds.

The studied flowers were extracted by two solvents i.e. petroleum ether and methanol by cold percolations and the yield of flower extracts in petroleum ether and methanol are 35 ml and 3.45 gm respectively.

In a study on the extracts of leaves of *Bauhinia variegata*, it was reported that the methanol extract was richest extract for phytoconstituents[4]. Except tannins of phenolic compounds, carbohydrates and flavonoids, it

contains all tested phytoconstituents viz. Alkaloids, glycosides, proteins and amino acid, triterpenoids of sterols, phenolic compounds and saponins and fats and fixed oil. The methanol extract was the richest extract for phytoconstituents while petroleum ether extract contains least phytoconstituents was reported on the study on the extracts of seeds of *Gmelina arborea*[10]. It was also reported that the methanol extracts of the leaves and flowers of *Cassia glauca* was the richest source of phytochemicals [12]

The anti-inflammatory activity of the different extracts of *Bauhinia variegata* flowers was compared with activity of standard drug Aspirin at 560 nm. From the comparison with the standard drug, it was observed that the concentration of 2000 µg/ml of methanol extract showed maximum activity (58.0%) at 560 nm while the other extract from Petroleum ether, in comparison with standard drug aspirin shows no activity. Table(2,3 and 4).

In an author work the anti-inflammatory activity of the different sample extracts of leaves of *Bauhinia variegata* was also compared with activity of standard drug Aspirin at 560 nm. From the comparisonWith standard drug it was observed that the n-Butanol extract shows maximum activity at 560 nm while other extract such as methanol, chloroform and petroleum ether show less activity[4]. In his study, he reported that petroleum ether extract showed more anti-inflammatory activity in comparison to that of methanol extracts of leaves of *Bauhinia variegata.*

It was also reported in a study on the anti-inflammatory activity of the different sample extracts of seeds of *Gmelina arborea* that methanol extract showed more anti-inflammatory activity at 560 nm in comparison to standard drug i.e. Aspirin while other extract of petroleum ether show less activity [10].

Singh[12] worked on the anti-inflammatory activity of the leaves and flower extracts of *Cassia glauca*. He compared the anti-inflammatory activity of the different sample extracts with the activity of standard drugAspirin at 560nm. From the study, he reported that the concentration of 3000 µg/ml of methanol extract showed the maximum activity at 560nm, as compared to acetone, chloroform and hexane[12].

**Table-1 Qualitative Phytochemical analysis of extract of *Bignonia venusta* flower.**

| Test performed | Pet. Ether Extract | Methanol Extract |
|---|---|---|
| **Test for Alkaloids** | | |
| Mayer's test | - | - |
| Hager's test | - | + |
| Wagner's test | - | + |
| **Test for carbohydrates** | | |
| Fehling's test | - | - |
| Molish's test | - | + |
| Benedict test | - | + |
| Barfoed's test | - | + |
| **Test for Steroids** | | |
| Salkowaski test | + | + |
| **Test for Saponins** | | |
| Foam test | - | + |
| **Test for phenolic compounds** | | |
| $Fecl_3$ - test | - | + |
| Vanillin HCl test | + | + |
| **Test for proteins and Amino acids** | | |
| Biuret's test | - | - |
| Ninhydrin test | - | - |

(-) Absence, (+) Presence

**Table-2 Standard drug used for Anti-inflammatory action and results**

| Sample Aspirin | concentration | Absorbance (at 560nm) | % Inhibition of hemolysis |
|---|---|---|---|
| 1 | Control | 0.711 | - |
| 2 | 1000 | 0.359 | 49 |
| 3 | 1500 | 0.323 | 54 |
| 4 | 2000 | 0.296 | 58 |

**Table-3 Effect of different extracts of bignonia on stability of erythrocyte membrane: (petroleum ether extract).**

| Sample | Concentration (µg/ml) | Absorbance (at 560nm) | % Inhibition of hemolysis |
|---|---|---|---|
| 1 | Control | 1.72 | - |
| 2 | 1000 | 1.92 | 0 |
| 3 | 1500 | 2.69 | 0 |
| 4 | 2000 | 2.08 | 0 |

**Table-4 Effect of different extracts of *Bignonia venusta* on stability of erythrocyte membrane: (Methanol extract).**

| Sample | Concentration (µg/ml) | Absorbance (at 560nm) | % Inhibition of hemolysis |
|---|---|---|---|
| 1 | Control | 1.47 | - |
| 2 | 1000 | 1.28 | 12 |
| 3 | 1500 | 1.17 | 20.4 |
| 4 | 2000 | 1.09 | 25.85 |

## Conclusion

The anti-inflammatory activity of the different extracts *Bignonia venusta* was compared with the activity of standard drug Aspirin at 560nm. From the comparison with the standard drug, it was observed that concentration of 2000µg/ml of methanol extract showed maximum activity (58.0%) at 560 nm while the other extract from petroleum ether, in comparison with standard drug aspirin shows no activity. The methanol extract of *Bignonia venusta* showed increase in the protection of the erythrocyte membrane against hypotonic haemolysis and less protection shown by petroleum ether.

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# Phytochemical Aspects of Indian Valerian 'Tagara' (*Valeriana Wallichii*) from Uttarakhand, Western Himalaya

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**Abstract-***Valeriana Wallichii* is also known as Tagara found in temperate regions of Himalayas. It is used as herb and well documented in Ayurvedic system as well as modern medicinal system. In the present study investigation on different phytochemical constituents screening was carried out from medicinal plant, *V. Wallichii*. Preliminary phytochemical screening reveals the presence of different bioactive compounds such as alkaloids, flavonoids, terpenes, saponin, tannin, phytosterol, gylcosides, steroids and phenolics. GC-MS and FTIR analysis showed that important phytochemical compounds, bond and important functional groups are present in *Valeriana wallichii.* There is need to study on the comprehensive phytochemical composition and pharmacological characteristics of *V. wallichii* along with analysis of the biomedical researches on this multipurpose herbaceous species in near future.

**Key Words:** GC-MS, FTIR, Phytochemical, *Valeriana Wallichii*

## Introduction

Indian Himalaya occupies a unique location with remarkable storehouse of biological resources in its diverse habitats and ecosystems that has made it one of the most biodiversity rich regions of India. It is the place of more than 8000 inhabited species of vascular plants, out of which 1748 are identified for their medicinal properties. WHO reports claimed that world's 80% of population totally depend on traditional system of medicine (Igoli et al., 2003). *Valeriana Wallichi* also known as Tagara in Ayurvedic medicinal system as Indian valerian belongs to valerianaceae family, is wild medicinal herb distributed at higher altitudinal region (upto 3000 m asl) of Himalayan regions of China, India, Nepal and Pakistan (Sahu et al., 2016). This plant is a perennial herb whose under part consists of a rhizome bearing many rootlets and flowers which are small and white or pink in color, the fruits are oblong-ovate, single seeded achienes.

Medicinal uses of this plant are used for patients suffering from restlessness (Balderer & Borbely, 1982), for nervous disorders, it reduces a high blood pressure and stress is used as an antidote for snake poisoning, cure disease of the blood liver and eye. It is used in neurological, psychological, and digestive

disorder in small dosage, it calms the mind, strengthens nerves (Singhal et al, 2013). Modern uses are in the treatment of disturbed sleep (insomnia), and paralysis. It has anti-inflammatory actions (subham et al, 2007), that is why, it is used to treat rheumatoid arthritis, it helps to treat abdominal pain, gas, abdominal heaviness and improves the strength of the muscle, heart and reduces blood pressure.

## Material and Methods

Roots of *Valeriana Wallichii* were collected from local market of Dehradun. They were cleaned first and crushed in mortar & pestle. Extraction of essential oil carried out by hydro distillation unit. The fresh leaves were washed with tap water and then rinsed with distilled water. It was then air dried, cut into pieces and pulverized into fine powder using a grinder. The dried powdered plant extracts were extracted in methanol in soxhlet apparatus for 4 hours. The extract was concentrated by using hot plate for 1 hour for the reduced to get crude extract.

**Preparation of plant extracts**

*Leaves samples of Valeriana Wallichii* were dried under shade and ground in powdered form. The powdered form was stored in a container for use .

**Method of extraction**

Continous hot percolation (successive solvent extractions) was done by applying Soxhlet apparatus, methanol is used with dried coarse powder of *V. Wallichii* .

**Extraction procedure for *valeriana wallichii***

Extracts of dried leaves of *Valeriana Wallichii* were obtained with methanol by using Soxhlet extraction. 10 grams of coarse powder of dried leaf w*as* sequentially extracted in a Soxhlet extractor using 100ml of methanol for four hours. The extracts were evaporated using rotary evaporator and 2ml were stored at $4^{o}$c. After that, it was filtered through the filter paper and concentrated by rotary evaporator.

**Test for saponins**

Leaves extract (About 10ml) was used to mix with 5ml of distilled water and shaken vigorously for a stable foam soap frothing which was mixed with olive oil and shaken, then observed for emulsion formation.

**Test for tannin**

2ml of the leaves extract was mixed with 3ml of 5% of solutions of $FeCL_3$ presence of black coloration indicated presence of tannin.

**Test for steroids**

1ml of the leaves extract was mixed with chloroform (2ml) then concentrated $H_2SO_4$ was added, Presence of red color in lower chloroform layer indicated presence of steroids.

**Test for phytosterol**

Leaves extracts were mixed with chloroform and then filtered. The filtrates were treated with few drops of concentrated $H_2SO_4$ shaken and allowed to stand. Appearance of golden yellow color indicates the presence of phytosterol.

**Tests for flavonoids**

Flavonoid content was determined by adding concentrated sulphuric acid. Few drops of 1% $Alcl_3$ solution were added to a portion of each fraction. Yellow coloration indicated the presence of flavonoids, which disappered on standing.

**Test for glycoside**

The Leaves extract was hydrolised with diluted HCl and extract was treated with ferric chloride solution and immersed in boiling $H_2O$ for 5minute. The mixture was then cooled and mixed with equal volume of benzene. Benzene layer was separated and treated with ammonia solution. Rose pink color formation in ammonia layer indicated the presence of glycoside.

**Test for phenol**

Phenol content of plant was found out by Folin-Ciocalteu reagent method. First, test tube was kept in the dark for 2h and the absorbance was quantified by UV- spectrophotometer at wavelength 750nm.

Leaves extract was dissolved in methanol (1ml) and 10% Folin-Ciocalteu reagent was arranged by putting in 10 ml of Folin-Ciocalteu reagent in water (90ml), then 5% $Na_2CO_3$ (3g) was prepared by putting $Na_2CO_3$ (3g) in water (50ml). Leave extract (200µL) was kept in the test tube and added 10% Folin-Ciocalteu reagent (1.5ml). Then all the test tubes were kept in a dark place for 5min. Finally 5% $Na_2CO_3$ (1.5mL) was added to the solution and mixed by hand and again the test is carried out.

**GC-MS analysis**

Methanolic extract of leaves samples excised from plant by using soxhlet apparatus. The leaves extract was then subjected to GC-MS analysis for identification of different phytocompounds.

**FTIR Analysis**

The ether and methanol extract of *Valeriana Wallichii* was subsequently subjected to FTIR analysis so as to identify diverse phytocompounds present in the extract.

## Results and Discussion

Phytochemicals are chemicals substances having particular physiological functions on human body, which protect it from any of health issues such as diseases, injury etc. These are the reasons for medicinal value of medicinal plants.

1. **Saponin**- It is secondary metabolites and its structure is composed of one or more hydrophilic glyciside . Saponin is stable foam produced when they are shaken in the solution and the soap like foam is indicated the presence of saponin in the methanolic sample of *Valeriana Wallichii*. Toxicity to fish and haemolysis of RBC are two distinct properties of this group. It is found to be antimicrobial and employed as expectorant.
2. **Tannin**- Tannins are an important group of plant polyphenolics which are present in a variety of plants and are utilised as both food and feed due to their potential biological activity. Black coloration in test tube was showed to the presence of tannin in the methanolic sample of *Valeriana Wallichii.* Beside, oxidant properties they also possess myraid physiological properties, for example anti-allergenic, anti-inflammatory, antimicrobial etc. It acts as antioxidant in edible substances specially fats and medical astringents.
3. **Steriods**- Steriods have important biological functions and important components of cell, membrane fluidity**.** All the steriods are manufactured in the cells.

4. **Phytosterol-** Phytosterol is a plant sterols and stanols similar to the cholesterol which occur in plants and vary in carbon side chains and presence or absence of a double bond. They represent physiologically active compounds.
5. **Flavonoids**-Flavonoids serve as an important source of antioxidant found in different medicinal plants. In addition, plant flavonoids which show an antioxidant activity In vitro, also function as antioxidant activity in vivo. It exhibits anti-inflammatory properties also.
6. **Glycoside-** Glycoside play a important role in plants. They are known to have cardiac activity
7. **Phenol**- Phenolics have multiple biological properties including antimicrobial and antioxidant activity, the antioxidant activity is mainly due to their redox properties. They are the best anti oxidants. Methanol extract contain the lowest amount of phenol.

**Table Constituents of *V. Wallichii* leaves**

| S. no. | Constituents | *V. Wallichii* leaves (Methanol extract) |
|---|---|---|
| **1** | Saponin | + |
| **2** | Tannin | + |
| **3** | Steroids | + |
| **4** | Phytosterol | + |
| **5** | Flavonoids | + |
| **6** | Glycoside | + |
| **7** | Phenol | + |

**GC-MS analysis-**

Ether extract of *Valeriana Wallichii* leaves was analyzed with the help of GC-MS analysis and major phytocompounds found to be present were 7-Nitro-1-Tetralone Oxime Acetate; 3-[N-Acetyl-4-Acetylanilino] Propionic acid; Dihydroxanthin; Flutamide; Ethanethioic Acid, S-Pentyl Ester; 1,3-Cyclopentadiene-1,3-Dicarboxylic Acid; 1,5-Dodecadiene; Heptane, 4-Azido. Methanol extract of *Valeriana Wallichii* leaves was analyzed with the help of GC-MS analysis and major phytocompounds found to be present were 1, 3-Dioxin-4-One, 5-Bromo-2, 6-Dimethyl; Flutamide; Acetamide, N-[4-Nitro-2-(Trifluoromethyl); Quinoxaline, 1,4-Diacetyl-1,2,3,4-Tetrah; Ethanethioic Acid, S-Butyl Ester; 2,5-Cyclohexadiene-1,4-Dione, 2,5-Diacet; Glycine, N-Acetyl-, Ethyl Ester; Ethanethioic Acid, S-(2-Methylbutyl), Valeric acid ,pentadecadienoic acid, aristolene, Est. These phytochemicals are responsible for various pharmacological actions such as antimicrobial, anti-inflammatory, antioxidant etc.

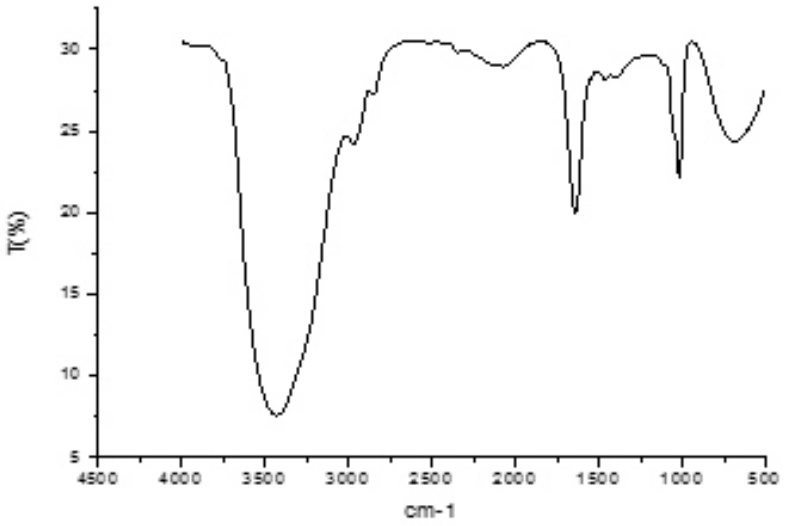


Fig.2 FTIR spectrum of (A) leaf methanol extract of *V. Wallichii*

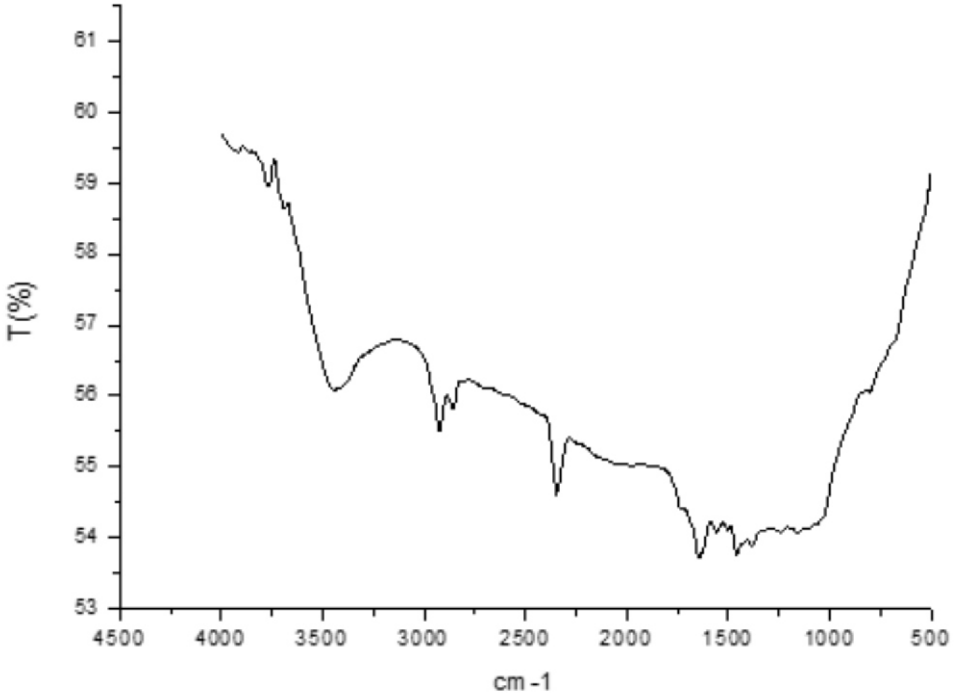


Fig.1 FTIR spectrum of (B) leaf ether extracts of *V. Wallichii*.

Fourier Transform Infrared spectroscopy is related to absorption of light in infrared region by the molecular atom. FTIR is basically used to observe the identification of bond and functional groups present on the surface of organic, inorganic a polymeric substance in frequency range of 4000-500cm-1. The broad peaks are obtained by methanolic extract of *V. wallichi* at 3441cm-1, 2077cm-1, 1641cm-1, 1018cm-1 and 648cm-1. These peaks are related to presence of O-H, C=C (alkene), C=O and C=C. The broad peaks are obtained by ether extract of *V. wallichi* at 3450$cm^{-1}$, 2850$cm^{-1}$, 2350$cm^{-1}$ and 1643$cm^{-1}$. These peaks are related to presence of O-H, C-H(alkene). C=O ($CO_2$), C=O (aldehyde ketonic) etc. *Valeriana wallichii* DC, is an medicinal plant used in Indian traditional system for treatment of central nervous system from many decades.

*Valeriana Wallichii* is categorized under the family *Caprifoliaeceae* few years ago but in recent years it was categorized under family *Valerianaceae*. It is also be suggested by Ayurvedic medicinal system that *Valeriana Wallichii* having important pharmacological properties including neuroprotective, sedative, anticonvulsant, hypnotic anxiolytic, antispasmodic and anti-inflammatory analgesic properties. The present investigation are in agreement with the previous finding of Devi et al., 2014, who suggest that presence of various phytochemicals constituents within Valeriana Wallichii make it effective medicinal plants against chronic disease such as cancer, AIDS, heart disease and diabetes etc.

Phytochemicals have important role to cure much chronic disease of present scenario. They are categoriesed into two types on the basis of its work on plant growth, metabolism and development. (Wadood et al., 2013). Primary phytochemical include amino acids, carbohydrates, lipids, nucleic acids etc. while secondary phytochemical include alkaloids, saponins, flavonoids, phenolics, terpenes and steroids etc. The presence of different phytochemical constituents gives to plant additional characteristics. Presence of flavonoids give rise to plant antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer, antiallergic and diuretic characteristics (Yadav et al., 2014; Chic et al., 2014).

## Conclusion

The present study concluded that due to various phytochemicals present in *Valeriana Wallichii,* it posseses effective medicinal values. Besides, its antioxidant, antimicrobial, anti-inflammatory, antileishmanial activity, sedative and hypnotic properties are also reported Studies on *Valeriana Wallichii* should further be carried out to develop new potential compound to modern drug. In addition, studies should be made to reveal the mode of action produced by *Valeriana Wallichii* which may be helpful in understanding its possible roles in modern medicines.

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# Phenylhydrazine Induced Haematotoxicity and Its Amelioration by Ethanolic Extract of *Momordica charantia*

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**Abstract**-It has been established that several plants serve as haemoprotective agents. The mechanism is that they bind to the oxidative radicals which is produced during the haemolysis of red blood cells and the binding restores the actual form of these cells. This way, the degenerative effect of plants as the blood is ameliorated by plants. In our study, phenyl hydrazine caused significant anaemia in Charles Foster rats which was recovered by ethanolic extract of *Momordica charantia.*

**Keywords:** *Momordica Charantia,* Haematological analysis, Anaemia

## Introduction

*Momordica charantia* showed strong antioxidant activity in *in-vitro* experiments. This activity was correlated with its poly - phenolic constituents which play important roles in its anti -oxidative effects. Polyphenols act as reducing agents and as antioxidants by several mechanisms as by scavenging of free radicals , mediation and inhibition of enzymes and chelation of transition metals (Chang *et al*., 2011 ; Singh and Singh, 2019 ; Meena *et al*., 2019 ).

We have seen the effect of phenyl hydrazine on haematological parameters of Charles Foster rats and recovery of anaemia by ethanolic extract of *Momordica charantia*.

## Material and Methods

### Chemicals

Ethanol and phenylhydrazine were purchased from Sigma Aldrich, USA. All other chemicals used in the study were of analytical grade and available locally.

### Plant Materials

*Momordica charantia* was purchased from local market of Lucknow city of Uttar Pradesh. These samples were authenticated by Botany division of CSIR-CDRI, Lucknow.(U.P.), India.

### Preparation of Plant Extracts

The material was dried at room temperature and ground into powder. Dry powder 4 Kg was macerated in 95% ethanol and kept at room temperature for 48 hours. The resulting extract was filtered and filtrate the was concentrated in an oven at a temperature of $40^0$ C for 24 hours. The extract was stored at $40^0$ C.

### Test Animals

The rats of both the sexes were obtained from National Laboratory, Animal Center, CSIR-Central Drug Research Institute, Lucknow and were allowed to acclimate for 7 days before the start of experiment. They were kept in a controlled environment at $24\pm3^0$C and 40-55 % relative humidity with a 12 hour dark and light cycle. The animals were fed a standard rodent pellet diet and water ad libitum. Animal studies were conducted as per regulations of Institutional Animal Ethics Committee.

### Induction of Anaemia

Basal haematological values were determined by using MS-9 haematology

analyzer. Phenyl hydrazine is an established model for induction of anaemia (Biswas *etal.,* 2005 ; Chang *et al.,* 2011 ). In our study, phenyl hydrazine at a dose of 10 mg / kg body weight for seven consecutive days was used to induce anaemia. After 7 days, haematological values were determined and rats with a reduction of 35-45% in haemoglobin, total red blood cell count and haematocrit were considered anaemic.

**Experimental Procedure**

The rats were divided into four groups of 10 animals ( 5 males and 5 females) in each group. They were subjected to the following treatments.

Group I Control-Distilled Water ( 7 Days) ( 1% Gum Acacia)

Group II- 10 mg/ Kg B.W. PHZ (7 Days), then 500 mg/ Kg *M. charantia* extract

Group III- 10 mg/ Kg B.W. PHZ (7 Days), then 1,000 mg/ Kg *M. charantia* extract

Group IV- 10 mg/ Kg B.W. PHZ (7 Days), then 2,000 mg/ Kg *M. charantia* extract

At the end of 21 days, all the rats were anaesthetized with diethyl ether and sacrificed.

**Body Weight**

Body weight was recorded at days 0, 7, 14 and 21 of the experiment.

**Food and Water Consumption**

Monitoring of 24 hours food and water consumption of the animals in all the groups were done at days 0, 7, 14 and 21 of the experiment. This was done by giving a measured quantity of water and pellet diet followed by estimation of the amounts remaining at the end of 24 hours. Average food and water consumption per animal was calculated for each group.

**Haematological Analysis**

Blood samples were collected at days 0, 7, 14 and 21 of the experiment through tail vein in EDTA coated vials. The haematological parameters-haemoglobin, total red blood cell count and haematocrit were analyzed by using MS-9 fully automated haematology analyzer. At the end of 21 days, all the vital organs were collected by using standardized surgical procedure. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidney and brain were quickly removed, cleaned with normal saline, weighed and preserved in 10 % formalin.

**Statistical Analysis**

All the data were analyzed and results are expressed as Mean + Standard Deviation. Data were analyzed with one-way ANOVA. Statistically significant effects were further analyzed. The statistical significance was determined at p o.o5.

## Results and Discussion

**Induction of Anaemia**

The rats which were given 10 mg / kg body weight for 7 days, significant decreases in haemoglobin ( 45 %), total red blood cell count ( 43 % ) and haematocrit ( 44 % ) were observed . This way, the anaemic conditions were recorded in all the rats.

**Body Weight**

After 7 days, there was decrease of 15-20 % in all phenyl hydrazine treated groups of rats. At the end of 21 days, the body weights were recovered and comparable to controls.

**Food and Water Intakes**

The animals treated with phenyl hydrazine at dose of 10 mg/ kg body weight for 7 days showed insignificant decreases in the consumption of food and water. After 7days of extract treatment , this was recovered and at the end of 21 days, insignificant increases were recorded.

**In vivo Haemaprotective Activity**

The haematological parameters in phenyl hydrazine treated animals were insignificantly decreased at the end of 7 days. After 7 days of treatment of different doses of extract , it recovered and was comparable to controls which after further treatment of extract for another seven days at different doses caused dose related increases of haematological parameters of all the animals.

## Conclusion

In phenyl hydrazine induced anaemic rats, different doses of *Momordica charantia* extracts ( 500 mg, 1,000 mg and 2,000 mg ) were evaluated. Rat is an established animal model for the induction of haemolytic anaemia (Mc Millan etal.,2005). Phenyl hydrazine generates ROS within both human and rat erythrocytes. The production of ROS was associated with extensive binding of oxidized and denatured haemoglobin to the membrane cytoskeleton. This way phenyl hydrazine induces haemolytic injury which is derived from oxidative alterations to red blood cell proteins rather than to membrane lipids .

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Singh , Jyoti and Singh, R. K. Antioxidant activity of ethanolic extracts of *Daucus Carota Leaf*. *World Journal of Pharmaceutical Research,* 2019,8(9):912-918.

# 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.

**Keywords:** HiOwna, Haematological, parameters, Immunomodulatory effects

## 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 *etal*., 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 $22+2^{0}$C 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 were 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.

## Results and Discussion

**Body Weght** :- There was significant increase in body weight of all the HiOwna fed groups of rats as compared to control and it was dose related.

**Food and Water Intakes** :- Food and water intakes in all the treated rats were well comparable to control and within physiological limits of normalcy.

**Haematological Parameters**

**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.

**Total Red Blood Cell Count**

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

**Haematocrit**

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

**Platelet Count**

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.

**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.

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 *et al*., 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 favourably modified cognition in children in children (Palani *et al*., 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. We have also found that HiOwna powder is having significant improvement in management and prevention of arthritis.

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# Standardization of Phytomarker for Determining Immunomodulatory Activities in *Ocimum sanctum* and *Curcuma longa*

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**Abstract-**Medicinal plants have been used for centuries and have become part of complementary medicine worldwide because of their health benefits. Some plants used directly in the treatment and prevention of infectious diseases or indirectly by stimulating the immune system. In recent years there is a great demand throughout the world to use herbal products in healthcare system. Plant materials and their products are becoming popular because of their wide biological activities. Standardization of herbal products is essential for their acceptance as remedies for various diseases and ailments. Marker based standardization is one of the widely accepted methods which is based on the analysis of phytochemical markers using HPTLC, HPLC etc. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker based standardization. Marker compounds are chemically defined constituents of the herbal drug with or without therapeutic activity and can be used for the quality assurance of finished product. In nature, there are various medicinal plants which are used as immunomodulator agents. In this paper we standardize the phytomarkers of some selected plants which are very essential for our daily life.

**Keywords:** Phytomarker, Immunomodulatory activities, *Ocimum sanctum, Curcuma longa*

## Introduction

Human survival is dependent upon their defensive immune mechanisms against External harms, pathogenic microbes and cancer. It is well known that the immune system helps the host to control microbes, allergic or toxic molecules and prevent cancer development[1]. Herbs with immunomodulatory properties are a moderately recent concept in phytomedicine. In addition to the enhancement of the humoral and cell-mediated immunity, the concept of immunomodulation initiates the activation of the "non-specific" immune responses which include the activation of the complement system, granulocytes, macrophages and natural killer cells. Hence activation of these essential immune cells initiates the production of various effect or molecules that take part in the modulation and enhancement of the immune responses[2,3]. The extreme manifestations of immunomodulating action of biologically active substances are immunosuppression and immunostimulation, hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world [4]. There are several medicinal plants employed in different system of medicine throughout the world to improve the immunological disorders. In India, use of plants as remedy can be traced back to 6000 BC[5,6]. In recent times modulation of immune response to cure various diseases has been a very interesting concept and the concept of rasayana in Ayurveda deals with the same. Ayurvedic system of medicine describes this concept of rasayana under which plants with rejuvenating activity have been described

which the emphasis on promotion of health by strengthening host defenses against different diseases. In addition, phytomarkers from natural sources have always been of great interest to scientists working on infectious diseases[7]or to improve immune function. *Ocimum sanctum* commonly known as 'Tulsi' has been extensively used in Ayurvedic system of medicine for various ailments.The compounds present in tulsi like ursolic acid exhibited potent effect against virus and has been shown to increase immunity, which may help fight viral infections. In a 4-week study in 24 healthy adults, supplementing with 300 mg of holy basil extract significantly increased levels of helper T cells and natural killer cells, both of which are immune cells that help protect and defend our body from viral infections [8]

*Curcuma longa* Linn. belonging to family *Zingiberaceae*, is a perennial herb extensively cultivated in all parts of the country, India is a largest country which produces the *curcuma longa linn* (about 90% of the total output of the world). It gives color and taste to the food and is well known for its health promoting effects. In ancient time, it was used as an anti-inflammatory agents to treat gas, colic, toothaches, chest pain etc. This spice was also used to help with stomach and liver problems, to heal wounds and lighten scar and as a cosmetic. The most active component of turmeric is curcumin which makes up to 2-5% of the spice, and is responsible for most of the therapeutic effects. Turmeric contains a wide variety of phytochemical including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, eugenol.The characteristic yellow color of turmeric is due to Curcuminoid first isolated by Vogel in 1842.[9]

In this paper, The standardization of phytomarkers through HPTLC and HPLC present in two plants Ocimum sanctum and curcuma longa is the major objective of this paper which are reported for their immunomodulatory activity. Two commonly used plants are *Ocimum sanctum* and *curcuma longa* used for this study which is easily available for everyone and everywhere. These plants possess immunomodulatory effects as reported earlier in various research papers.

## Material and Methods

### Plant materials

The leaves of *Ocimum sanctum* and *rhizome of Curcuma longa* were collected from the herbal garden of The Himalaya Drug company, Dehradun. Selected leaves/rhyzomes were washed thoroughly with water, and then air dried under shade and grounded using a pestle and mortar.

### Experimental

The instrument used in the present study was CAMAG-HPTLC system with Wincat software and SHIMAZDU-HPLC system with LC-solution software.

### Method of HPTLC

**Sample preparation:** Measure accurately 5gm of samples of *Ocimum sanctum and Curcuma longa* separately in a 250ml flat bottomed flask add Methanol and reflux it by immersing in a water bath at $80^0$C for 30 minutes . Filter the extract through whatman no.1 filter paper into an conical flask.

Standard: 1) Oleanolic acid- 10mg in 10ml methanol
2) Curcuminoid 10mg in 100ml methanol

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

### Mobile phase

1 *Ocimum sanctum*- Toluene:ethyl acetate:acetic acid( 5.5:4.5:0.2)[10]
2 *Curcuma longa* - Chloroform: Methanol : glacial acetic acid ( 94:5:1)[11]

Used camag twin trough development tank (10×10cm). Visualized the dried plate under UV 254 nm and 366nm using reprostar-3. Exposed the plate of tulasi extract at white light after derivatized with vanillin sulphuric acid reagent.

**Estimation of Total oleanolic acid by HPLC**

HPLC Conditions required for analysis

1.0 Column : C18
2.0 Mobile Phase: Methanol: Sodium phosphate buffer (89:11)
3.0 Flow Rate: 1.0ml/min
4.0 Detection: 210nm
5.0 Volume of injection: 20µl of standard and sample solution

**Standard oleanolic acid Solution (0.101mg/ml)**

Weighed accurately 10.1mg of standard in 10ml of volumetric flask. Added 7-8 ml of methanol and dissolved. Made the volume up to the mark with methanol. Took 1 ml of this solution in 10 ml of volumetric flask and made up the mark with methanol.

**Sample solution ( 10.03 mg/ml)** : Weighed accurately 1g of the Tulsi powder in a 250ml flat bottom flask and add 80ml of methanol. Refluxed on a water bath at $80^0$C for 30 minute. Transferd the extract into a 100ml volumetric flask without filtering.

**Method of Analysis**

Stabilized the instrument with the mobile phase and injected 20µl of working standard solutions. Recorded the chromatogram. Injected 20µl of the sample solution and recorded the chromatogram. Calculated the AUC of the of the two major adjacent out of which the first peak corresponds to oleanolic acid and second peak corresponds to ursolic acid.

Calculation: % total oleanolic acids w/w content can be calculated using the formula

$$\frac{\text{Total area of both peaks in Sample}}{\text{Total area of both peaks in Standard}} \times \frac{\text{Standard concentration}}{\text{Standard concentration}} \times \%\ \text{Purity of standard}$$

**Estimation of Curcumin in *Curcuma longa***

HPLC Conditions required for analysis:

1.0 Column : C18
2.0 Mobile Phase : Acetonitrile: 0.1% orthophosphoric acid in purified water (50:50)
3.0 Flow Rate: 1.0ml/min
4.0 Detection: 420nm
5.0 Volume of injection: 20µl of standard and sample solution

**Standard Solution**

(0.0105mg/ml) Weighed about 10.5 mg of standard of Curcumin in a 10ml of volumetric flask, and dissolved by sonication in methanol. Made the volume up to the mark with methanol. Took 1 ml of the above stock solution into a 100ml volumetic flask, and made up the volume up to the mark with methanol and filtered the solution through 0.20 µm syringe filter.

**Sample solution**

(1.0mg/ml) Weighed about 100mg of the sample in a 250ml flat bottom flask and add 80ml of methanol. Refluxed on a water bath at $80^0$C for 30 minute. Transfered the extract into a 100ml volumetric flask without filtering. Made the volume up to the mark with methanol and shook well allow the residue to settle to settle for 5 minutes. Filtered the solution through 0.20 µm syringe filter.

Calculation: % content of Curcumin

$$\frac{\text{AUC of the major peak in the Sample}}{\text{AUC of the major peak of standard}} \times \frac{\text{Std conc(mg/ml)}}{\text{Sample conc(mg/ml)}} \times \%\ \text{Purity of standard}$$

## Results and Discussion

Oleanolic acid and ursolic acid are closely related isomers occurring in Tulsi. Since they are structurally related, their retention times are very close and often appear as less resoluted peaks. Hence, they can be collectively estimated as total oleanolic acids comprising both oleanolic acid and ursolic acid peaks. HPTLC methods were developed for estimation of oleanolic acid in *Ocimum sanctum* using different solvent system. The proper solvent system selected for estimation of oleanolic acid was toluene:ethyl acetate:glacial acetic acid in the ratio of 5.5:4.5:0.2.( Fig. 1).

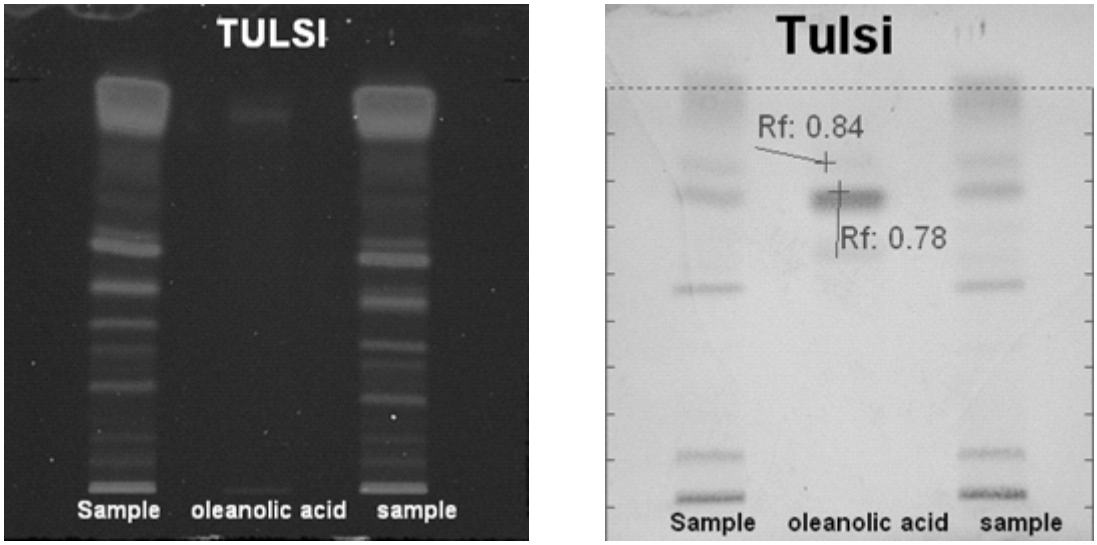


Figure 1 HPTLC chromatogram of *Ocimum sanctum*.

The validated HPLC method was applied to quantify the markers in methanolic extracts of *Ocimum sanctum*. The methanolic extract of the sample was found to have more amounts of active constituents. The amount of total oleanolic acid in methanolic extract was found 2.13% w/w. The method developed was specific, sensitive, precise and accurate. Therefore, this validated method can be used for routine quality control analysis for *Ocimum sanctum* extracts and formulations.

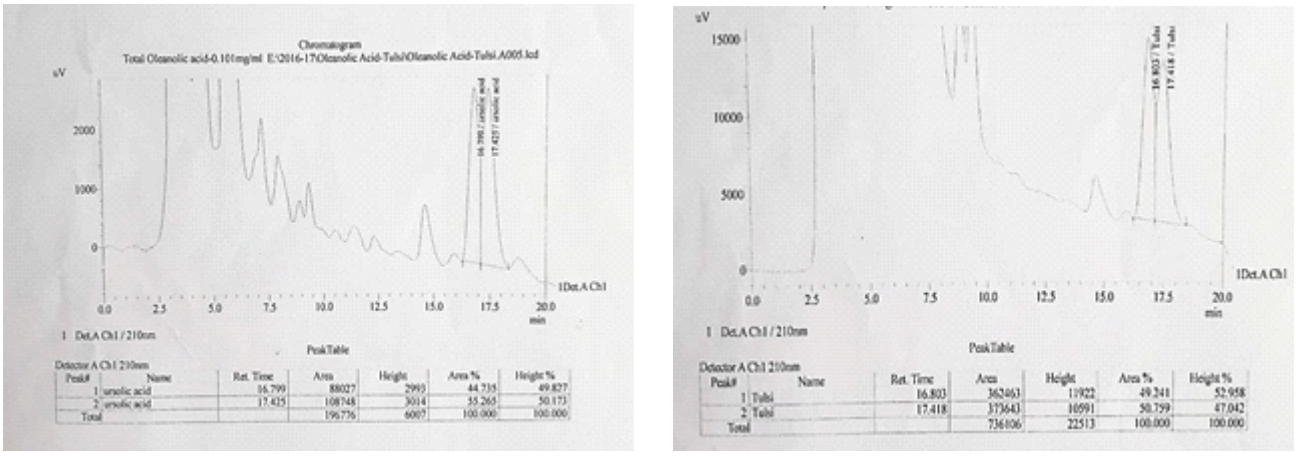


Figure 2 HPLC chromatogram of *Ocimum sanctum*.

In Figure 3 the HPLC peak for *Ocimum sanctum* at RT(16.803) and RT(17.418) corresponds to the peak of ursolic acid and oleanolic acid. Both the peak represent the total oleanolic acid.

**Estimation of total oleanolic acid in *Ocimum sanctum* leaves:(0.101 /10.03) x (736106 /196776) x 56.66% = 2.13% [w/w]**

Turmeric contains many plant substances, but one group, curcuminoids, has the greatest health promoting effects. Three notable curcuminoids are Curcumin( 60-70%), demethoxycurcumin(20-27%) and bis-demethoxycurcumin(10-15%). Of these, Curcumin is the most active and most beneficial to health. HPTLC methods were developed for estimation of Curcumin using different solvent system. The suitable solvent system selected for the separation of curcuminoid was Chloroform: Methanol : glacial acetic acid (94:5:1) (Figure-3).

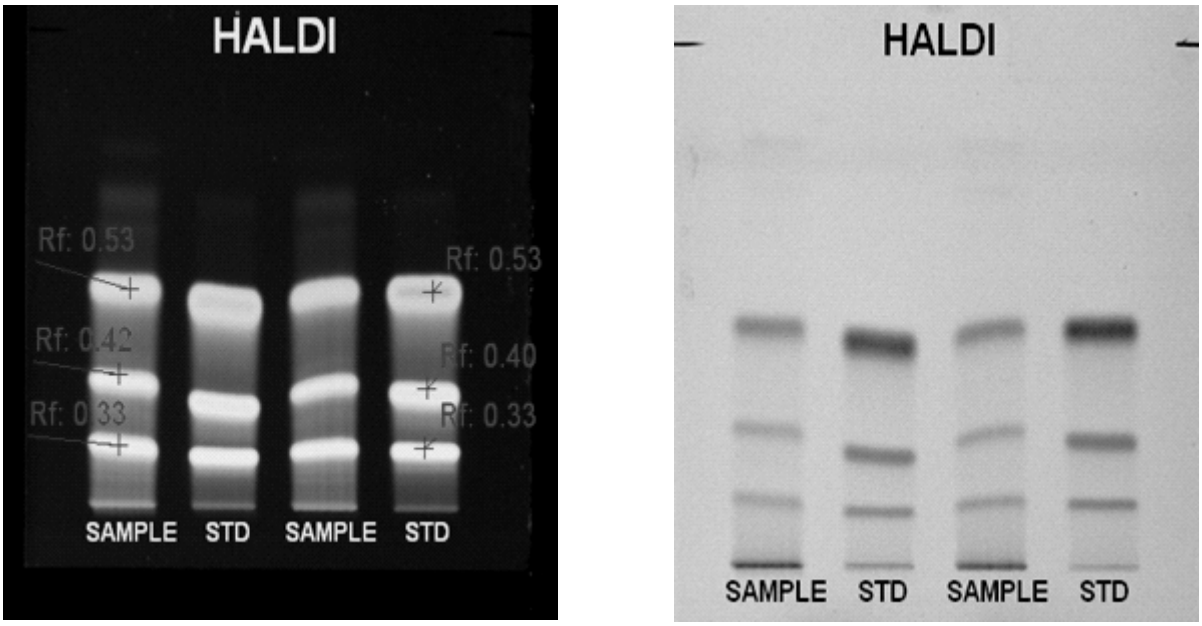


Figure 3 HPLC chromatogram of *Curcuma longa*.

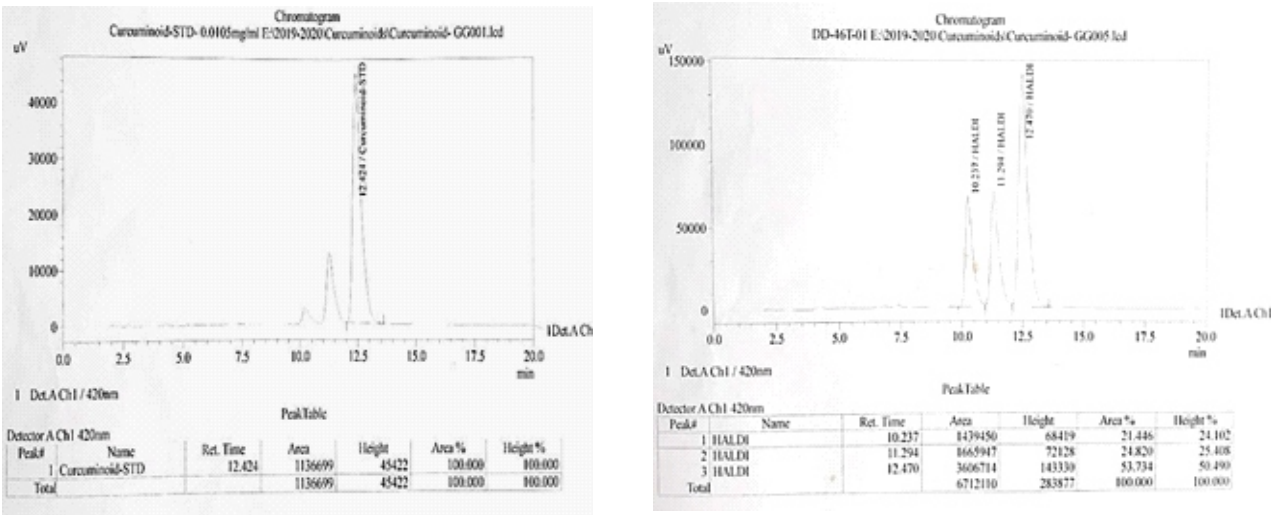


Figure 4 HPLC chromatogram of *Curcuma longa.*

In Figure 4 the HPLC chromatograph for *curmuma longa* shows the peak at RT(10.237) for Curcumin, RT (11.294) for demethoxycurcumin and RT(12.470) bis-demethoxycurcumin. **Estimation of curcumin in *Curcuma longa*: (0.0105 /1.0) x (3606714 /1136699) x 96.67% = 3.22% [w/w]**

Table-1 The % calculation of Phytomarkers

| Sr. No | Plant Name | Phytomarker | % w/w | Limit of IP |
|---|---|---|---|---|
| 1 | *Ocimum sanctum* | Total oleanolic acid | 2.13% | NLT- 2.0% |
| 2 | *Curcuma longa* | Curcumin | 3.22% | NLT- 1.5% |

## Conclusion

Immunity is our body's natural defence against disease causing bacteria and virus. It can considerably reduce the odds of getting sick. Summer season is approaching and our immune system is getting compromised slightly due to the change in weather. It is only due to the weak immunity that people are getting affected with the widespread coronavirus and other such panedemics.

*Ocimum sanctum and Curcuma longa* aids in making our immunity stronger, the main life saving marker in Tulsi is Oleanolic acid (2.13%) and in turmeric is Curcumin(3.22%). According to IP its limit is NLT-2% for oleanolic acid and NLT- 1.5%for Curcumin. The results of this investigation revealed that the *Ocimum sanctum* and *curcuma longa* contains good quantity of immunobooster phytomarker which gives us protection against the various viral infections.

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# Formulation and Evaluation of Anti-inflammatory Herbal gel using *Sarcosteema acidum*

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**Abstract**-Inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases. Therefore, the uses of anti-inflammatory agents are helpful in the therapeutic treatment of these pathologies. In this context, medicinal plants are widely used in folk medicine of many countries to treat different inflammatory conditions and, in particular, skin inflammations. Present work was designed to formulate herbal gel using extract of *Sarcosteema acidum* stem which provide better efficacy and to ensure biological evaluation of prepared herbal gel for safety and efficacy.

**Keywords**: Inflammation, Herbs, *Sarcosteema acidum,* Formulation, Evaluation, Gel

## Introduction

Drug delivery through the skin has been a promising concept for a long time because the skin is easy to access, has a large surface area with vast exposure to the circulatory and lymphatic networks and the route is non-invasive.

Gel consists of a natural or synthetic polymer forming a three-dimensional matrix throughout a dispersion medium or hydrophilic liquid. After application, the liquid evaporates leaving the drug entrapped in a thin film of the gelforming matrix physically covering the skin.[1,2] Many anti-inflammatory drugs (both NSAIDs and corticosteroids) have been developed but their safety profile studies have shown that none of them is clearly safe. They show wide ranges of adverse effects. Due to adverse reactions of synthetic and chemical medicines being observed round the globe, herbal medicines have made a comeback to improve our basic health needs. Many plants and herbs such as ginger, turmeric, olive oil, have been shown to exhibit potent anti-inflammatory effect.[3,4,5]

The presence of a network formed by the interlocking of particles of the gelling agent gives rise to the rigidity of a gel. The nature of the particles and the type of form that is responsible for the The plant *Sarcostemma acidum* commonly known as somlata is herb. The plant has the height of 2-4 feet and mostly occurs in India, Pakistan, Bhutan, Africa. The plants have presence of latex in stem.[6,7]

There is utter need to formulate safe and effective anti-inflammatory formulation for the treatment of inflammation. The plant *Sarcosteema acidum* is selected because as per the literature, it was reported to consist of anti-inflammatory action. The formulation of the same will be beneficial for the treatment of several types of inflammation. In the present work, the formulation and evaluation of herbal gel of *Sarcosteema acidum* for antiinflammatory activity is planned. No work has been reported on formulation of the herb till date.

## Material and Methods

### Selection, Collection and Authentication of Plant material

The plant *Sarcosteema acidum* was selected due to its importance in traditional system of medicine in the treatment of medicine. The dried stem and roots of herb *Sarcosteema acidum* was collected from the local area of Chopda, MH. The plant part (stem and roots) was authenticated by Dr. H. Ansari, Assistant Professor Botany Department, Science College, Chopda, (MH), a voucher specimen no. SA/S/2019-12 was coded for further reference.

### Extraction of Plant material

The air dried and crushed stem and roots of *Sarcosteema acidum* (about 250 gm) was taken and extracted with ethanol as a solvent using soxhlet apparatus for 72 hr. After extraction the solvent was cooled and was filtered by whatsman filter paper size No.1. The extract was dried under reduced pressure using rotatary evaporator and was concentrated.[8,9]

### Formulation of *In situ* Herbal gel[10,11]

The herbal gel of the stem and root extract of *Sarcosteema acidum* was prepared. The carbopol 934 was dissolved slowly in de-mineralized water with continuous stirring for 1hr to avoid agglomeration. The beaker, was kept aside. In another beaker xanthum gum and propylene glycol was taken and added. Both were dissolved in 10 ml of de-mineralized water with contious stirring for 10 min to insure homogenous solution. In third beaker HPMC K 100M was taken and 12 ml of de-mineralized water was added with continuous stirring for 10 minutes. The solution of second beaker was added to beaker containing carbopal 934 with continues stirring for 10min and the pH was adjusted to 7.4. In the above mixture, the HPMC K 100 M solutions were added with continue stirring for 10 min. The stirring was done to achieve clear and consistent gel base. To the clear gel base weighed quantity of extracts as specified in table-5.1 was added with continuous stirring and volume was adjusted with de- mineralized water. Eight different herbal gel formulations were prepared using ethanolic extracts of stem and roots of *Sarcosteema acidum* (EESAS) and (EESAR) as mentioned in Table -1.

**Table-1 Formulation of different batches using stem & roots of *Sarcosteema acidum***

| FC | EESAS | EESAR | C | HPMC | XG | PG | MP | PP | DMW |
|---|---|---|---|---|---|---|---|---|---|
| HG1 | 1 | - | 1 | - | 0.5 | 10 | 0.2 | 0.5 | 100 |
| HG2 | 1 | - | - | 1 | 1 | 10 | 0.2 | 0.5 | 100 |
| HG3 | 1 | - | 1.5 | - | 1.5 | 10 | 0.2 | 0.5 | 100 |
| HG4 | 1 | - | - | 1.5 | 2 | 10 | 0.2 | 0.5 | 100 |
| HG5 | - | 1 | 1 | - | 0.5 | 10 | 0.2 | 0.5 | 100 |
| HG6 | - | 1 | - | 1 | 1 | 10 | 0.2 | 0.5 | 100 |
| HG7 | - | 1 | 1.5 | - | 1.5 | 10 | 0.2 | 0.5 | 100 |
| HG8 | - | 1 | - | 1.5 | 2 | 10 | 0.2 | 0.5 | 100 |

**Evaluation Parameters of Herbal gel**

The prepared formulations were evaluated for the following parameters:[10-11]

**Physical evaluation**

The physical evaluation of the formulation was done by evaluating clarity and transparency which was determined visually. The samples were observed in light at white background.

**Determination of pH of Formulation**

The pH meter was calibrated first and zero reading was recorded. The samples were taken in the beaker and the readings were taken from calibrated electrode. The procedure was repeated and three average reading was recorded.

**Determination of Gelling capacity**

Visual method was employed for determination of the gelling capacity. Near about 100µl of gel sample was taken in a vial. To the above freshly prepared artificial tear fuild was added and the solution and equilibrated at $35^0$C. The procedure of gel formulation was assessed and time taken for the formation of gel was recorded.

**Determination of Gelation temperature**

Test-tube-inverting method was used to determine the gelation temperature of the samples. About 2 ml of the formulated gels was taken in a test tube and was kept on water bath. The temperature was kept at room temperature and increase gradually. The formulation was examined after every two minutes. The temperature at which the gel stops flowing was recorded. Three times the experiment was repeated and every reading was reported.

**Determination of Viscosity of gel**

The viscosity of the gel formulation was determined by Brookfield viscometer using spindle no. 01 at 20 rpm at temperature 4 °C and 37°C. About 15ml of the gel formulation was taken in beaker and spindle was immersed in the formulation. The reading was recorded at initial and after rotation at different temperature. The reading was recorded thrice.

**Determination of Syringeability**

The syringeability studies was done on the gel formulation. It was done to check the flowing ability through 21 gauge needle.

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**Determination of Syringeability**

The syringeability studies was done on the gel formulation. It was done to check the flowing ability through 21 gauge needle. The syringeability was checked for the ability of the flow property. The gel was kept at cold temperature and 1ml of gel was filled and the ability of gel to flow at normal pressure was observed.

**Determination of Extrudability**

The extrudability property of gel was determined to check the amount extruded from collapsible tube. Near about 20g of gel was placed in collapsible tube and sealed. At the crimped end of collapsible tube the formulation was firmly pressed. To prevent the rolling back of gel the clamps were applied. The cap of collapsible tube was taken off and formulation was forced out. The formulation gets extruded which was collected and weighed in weighing bottle.

**Determination of Spreadibility**

The spreadibility was determined for all the gel formulation. The gel was placed on the glass slide and the empty glass slide was placed on the top of gel containing slide. The formulation was placed in such as way that it was placed between two slides. The occupied distance of the slides was observed to be of 7.5 cm. The gel was placed between slide and pressed, formed thin uniform layer. The weight kept on the gel was removed. The excess gel observed in the slides was removed. The two slides were fixed and on the upper glass slide the 20 ±0.5 g of the weight was tied. Due to weight the both the slides were separated which was recorded as time to complete the separation distance of 7.5 cm. The three readings were recorded and mean time was taken.

The spreadabilty was calculated as **S= m X l/t**

l is the length of slide (7.5 cm), m is the weight which is tied to slides and t is the time taken in second.

**Determination of Drug content**

The content of the formulation was estimated using UV-Visible spectrophotometer. Near about 1g of the gel formulation was taken in 50 ml of volumetric flask. The solution was made up to mark with methanol. The solution was shaken and filtered though whatman filter paper. The 0.1ml of the filterate was further diluted to 10ml with solvent and estimated at 560 nm.

## Results and Discussion

*Sarcosteema acidum* commonly known as somlata belongs to family Asclepidiaceae have been evaluated for may pharmacological activity and the same has been reported in literature. The plant species have been phytochemically screened and numbers of chemical constituents have been reported.

The present work shows the results of formulation and evaluation of herbal gel containing two extracts of *S. acidum* The dried stem and root powder of *Sarcosteema acidum* was extracted with ethanol. The percentage yield of extract thus obtained has been mentioned in table -2.

**Table -2 Percentage yield of Extract**

| Sample Code | Weight of crude drug taken (gm) | Amount of extract obtained (gm) | % Yield of extract (w/w) |
|---|---|---|---|
| EESSA | 100 | 10.28 | 10.28 |
| EESAR | 100 | 9.26 | 9.26 |

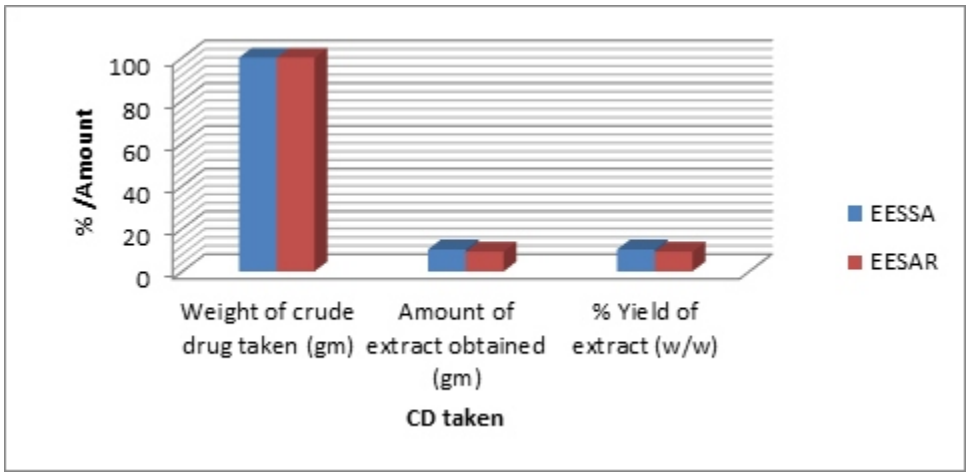


**Figure-1 Percentage yield of Extract**

### Evaluation of In situ Herbal gel

In the present study, an attempt was made to develop and evaluate herbal gel formulations of ethanlic extract of stem and root extract S. acidum. HG1 to 8 was formulated using different concentration of extract. The formulated herbal gel was evaluated for various parameters viz., physical appearance, pH, gelling capacity, gelation temperature, viscosity, Syringeability study, Extrudability, Spreadability and drug content. The result of evaluation of parameters of formulated herbal gel was mentioned in table-3. Drug release profile was studied for all the formulated batches.

**Table -3 Evaluation parameters of herbal gel**

| Evaluation Parameters | Formulations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HG1 | HG2 | HG3 | HG4 | HG5 | HG6 | HG7 | HG8 |
| Clarity | Unclear | Clear | Clear | Clear | Clear | Clear | Clear | Unclear |
| pH | 7.14 | 7.07 | 7.10 | 7.05 | 7.01 | 6.98 | 7.03 | 7.12 |
| Gelling capacity | + | ++ | ++ | +++ | ++++ | ++++ | +++ | +++ |
| Gelation temperature | 25.22 | 28.22 | 30.26 | 30.42 | 32.81 | 31.92 | 30.83 | 24.12 |
| Viscosity (Poise) | 0.3912 | 0.3810 | 0.3819 | 0.3879 | 0.3984 | 0.3817 | 0.3811 | 0.3310 |
| Syringeability study | E | E | E | E | E | E | E | E |
| Extrudability (%) | 94.18 | 96.58 | 97.57 | 98.18 | 99.83 | 97.51 | 98.21 | 92.18 |
| Spreadability (gcm/sec) | 57.40 | 62.40 | 69.34 | 70.48 | 72.49 | 69.39 | 67.41 | 58.40 |
| Drug content (%) | 88.32 | 91.32 | 94.22 | 95.92 | 99.82 | 92.10 | 89.48 | 87.22 |

++++= Excellent; +++=Very good, ++= Good, +=Satisfactory

The results of drug content of herbal gel for ethanolic extracts were indicated in Figure -2. The drug content was observed maximum in HG5 ehere as lowest in HG8, therefore the formulation code HG8 is chosen for further studies.

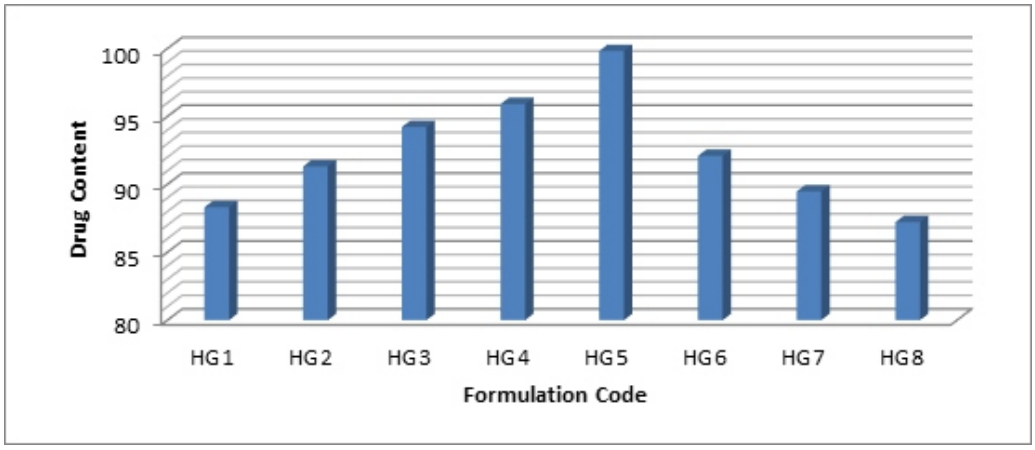


**Figure-2 Drug content of herbal gel**

## Conclusion

In the present work the herbal gel was formulated for effective and safe treatment used for the treatment of inflammation. The formulated herbal gel has passed the evaluation tests and are successful formulation for drug delivery. The *S. acidum* is medicinally important plant grown wildly in India and is useful in the treatment of inflammations by tribal's and rural people of India. Hence, the present plant is s selected to formulate herbal gel using *S. acidum* as active ingredients for the treatment of inflammation.

The stem and root of plant were collected; dried and powdered plant material was extracted with ethanol. The percentage yield of ethanolic extract of stem and root was found to be 10.28 % w/w and 9.26% w/w respectively. Eight different batches of formulation (HG-1, HG-2, HG-3, HG-4, HG-5, HG-6, HG-7, HG-8) were prepared using ethanolic extract of *S. acidum* stem and roots.

HG-1 HG-4 was formulated using ethanolic extract of stem and HG-5 HG-8 was formulated using ethanolic extract of root. In the above mentioned both the extract of different proportion of Carbopol, HPMC and xanthum gum was added with different concentrations.

Various evaluation parameters such as physical appearance, pH, gelling capacity, gelation temperature, viscosity, Syringeability study, Extrudability, Spreadability and drug content were performed to evaluate the formulated herbal gel. From the results observed it was concluded that all the prepared herbal gel i.e., HG-5 (ethanolic extract of root) has good clarity and transparency. The pH so obtained was within the limit as for most of the preparation intented to be used for skin. All the formulations were tested for pH value which was observed to be neutral in almost all cases 6.98-7.14 pH which has indicated that formulation has no skin irritation during application.

The gelling capacity and gelation temperature were found within the limit. The viscosity was found to be in limit. The ideal viscosity readings were reported between 0.38 and 0.39 poise which indicated the optimum herbal gel formulation using HPMC polymers.

The results of spreadibility showed that the formulations spreadable and were easily extrudable. This has indicated the excellent property of gel.

The drug content was found to be maximum of 99.82 (HG-5) followed by 98.21 (HG-7). From the data obtained, it was concluded that the herbal gel formulated using ethanolic extract of roots of *S. acidum* was found to be more potent and efficacious than ethanolic extract of stem of *S. Acidum.*

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# Modulatory Effect of Paste of Dried seeds of *Prunus amygdalus* on Ovotoxicity Induced Postmenopausal Complications in Rat

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**ABSTRACT-**Menopause is a natural process in women's reproductive life at the age of 40 to 50 years and leads to deficiency of estrogen causing various complications in women's health. *Prunus amygdalus* nuts are abundant source of various phytoestrogens and produces effect similar to that of estrogen and can be used in menopausal women in place of HRT.

In the present study ovotoxicity and menopause was induced in female rats by 4-vinyl cyclohexane diepoxide (165 mg/kg, i.p. five times a week for 15 days). After induction menopause the animals were treated with the paste of dried seeds of *Prunus amygdalus* at the doses of 100, 200 and 400 mg/kg, p.o. for 45 days. Blood estrogen level was checked before and after administration of VCD to confirm induction of menopause. After the treatment period of 45 days blood was collected from the animals, centrifuged and serum was separated and used for the estimation of estrogen, cholesterol, triglyceride, HDL, LDL and VLDL. Ovaries were isolated from the treated animals, kept in ice cold tris buffer, homogenized and centrifuged. The supernatant was used for the estimation of malondialdehyde (MDA) and antioxidant enzyme (superoxide dismutase and glutathione peroxidase). Results indicate that administration of seeds of *Prunus amygdalus* at different dose levels do not have any effect on serum estrogen level but significantly improved lipid profile and decreased atherogenic index in postmenopausal rats. A significant decrease in the MDA and increase in antioxidant enzymes level was observed in postmenopausal rats after treatment with paste of dried seeds of *Prunus amygdalus*. Results conclude that administration of seeds of *Prunus amygdalus* for a long period of time may improve the cardiovascular complications of women's health after menopause.

**Keywords**: Estrogen, Menopause, Phytoestrogen, *Prunus amygdalus*, 4-vinyl cyclohexane diepoxide.

## Introduction

Menopause is defined as 1 year without menses and characterised by the failure of ovaries to produce estrogen. This failure often begins in the late 30s, and most women experience near-complete loss of production of estrogen by their mid-50s. The transition from normal ovarian function to ovarian failure is described as the menopausal transition. Menopause can occur at different times (Mirghafourvand M.*et al*.,2014). Normally most women receive menopause status at age 45 and 55. At the age of 51, permanent amenorrhea lead to decline level of estrogen in women(Ghazanfarpour M.*et al*.,2015).The decreased level of estrogen in women shows symptomatic changes, such as sleep disorders, night sweats, hot flashes, mood changes, sexual complaints, urogenital symptoms and dementia (Henderson, V.W 2008).The dropping level of estrogen and progesterone also affects the brain and skin tissue which causes psychological disturbances(Elavsky E and Mc Auley, 2009).

During the end of reproductive age, the majority of women generally uses estrogen based therapy to

keep down the physiological changes in body. Hormone Replacement Therapy is used as primary treatment for relieving the symptoms of menopausal related disorders, such as osteoporosis and coronary heart disease (Holloway D, 2011). The regular intake of estrogen and progesterone lead to increase risk of breast cancer, ovarian disease, venous thromboembolism, pulmonary embolism, stroke, heart disease, endometrium cancer and vascular dementia (Cauley JA.*et al*.,2001). Studies from literature indicates the use of plant based oestrogens also known as phytoestrogens are useful to treat the menopausal complications without adverse effects. Phytoestrogens are non-steroidal, polyphenolic plant derived metabolite that mimic or modulate actions of estrogen, having structure similar to 17β-estradiol (Whitten PL.*et al*.,1997). Phytoestrogen act through binding to estrogenic receptors (ER)-alpha and beta.They also show some antioxidant property.Phytoestrogens are considered natural therapeutic alternative to synthetic hormonal therapies, which are not associated with chronic indications and adverse effects. According to literature survey it is known that the fruit of*Prunus amygdalus*(almond) family *Rosaceae*. Almonds contains high number of phytoestrogens which are beneficial for human health.It contains large amount of phytoestrogens such as flavonols, flavanones, phenolic acids, anthocyanins(Spiller GA. e*tal*., 2012). It also contains large amount of vitamins such as vitamin E, folic acid, vitamin B6, proteins, etc which are responsible for its beneficial effects on health (Frison-Norrie S. *et al*., 2002). Traditionally almonds have a consistent LDL cholesterol lowering effect in healthy individuals and in individuals with high cholesterol and diabetes. It also possess a memory enhancing activity.Vitamin E present in almonds is known to be potent antioxidant properties and free radicals may contribute to the pathological processes of cognitive impairment. Literature survey showed that almonds improved the immune response due to production high level of cytokines such as INF- α, INF- γ, IL-12 and TNF- α etc. (Adriana Arena, *et al,*2010). One of the other almonds health benefits is that it helps in maintaining a proper blood pressure and indirectly helps in prevention of diseases caused due to high blood pressure **(**Phillips KM. *et.al*., 2012).

## Materials and Methods

### Reagents

Estradiol and 4-vinyl cyclohexene diepoxide were purchased from Sigma Aldrich. Other chemicals were obtained from local sources and were of analytical grade. Diagnostic kits were used for the estimation of all biochemical parameters.

### Animals

Female Wistar rats weighing between 150-200g and 6-7 weeks of age were used for study. The rats were housed in standard cages and were allowed to have free access to food and water ad libitum under controlled room temperature (24±2°C) in a 12-h light-dark cycle. The guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) of the Government of India were followed and prior permission was taken from the Institutional Animal Ethical Committee(273/PO/Re/S/2000/CPCSEA/002/2015-2017) for conducting the animal experimental studies.

### Induction of ovotoxicity and Menopause

Ovotoxicity was induced in mice by the Accelerated Ovarian Failure (OVF) method. An ovotoxic chemical, 4-vinylcyclohexene diepoxide (VCD) was injected at a dose of 160 mg/kg, i.p. five times per week for 15 days in female mice. The blood estrogen level is checked in normal female mice before injecting 4-vinylcyclohexene diepoxide (VCD) and after 15 days of injecting the chemical in order to confirm the induction of menopause (Hoyer PB., *et.al.*2001).

### Preparation of test sample and dose selection

The nuts of *Prunus amygdalus*were purchased from the local market and authenticated at the Botany Department of forest Research Institute, Dehradun, India. The fine paste of the PA nuts was prepared in distilled water and sonicated for 20 min to obtain a fine suspension.

Then, the paste was administered orally to the mice at three doses of 100, 200 and 400 mg/kg/day. The above dose levels were selected by the conversion of conventional human dose into animal dose. The human dose of PA was five to six nuts daily (approximately 6 g).

**Treatment protocol**

The ovotoxic animals were divided into six groups each group having six animals and one group of normal animal was taken into the study. The first group of ovotoxic animals received normal saline (1 ml/kg,p.o.). The ovotoxic animals received the following treatments for a period of 45 days.

**Group 1**: Normal animals received normal saline (1mg/kg p.o.)

**Group 2**: Ovotoxic animals received normal saline 1ml/kg, p.o.

**Group 3**: Ovotoxic animals received the paste of seeds of *Prunus amygdalus* (100 mg/kg, p.o.) for 45 days.

**Group 4**: Ovotoxic animals received the paste of seeds of *Prunus amygdalus* (200 mg/kg, p.o.) for 45 days.

**Group 5**: Ovotoxic animals received the paste of seeds of *Prunus amygdalus* (400 mg/kg, p.o.) for 45 days.

**Group 6:** Ovotoxic animals received estrogen therapy (60/mg/kg) for 45 days.

At 0 day and after the treatment period of 15 and 60 days, hormonal and biochemical parameters and oxidative stress parameters were studies. The blood was collected from the animal by retro orbital puncture under mild ether anaesthesia, it was centrifuged at 2500 rpm in cooling centrifuge and serum was separated to evaluate the biochemical parameters using autoanalyser and spectrophotometer. Ovaries were was quickly removed and place in ice cold 10% formaldehyde solution. The ovarian tissue was weighed and homogenized in 0.1M phosphate buffer (pH 7.2). The homogenate was centrifuged at 300 in the homogenizer (Remi motors, Mumbai) for 10 min and the resultant cloudy supernatant liquid was used for estimation of oxidative stress.

**Estimation of Estrogen**

The estimation of estrogen was carried out from serum using an ELISA kit(MACDONALD et al., 1979).

**Estimation of lipid profile for evaluation of cardiovascular risk factors**

Serum cholesterol, triglyceride, high density cholesterol (HDL-cholesterol) level were estimated by CHOD-PAP method with LCF(Castelli et al., 1977), Enzymatic Colorimetric Method or GPO (Rust et al., 2006), and Phosphotungstic Acid Method(Miller et al., 1977), respectively using ERBA diagnostic kits. The concentration of VLDL, LDL and atheraogenic index was obtained by using the formula.

LDL= Triglyceride/5

VLDL= TC-HDL

Atherogenic Index= TC-HDL/HDL

**Estimation of lipid peroxidation and superoxide dismutase and reduced glutathione from ovaries**

Estimation of lipid peroxidation was done from the tissue supernatant by the method of Slater and Sawer, 1971 (Slater and Sawyer, 1971). Superoxide dismutase and reduced glutathione were estimated by the method of Mishra et. al 1972 and Moron et.al, 1979 respectively using supernatant from ovaries.

**Statistical analysis**

The statistical analysis was carried out using Graph Pad Prism 4.0 software. All values were expressed as Mean ± SEM. Multiple comparison between different groups was evaluated statistically using analysis of variance (ANOVA) followed by Dunetts Multiple comparison test. Difference level at $P< 0.05$ was considered as statistically significant condition.

## Results

**Effect of paste of dried seeds of *Prunus amygdalus* on serum level of estrogen in ovotoxicity induced postmenopausal complications in rats**

Result summarized in table no.3.1 indicates the effect of VCD on the estrogen level in mice. Result shows that administration of VCD at a dose of 160mg/kg for 15 days (5 days a week) caused significant (P<0.01) decrease in estrogen level as compared to normal rats. The result obtained from the study confirmed induction of menopause and ovarian failure in rats. Result presented in table-1 reveals that the seeds of *Prunus amygdalus* does not have any significant effect on serum estrogen level in menopausal mice. Treatment of ovotoxic post menopausal rats by the seeds of *Prunus amygdalus* at a dose of 400mg/kg and 200mg/kg for 45 days caused slight increase(84.6 ±0.75&79.6 ± 0.64 respectively) in estrogen level in ovotoxic animals. At the dose level of 100mg/kg however the seed powder does not have significant effect on estrogen level (65.56 ± 0.4) in ovotoxic animal. However in estrogen treated animals, the level of estrogen was significantly improved.

**Effect of seeds of *Prunus amygdalus* on cardiovascular parameters (Cholesterol, triglycerides, HDL, LDL, VLDL and atherogenic index) in ovotoxicity induced postmenopausal complications in rats**

Results shown in table no. 2 reveals that after VCD administration, the level of cholesterol, triglycerides, LDL and VLDL were significantly (P<0.001) increased in postmenopausal women indicating impairment of lipid profile. Treatment of the ovotoxic animals by the seeds of *Prunus amygdalus* at a dose of 400mg/kg and 200mg/kg for 45 days caused significant (P<0.001 and P<0.01 respectively) decrease in blood cholesterol, triglyceride, LDL and VLDL levels. Treatment with 100 mg/kg caused moderate decrease ((P<0.05) in the level of above mentioned parameters. HRT by estrogen at a dose of 60mg/kg for 45$^{th}$ days caused most significant (P<0.001) decrease in lipid profile in postmenopausal rats. HRT maintain the estrogen level in the menopausal animalsand restored the lipid profile to the normal value.

Results indicate that VCD administration severely decreased the level of HDL in rats. Administration of seeds of *Prunus amygdalus* to the postmenopausal ratsat a dose of 200mg/kg and 400 mg/kg for 45 days significantly (P<0.001) increased (32.125±0.18 and 37.29± 0.6 respectively)the level of HDL ac compared to positive control animals. Estrogen therapy also normalized the level of HDL from 20.61±0.15 to 38.65±1.25. Atherogenic index was also increased significantly (P<0.001) in female rats after induction of menopause. Table 3 shows a highly significant (P<0.001) decrease in the level of AI in the group administered with the paste of *Prunus amygdalus* at a dose of 200 mg/kg and 400 mg/kg. Estrogen therapy also normalized the AI level.

**Effect of paste of dried seeds of *Prunus amygdalus* on the level of lipid peroxidation, superoxide dismutase and reduced glutathione in ovotoxicity induced postmenopausal rats**

Result summarized in table-4 shows that administration of VCD significantly (P<0.001) increased the level of malondialdehyde (MDA), an indicator of lipid peroxidation and decreased the levels of superoxide dismutase (SOD) and reduced glutathione (GSH)in the ovaries of female rats indicating increased oxidative stress. Treatment with the paste of dried seeds of *Purus amygdalus*at a dose of 400mg/kg, p.o. for 45 days caused a remarkable decrease (P<0.001) in the level of MDA in postmenopausal rats as compared to ovotoxic

control animals. At a dose of 200mg/kg the paste of seeds also caused significant (P<0.01) decrease (54.15±1.28) in MDA level in postmenopausal rats. At a dose level of 100 mg/kg seeds of *Prunus amygdalus* does not exhibit any effect on MDA. Estrogen therapy given for 45 days at a dose of 60 mg/kg also caused significant ((P<0.001) decrease (48.05±1.92) in the level of TBARS which indicates decrease in free radicals formation in postmenopausal rats. Treatment of the ovotoxic rats for 45 days by the paste of seeds of *Prunus amygdalus* (400mg/kg and 200mg/kg) significantly (P<0.001) increased (213.09±2.1 and 208.54±0.64respectively) the level of reduced glutathione in ovotoxic animals as compared to the ovotoxic control group (99.6±1.52). However treatment of ovotoxic animal by the seeds of *Prunus amygdalus* at 100 mg/kg do not have any significant effect on ovarian reduced glutathione level in ovotoxic animals. The paste of seeds of *Prunus amygdalus at a dose of* 400mg/kg and 200mg/kg also caused significant (P<0.001 and P<0.01) increase in level of SOD in ovotoxic animal as compared to positive control group. Estrogen therapy for 45 days caused significant increase ((P<0.001) in the level of reduced glutathione and SOD in ovotoxic postmenopausalrats.

## Discussion

The present study was aimed to evaluate the effect of paste of dried seeds of *Prunus amygdalus* on ovotoxicity induced postmenopausal complications in rats. Study was mainly designed to evaluate the effect of *Prunus amygdalus*seeds at different dose level on cardiovascular parameters, estrogen level and oxidative stress in ovotoxicity induced postmenopausal complications in female rat.VCD is an ovotoxic chemical, used for induction of ovotoxicity and menopause in experimental animals. It acts by gradual degeneration of primary follicles and thereby induces menopause similar to the natural menopause in humans (Hoyer PB., *et.al.*2001). Results of the study reveals that VCD caused a destruction and gradual decline in the no. of oocytes as indicated by changes in vaginal cytology and decreased estrogen level. The process of decline in the serum estrogen level in rats was gradual and was similar to natural menopause in humans.

In the present study the paste of dried seeds of *Prunus amygdalus* does not have any significant effect on serum estrogen level. So it can be assumed that the phytoconstituents present in *Prunus amygdalus* does not have any effects on estrogen synthesis. The phytoestrogens of *Prunus amygdalus* only binds with estrogen receptor and produces estrogen-like effects, thereby decreases menopausal complications.

In women estrogen has direct metabolic actions on many non reproductive tissues such as bone, vascular endothelium, liver etc. The level of oestrogen has its one of the major effects on serum lipid proteins and triglycerides. Oestrogen reduces the level of total serum cholesterol. It increases the level of good cholesterol,.i.e. HDL (high density lipoprotein) and reduces the levels of lipoprotein A and low density lipoproteins(LDL) which is mediated by ERα**.** Oestrogen also enhances lipolysis and reduces lipogenesis which is mediated by increase in the hepatic expression of apoprotein genes and LDL receptors while decreases the transcription of lipoprotein lipase (LPA) gene which is mediated by ERα. After menopause the level of oestrogen decreases that causes increase in the LPL activity that further enhances the accumulation of free fatty acids on abdomen. There is a greater chance of development of central obesity if expression of ERα and ERβ is reduced on proliferating adipocytes which is associated with an increase atherogenic profile that results in increased risk of heart disease.

In postmenopausal women due to low level of estrogen, the level of TG, cholesterol, LDL and VLDLgets elevated and HDL level is reduced and is responsible for increasing the chances of cardiovascular diseases in postmenopausal women. In the present study, in ovotoxic postmenopausal animals the serum level of TG, cholesterol. LDL and VLDL were increased significantly whereas significant decrease was observed in the levelof HDL indicating abnormalities in serum lipoprotein level.

Treatment of ovotoxic animals with paste of dried seeds of *Prunus amygdalus*for a long period caused significant improvement in lipid profile and decrease in atherogenic index, thereby decreasing the chances of cardiovascular complications. Studies indicates that phytoestrogens exerts their cardioprotective action by regulating the vascular tone and altering the lipid metabolism. The vascular tone is regulated by mechanisms involving endothelial (dependent or independent) vasodilator systems (NO) and it also mediates vasodilatory action by inhibiting the vasoconstriction mechanisms (Doshi sejal B.*et.al.,*2013). We can correlate the findings of our studies with such studies on phytoestrogens and assume that the improvement in lipid metabolism by Prunus amygdalus is due to phytoestrogen present in it (WILMINK et al., 2000).

In menopause declining level of estrogen, loss of protective effects of estrogen and deficient levels of endogenous antioxidants leads to increased oxidative stress in the body that is responsible for various complications related to menopause such as atherosclerosis, cardiovascular diseases, etc. In menopause the disturbances between the formation and elimination of free radicals leads to various changes in the body like dryness, reduced collagen content, elasticity and fragility etc (Hall G and Phillips TJ, 2005).

In the present study, high level of malondialdehyde, which is a product of lipid peroxidation and decreased levels of endogenous antioxidant enzymes in postmenopausal rats indicated increased oxidative stress in the body.The paste of dried seeds of *Prunus amygdalus* caused significant decrease in lipid peroxidation and increase in the level SOD and GSH in postmenopausal rats. It may be assumed that the phytoestrogens present in the seeds of *Prunus amygdalus* are responsible for decreasing oxidative stress. The phytoestrogens by binding to estrogen receptors produces antioxidant effect and decreases oxidative stress in the body.

## Conclusion

From the results it can be concluded that consumption of the seeds of *Prunus amygdalus* for long period of time and on regular basis can protect the females from menopause induced health problem including cardiovascular diseases. However future study are required to establish the effect of seeds of *Prunus amygdalus* on various other complications of menopause such as depression, hot flushes, anxiety, dementia, osteoporosis etc. in postmenopause rats.

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**Table -1 Effect of paste of dried seeds of *Prunus amygdalus* on serum level of estrogen in ovotoxicity induced postmenopausal complications in rats**

| Treatment (i.p/ p.o) | Oestrogen Level ( pg/ml) | | |
|---|---|---|---|
| | 0th Day | 15th Day | 60th Day |
| Normal control (normal saline 1ml/kg p.o) | 165.85±0.9 | 162.4±0.6 | 168±0.42 |
| Positive control (VCD 160 mg/kg i.p, 5 times a week for 15 days) | 155.06±0.4 | 27.80±0.15 [a] | 30.4±0.6 |
| VCD+ Paste of dried seeds of *Prunus amygdalus* (100 mg/kg, p.o) | 154±0.8 | 30.4±0.44 [a] | 65.56 ± 0.4 |
| VCD+ Paste of dried seeds of *Prunus amygdalus* (200mg/kg,p.o) | 160.9±0.36 | 29.85±0.64 [a] | 79.6 ± 0.64* |
| VCD+ Paste of dried seeds of *Prunus amygdalus* (400mg/kg, p.o) | 169.95±0.32 | 38.6±0.75 [a] | 84.6 ±0.75* |
| VCD+ estradiol (60mg/kg, p.o) | 163±0.94 | 29.02±0.95 [a] | 158.27 ± 0.95*** |

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N= 6
Values are expressed as Mean ± SEM, *P< 0.05, **P< 0.01, ***P< 0.001 when Groups Compared: II:III, II:IV, II:V, II:VI on 60th day of the treatment.
[a]P<0.001 when results of day 15 were compared with day one of the study.

**Table -2 Effect of paste of dried seeds of *Prunus amygdalus* on serum level of Cholesterol, HDL, LDL and VLDL in ovotoxicity induced postmenopausal complications in rats.**

| Treatment (i.p/ p.o) | Cholesterol (mg/dl) | HDL (mg/dl) | Triglyceride (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|
| Normal control (normal saline, 1ml/kg p.o) | 60.505 ± 2.6 | 40.7 ± 0.98 | 69.7± 1.25 | 13.94±0.25 | 24.505±1.62 |
| Positive control (VCD 160mg/kg i.p, 5 times a week for 15 days) | 96.54 ± 1.2[a] | 20.61±0.15 [a] | 182.5± 0.32 [a] | 36.5±0.064 [a] | 75.54±1.05 [a] |
| VCD +Paste of seeds of PA (100mg/kg, p.o) | 80.26±1.35* | 23.62±0.15 | 153.3±0.12* | 30.6± 0.024* | 118.7±0.096* |
| VCD+ Paste of seeds of PA (200mg/kg,p.o) | 66.25± 2.0*** | 32.125±0.18** | 136.5± 1.56** | 27.3±0.312** | 109.2±1.28** |
| VCD+ Paste of seeds of PA (400mg/kg, p.o) | 64.05±1.59*** | 37.29± 0.6*** | 85.5± 0.25*** | 17.1±0.05*** | 68.4±0.02*** |
| VCD+ Estradiol (60mg/kg, p.o) | 63.19 ± 2.5*** | 38.65±1.25*** | 76.15±0.16*** | 15.23±0.032*** | 60.92±0.128*** |

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N= 6

Values are expressed as Mean ± SEM, *P< 0.05, **P< 0.01, ***P< 0.001 when Groups were compared with the positive control group.

[a]P<0.001 when results of positive control group were compared with the normal animals.

**Table - 3 Effect of paste of dried seeds of *Prunus amygdalus* on atherogenic index in ovotoxicity induced postmenopausal complications in rats.**

| Treatment (i.p/ p.o) | Atherogenic Index |
|---|---|
| Normal control (normal saline, 1ml/kg p.o) | 56.84 ± 0.43 |
| Positive control (VCD 160mg/kg i.p, 5 times a week for 15 days) | 94.44 ± 0.12[a] |
| VCD +Paste of seeds of PA (100mg/kg, p.o) | 83.76 ± 0.51 |
| VCD+ Paste of seeds ofPA (200mg/kg,p.o) | 63.26 ± 0.61** |
| VCD+ Paste of seeds of PA (400mg/kg, p.o) | 61.50 ± 0.11** |
| VCD+ Estradiol (60mg/kg, p.o) | 59.78 ± 0.8 *** |

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N= 6

Values are expressed as Mean ± SEM, *P< 0.05, **P< 0.01, ***P< 0.001 when Groups were compared with the positive control group.

[a]P<0.001 when results of positive control group were compared with the normal animals.

**Table 3.4. Effect of paste of dried seeds of *Prunus amygdalus* on serum level of MDA, SOD and GSH in ovotoxicity induced postmenopausal complications in rats.**

| Treatment (i.p/ p.o) | MDA (mol/l) | SOD (IU/l) | GSH (µg of Tissue/ml) |
|---|---|---|---|
| Normal control (normal saline, 1ml/kg p.o) | 45.06±2.3 | 114.14±1.96 | 230±0.09 |
| Positive control (VCD 160mg/kg i.p, 5 times a week for 15 days) | 93.75±1.46[a] | 60.19±2.01[a] | 99.6±1.52[a] |
| VCD +Paste of seeds of PA (100mg/kg, p.o) | 78.16±0.52* | 74.34±0.98 | 102.81±2.2 |
| VCD+ Paste of seeds of PA (200mg/kg,p.o) | 54.15±1.28*** | 98.84±0.39** | 208.54±0.64*** |
| VCD+ Paste of seeds of PA (400mg/kg, p.o) | 51.625±0.49*** | 111.13±0.28*** | 213.09±2.1*** |
| VCD+ Estradiol (60mg/kg, p.o) | 48.05±1.92*** | 112.55±1.56*** | 222.98±2.96** |

The statistical significance of difference between means was calculated by ANNOVA followed by t-test for unpaired comparison. N= 6

Values are expressed as Mean ± SEM, *P< 0.05, **P< 0.01, ***P< 0.001 when Groups were compared with the positive control group.

[a]P<0.001 when results of positive control group were compared with the normal animals.

# In vitro antimicrobial activity of Lemon grass oil by MIC and agar well diffusion method

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**Abstract-**Food preservatives are used to increase the shelf life of food and to maintain the quality for longer time. In the last scenario no herbal ayurvedic preservative had been considered with respect to the use of chemical preservative. Due to increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils of antimicrobial properties.

Essential oils from medicinal plants are potential source of novel antimicrobial compounds especially against food spoilage pathogens. The aim of this project was to study antimicrobial activity of essential oil from Lemongrass (*Cymbopogan citratus)* oil against food spoilage organisms *Escherichia coli*, *Micrococcus luteus, Staphylococcus aureus* and *Bacillus cereus* and fungus *Aspergillus brasilliensis, Candida albicans, Chaetomium globosum* and *Penicillium funiculosum* using agar well diffusion method. The antimicrobial activity was evaluated by measuring the zone of inhibition. The oil at 30% concentration completely/partially inhibited the growth of food spoilage pathogens. The successful effectiveness of Lemon grass oil could also play a major role in replacing the chemical preservative.

**Keywords:** Lemon grass oil, antimicrobial activity, Agar well diffusion assay

## Introduction

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent . Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens .

The use of natural antibiotics agents are the best alternative to synthetic or chemical antibiotics. It prevents development of antimicrobial resistance in bacteria and fungus and also devoid side defects. The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against

currently available antibiotics. However, traditional plants have been proved to be better source for novel antimicrobial drugs. Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine. Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savoury and fennel have been successfully used either alone or in combination with other preservation methods .

Lemon grass belongs to the section of *Andropogan* called *Cymbopogon* of the family Germineae. A very large genus of the family including about 500 described species out of which eight species occur in Iraq. Due to the production of lemon grass oil as major component, two of the species i.e. *Cymbopogon citratus* commonly known as lemongrass is an herb which belongs to the grass family of *Poaceae*. It is utilized for its distinct lemon flavour and citrusy aroma. It is a tall, perennial grass which is native to India and tropical regions of Asia. It is a coarse and tufted plant with linear leaves that grows in thick bunches, emerging from a strong base and standing for about 3 meters in height with a meter-wide stretch.

The genus *Cymbopogon* comprises of 55 species of grasses, two of which are referred to as lemongrass. These are *Cymbopogon citratus,* which is famously preferred for culinary use and *Cymbopogon flexuous,* which is used in the manufacturing of fragrances because of its extended shelf life, owing to the low amount of myrcene in that variety. Lemongrass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavour. Lemongrass oil, used as a pesticide and preservative, is put on the ancient palm-leaf manuscripts found in India as a preservative. One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral (3,7-dimethyl-2,6-octadien-1-al). The volatile oil from the roots contains 56.67% longifolene-(V4) and 20.03% selina-6-en-4-ol.


**Fig.1 Leaf of lemongrass**


**Fig.2 Lemongrass oil**

*Cymbopogon citratus* has been cultivated over many years for medicinal purposes in different countries through-out the world. Lemongrass is an aromatic storehouse of essential nutrients providing an array of health benefits. It is a source of essential vitamins such as vitamin A, vitamin B1(thiamine),vitamin B2 (riboflavin), vitamin B3(niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folate and vitamin C. It also provides essential minerals such aspotassium,calcium, magnesium, phosphorous,manganese, copper, zinc and iron, which are required for the healthy functioning of the human body. It offers no harmful cholesterol or fats. The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties. It can be used in cleaning wounds and treatment of skin diseases such as ringworm. It can also be used in food poisoning, staphylococcal infections, and other common infections.

The oil has been found to posses bactericidal and anti fungal properties, which is comparable to penicillin in its effectiveness .

Antimicrobial activity of the Cymbopogon citratus (lemongrass) essential oil against food-borne pathogens was determined to investigate its potential for reducing microbial population of food products. Previous reports suggest that lemongrass essential oil is a safe natural flavour complex, preservative, and food spoilage inhibitor capable of reducing the risk of diseases associated with contaminated products.

The aim and objectives of this work is to determine therapeutic potentials of the plant extract on some pathogenic microorganisms. *Escherichia coli and* three strain of Gram-positive bacteria namely; *Micrococcus luteus, Bacillus cereus Staphylococcus aureus and against some fungus namely; Candida albicans* (ATCC No-3471), *Aspergillus brasilliensis, Penicillium funiculosum* and *Chaetomium globosum*. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Hence the present study was carried out to find out the antibacterial activity of Lemon grass oil.

**Materials and Method**

**Procurement of lemongrass oil**

The essential oil of lemongrass was procured from SIGMAALDRICH, India.

**Test organisms**

The test organisms used in this study was taken from National Institute of Chemical Laboratory (NCIM), Pune. The organisms used in the study were- *Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Bacillus cereus, Aspergillus brasilliensis, Candida albicans, Chaetomium globosum* and *Penicillium funiculosum*

**Propagation and maintenance of test organisms**

The bacterial test organisms were streaked on the Nutrient Agar slants and were incubated overnight at 37˚C and fungus test organisms were streaked on the Chloramphenicol Yeast Glucose Agar slants and were incubated for 5 days at 22˚C.

**Preparation of concentrations of lemongrass oil**

The 30% concentrations (v/v) of lemongrass oil were prepared aseptically in sterile tween-80.

**Antimicrobial activity**

The testing of the bacterial and fungal cultures for the inhibitory effect of essential oil of lemon grass for 30% concentration was performed by using agar well diffusion method.

**Agar Well Diffusion Assay (Zone of Inhibition Evaluation)**

Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 0.5 McFarland density of bacterial and fungal culture was adjusted using normal saline (0.85% NaCl) using densitometer to get bacterial and fungal population of 1.0 x $10^8$ cfu/ml. 100µl of each of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA (Mueller- Hinton agar), mixed well and poured into sterile Petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 100 µl of respective essential oil were pipette out into the well in assay plates. Bacterial plates were incubated overnight at 37°C and fungal plates were incubated for 5 days at 22˚C. Following incubation, petri-plates were observed for the inhibition zones, diameters of which were measured by using Vernier Callipers.

The determination of MIC of the essential oil of lemongrass on the test bacterial strain was done using broth dilution method as explained by Hammer *et al.* with different concentrations of oil . The range of MIC taken was 1 µg/mL to 128 µg/mL. The cultures of the test strains were prepared by inoculating the test strain in sterilized test tube containing 5 mL nutrient broth. The tubes were incubated 24 hours for bacteria and 48 hours for fungus at 37°Cand 25°C respectively. The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of the test compound to kill the microorganisms. The test tubes containing 10 mL of sterilized tryptic soy broth (TSB) with 0.5% (v/v) tween-80 were inoculated with different concentration of lemon grass oil ranging from 1-256 µ mL). TSB with 0.5% (v/v) tween 80 without oil was used as positive growth control. An aliquot of bacterial suspension (30µL) to each tube was added uniformly. The tubes were incubated for 24 hours (bacteria) 48 hours (fungus). The tubes were observed for turbidity after the period of incubation. The lowest concentration at which no visible growth occurs in either culture tubes was taken as MIC. Then the tubes showing no increased in the turbidity at each time interval hours were streaked on nutrient agar plates

## Results and Discussion

Lemon grass oil possesses a promising antimicrobial activity against the test organisms. The results obtained from the Agar diffusion assay and broth dilution method support the general indication that test organisms used are sensitive to the oil. Similar observations were made by Onawunmi and Ongulana and Cimanga *et al*. *P. aeruginosa* were found resistant at all the concentration of lemongrass oil including neat. Similar results were reported by Pereira et al, Marta War *et al* Torris *et al* , Alam *et al*, and Onawunmi *et al*. Lemongrass oil at concentration of 30% was found the most effective essential oil against all tested microorganisms.

All the selected microorganisms showed difference in their sensitivity at three different concentrations (5%, 15% and 30%). The strongest inhibition activity was observed in Lemongrass essential oil against *staphylococcus* and *Candida albicans*.

The activity of lemongrass oil (at 30%) was found in the series of *C. globosum* > *C. albicans* > *A. brasilliensis* > *S. aureus* > *B. cereus* > *E. coli* > *M. luteus* > *P. funiculosum*.

**Table-1 Antimicrobial activity of lemon grass oil against various selected microorganisms**

| Test Organisms | Zone of Inhibition (mm)- Lemongrass oil | | |
|---|---|---|---|
| | 5% | 15% | 30% |
| *Escherichia coli* | 13.76 | 19.20 | 23.14 |
| *Micrococcus luteus* | 13.44 | 17.45 | 20.47 |
| *Staphylococcus aureus* | 16.52 | 20.21 | 28.15 |
| *Bacillus cereus* | 14.56 | 20.48 | 26.20 |
| *Aspergillus brasilliensis* | 17.49 | 21.25 | 30.12 |
| *Candida albicans* | 18.88 | 23.45 | 31.25 |
| *Chaetomium globosum* | 19.25 | 24.60 | 34.25 |
| *Penicillium funiculosum* | 9.68 | 12.22 | 15.17 |

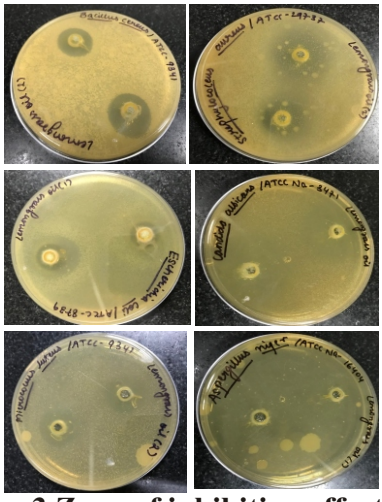

**Fig. 3 Zone of inhibition effects of lemongrass essential oil**

**Table-2 MIC (Minimum Inhibitory Concentration) of lemongrass oil against various selected microorganisms**

| Test Organisms | MIC-Lemongrass oil (µg/mL) | | |
|---|---|---|---|
| | 5% | 15% | 30% |
| *Escherichia coli* | 128 | 32 | 16 |
| *Micrococcus luteus* | 128 | 64 | 16 |
| *Staphylococcus aureus* | 64 | 16 | 8 |
| *Bacillus cereus* | 64 | 16 | 8 |
| *Aspergillus brasilliensis* | 64 | 16 | 8 |
| *Candida albicans* | 64 | 32 | 16 |
| *Chaetomium globosum* | 32 | 16 | 8 |
| *Penicillium funiculosum* | 16 | 8 | 4 |

## Conclusion

The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against currently available antibiotics. However, traditional plants have been proved to be better source for novel antimicrobial drugs.

The major concern is extensive uses of chemical food preservatives. The present scenario shows overuse of chemical preservatives in food products, this may not only lose the natural property of food but also affect the consumer's health.

Due to increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils of antimicrobial properties

The remarkable effect of Lemon grass oil on various test organisms are demonstrable indications of the oil as an antimicrobial agent. Thus, a study had been carried out to show that an herbal product Lemongrass oil is much potential against food spoilage organisms.

The study also recommends a new innovation and a challenging target in the food sector, vanishing the chemical substitutes, also protecting the naturality of the food product.

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# About Plants (Shown on the cover page)

## *Ocimum sanctum*- Tulsi

| | | |
|---|---|---|
| Kingdom | : | Plantae - Plants |
| Subkingdom | : | Tracheobionta - Vascular plants |
| Superdivision | : | Spermatophyta - Seed plants |
| Division | : | Magnoliophyta - Flowering plants |
| Class | : | Magnoliopsida - Dicotyledons |
| Subclass | : | Asteridae |
| Order | : | Lamiales |
| Family | : | Lamiaceae - Mint family |
| Genus | : | Ocimum L. - basil P |
| Species | : | Ocimum sanctum L. - holy basil P |

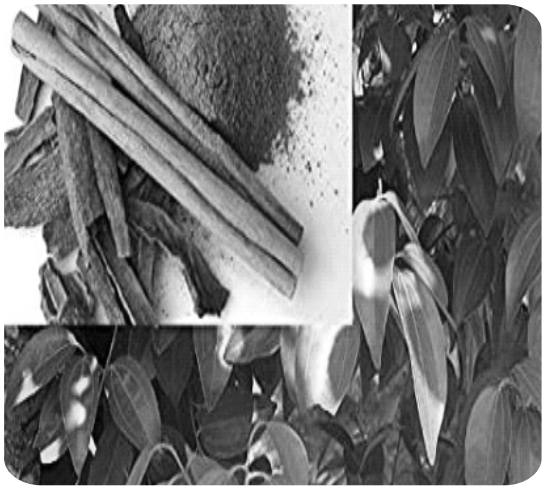
●Tulsi is also known as "**the elixir of life**" since it has intense antiviral and antibacterial property and promotes longevity.

●Different parts of the plant are used in Ayurveda and Siddha systems of medicine for prevention and cure of many illnesses and everyday ailments like common cold, headache, cough, influenza, earache, fever, colic pain, sore throat, bronchitis, asthma, hepatic diseases, malarial fever, as an antidote for snake bite and scorpion sting, flatulence, migraine headaches, fatigue, skin diseases, wound, insomnia, arthritis, digestive disorders, night blindness and diarrhoea.

● The leaves are good for nerves and to sharpen memory. Chewing of tulsi leaves also cures ulcers and infections of mouth. A few leaves dropped in drinking water or food stuff can purify it and can kill germs in it.

●Holy Basil is so good for boosting up the immune system. It protects from nearly all sorts of infections from viruses, bacteria, fungi and protozoa. Recent studies show that it is also helpful in inhibiting the growth of various types of viruses and HIV and also carcinogenic cells.

# *Cinnamomum zeylanicum-Dalchini*



## Cinnamomum

| | | |
|---|---|---|
| Kingdom Plantae | : | Plants |
| Subkingdom | : | Tracheobionta - Vascular plants |
| Superdivision | : | Spermatophyta - Seed plants |
| Division | : | Magnoliophyta - Flowering plants |
| Class | : | Magnoliopsida - Dicotyledons |
| Subclass | : | Magnoliidae |
| Order | : | Laurales |
| Family | : | Lauraceae - Laurel family |
| Genus | : | Cinnamomum Schaeff. - cinnamon P |
| Species | : | Cinnamomum zeylanicum Garcin ex Blume [excluded] P |

- Natural aqueous extract from a cinnamom bark has antiviral activity against enveloped viruses including influenza A,Parainfluenza virus and HSV-1 viruses as well as antibacterial activity.
- Cinnamom is loaded with Antioxidants and is a good immunomodulator
- Cinnamom has anti-Inflammatory **Properties**
- Cinnamom may Cut the Risk of Heart Disease
- Cinnamom Improve Sensitivity to the Hormone Insulin
- Cinnamom Lowers Blood Sugar Levels and has a Powerful Anti-Diabetic Effect.
- Powerful antiviral properties
- *in-vitro* and *in-vivo* studies in animals and humans from different parts of the world have demonstrated numerous beneficial health effects of CZ, anti-microbial activity, reducing cardiovascular disease, boosting cognitive function and reducing risk of colonic cancer.

# *Curcuma longa*-Turmeric

Turmeric

| | | |
|---|---|---|
| Kingdom Plantae | : | Plants |
| Subkingdom Tracheobionta | : | Vascular plants |
| Superdivision Spermatophyta | : | Seed plants |
| Division Magnoliophyta | : | Flowering plants |
| Class Liliopsida | : | Monocotyledons |
| Subclass | : | Zingiberidae |
| Order | : | Zingiberales |
| Family Zingiberaceae | : | Ginger family |
| Genus Curcuma L. | : | Curcuma P |

- Turmeric has a Good antiviral and antibacterial property it is an Immunomodulator its most active compound curcumin have many scientifically-proven health benefits, such as the potential to prevent heart disease, Alzheimer's and cancer.
- It's a potent anti-inflammatory and antioxidant and may also help improve symptoms of depression and arthritis.
- Turmeric is hailed for helping a host of conditions: high cholesterol, hay fever, depression, gingivitis, premenstrual syndrome and even hangovers. In Ayurvedic medicine, it is believed to act as an antiviral, antibacterial and antiparasitic, and has long been used to help with diabetes, pain, rheumatism, osteoarthritis, memory and skin conditions like eczema.

## *Withania somnifera*-Ashwagandha

# Ahwagandha

| | | |
|---|---|---|
| Kingdom Plantae | : | Plants |
| Subkingdom Tracheobionta | : | Vascular plants |
| Superdivision Spermatophyta | : | Seed plants |
| Division Magnoliophyta | : | Flowering plants |
| Class Magnoliopsida | : | Dicotyledons |
| Subclass | : | Asteridae |
| Order | : | Solanales |
| Family Solanaceae | : | Potato family |
| Genus Withania Pauquy | : | Withania P |
| Species Withania somnifera (L.) Dunal | : | Withania P |

- Ashwagandha is Antiviral and antibacterial
- Ashwagandha boosts Immunity and protect from infections
- Ashwagandha can reduce blood sugar levels(antidiabetic)
- Ashwagandha possesses anti-cancer properties and supports apoptotic mechanisms
- Ashwagandha can reduce cortisol levels in the blood
- Ashwagandha may help reduce stress and anxiety and improve memory
- Ashwagandha may reduce symptoms of depression
- Ashwagandha can boost testosterone and increase fertility in men
- Ashwagandha may increase muscle mass and strength.
- Ashwagandha can be used as an antiviral herbs. Antiviral activity of Withania somnifera extract has been reported earlier on Herpes simplex virus Type-1.

## Fourth Coming Events

**11th International Conference on Herbal therapy and Acupuncture**
**July 13-14, 2020 in Sydney, Australia.**
Website: **https://herbaltherapy.naturalproductsconference.com/**

**7th world congress on Natural Products, Medicinal Plants and Traditional Medicines**
**July 15-16, 2020 at Singapore**
Website: **https://naturalproductsconference.com/**

**29th International Conference on Plant Genomics**
**July 27-28, 2020 Madrid, Spain**
Website: **https://plantgenomics.environmentalconferences.org/**

**"Global Pharma Summit and Expo"**
**July 29-31, 2020 in Shanghai, China.**
Website: **https://globalpharmasummit.conferenceseries.com/**

Phytochemicals in Nutrition and Health Advances and Challenges
22-25, September, Bari, Italy
Website: http://bari2020.phytochemicalsociety.org/

**"22nd International Conference on Medicinal Chemistry, Drug Discovery & Drug Delivery"**
**September 25 - 26, 2020 at Montreal, Canada**
Website: **https://medicinalchemistry.pharmaceuticalconferences.com/**

**Sixth International Ayurveda Congress "*Roadmap for Globalization of Ayurveda"***
**1 to 4 October 2020 in Kathmandu, Nepal**
Website: **https://internationalayurvedacongress.com/nepal-2020/**

**17th International Conference on Pharmaceutical Formulations & Drug Delivery**
**October 30-31, 2020 at Vancouver, Canada**
Website: **https://formulations.pharmaceuticalconferences.com/**

**3rd World Conference on Pharmaceutical Research and Drug Management**
**November 11-12, 2020, Paris, France**
Website: **https://drug.pharmaceuticalconferences.com/**

**9th World Congress on Public Health, Epidemiology & Nutrition**
**August 17-18, 2020 Prague, Czech Republic.**
Website: **https://publichealth.conferenceseries.com/**

**International Conference on Nutrition and Health Care**
**October 26-27, 2020 in Osaka, Japan**
Website: **https://healthcarecongress.healthconferences.org/**

**Global Pharmaceutical and Pharma Industry Conference**
**October, 12-13, 2020 Rome, Italy**
Website: **https://pharmaconference.conferenceseries.com/**

**World Congress on Pharma and Clinical Trials**
**October 21-22, 2020 at Frankfurt, Germany.**
Website: **Https://clinicaltrial.pharmaceuticalconferences.com/**

**8th International Conference and Expo on Pharmacognosy, Medicinal Plants and Natural Products October 21-22, 2020**
**Shanghai, China. Pharmacognosy 2020**
Website: **https://pharmacognosy.pharmaceuticalconferences.com/**

**11th Annual Congress on Drug Formulation and Analytical Techniques**
**November 26-27, 2020 Istanbul, Turkey**
Website: **Https://drugformulation-bioavailability.pharmaceuticalconferences.com/**

**"2nd World Congress on Drug Discovery and Drug Design"**
**November 09-10, 2020 in Tokyo, Japan**
Website: **https://drugdiscovery.insightconferences.com/**

**Fourth International Symposium on Phytochemicals in Medicine and Food**
**Nov.30-Dec.04, 2020, Xi'an, China**
Website**: https://381313990.wixsite.com/4-ispmf**

"**2nd International Conference on Bio-Pharmaceuticals**"
**December 02-03, 2020, Auckland, New Zealand**
Website: **https://bioasia.pharmaceuticalconferences.com/**

**International Conference on Biotechnology and Healthcare**
**December 07-08, 2020 Auckland, Newzealand**
Website: **Https://biotechnology.biotechnologycongress.com/**

# Instructions to Contributors

The Universities' Journal of Phytochemistry and Ayurvedic Heights is a bi-annual Journal publishing free of charge the research work on herbs, natural products, phytochemicals and indigenous traditional system on Indian medicines for Human Health Protection, Nutrition and Plant Defence. It is open to research workers in India and abroad. Original articles, Research articles, short communications and Letters to the Editor on relevant topics are published in English. The copyright lies with the publisher. Publication of paper will be in clear understanding that they have not been submitted for publication in any Other journal.

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1. The typed manuscript should not exceed 10 pages and tables and graphs should not exceed 10% of the typed manuscript.
2. Manuscript should be typed on one side of the paper with double spaces. The usual format of a manuscript comprises of Abstract (not exceeding 150 words), Introduction, Keywords, Material and Methods, Observations, Results, Discussion, Acknowledgements, References and Tables & Figures.
3. Tables & Figures should be double spaced on separate pages, numbers consecutively in Roman numerals and placed at the end Of manuscript.
4. Those students who want their papers to be evaluated, corrected and formatted may address to the Director Helping Board.
5. The reprint of their articles will be supplied to the authors (First author) free of charge. Orders for additional reprints should be made along with the manuscript. They will be charged for postage and packing.
6. It is emphasized that the manuscripts should be neatly typed, double spaced throughout including tables on pages of uniform size with at least 2.5 cms margin on all sides. Author(s) should not break or hyphenate the words. Authors are required to include:

   i. The chemical structure, formula and proprietary name of novel or ill defined compounds.
   Ii. The w/w yield of prepared extracts in terms of starting crude material.
   iii. Complete formulation details of all crude drug mixtures.
   iv. The herbarium specimen number of plant(s) studied in case of less well known plants be cited using the collector and collection number (eg. Doe 123) as well as indicating the names of the herbarium institution where it has been deposited.
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# Uttarakhand State Council for Science & Technology

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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