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# Universities' Journal of Phytochemistry and Ayurvedic Heights

## Digital Health-An Ayurveda Perspective

*Mangifera indica*
( Mango)

*Momordicha charantia*
(Karela)

*Datura stramonium*

*Prunus cerasoides*
(Padam-Himalayan wild cherry)

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# DIGITAL HEALTH-AN AYURVEDA PERSPECTIVE

**Digital Health is possible only after merging with Technology**

Today the blend of Ayurveda and Artificial Intelligence can bring about many advancements in science and healthcare, thereby benefiting mankind. The amalgamation of Ayurveda and Artificial intelligence can potentially provide solutions to most problems by helping in the early detection of diseases, making accurate diagnoses and outlining prognoses and personalized medicines, and enhancing the community's health literacy.

Today, Ayurveda has started to use modern state-of the-art techniques to prove the scientific explanation behind the concepts written in ancient manuscripts. Artificial intelligence would be helpful in different aspects to enhance the pace of research on Ayurveda and its worldwide recognition.It will also improve the infrastructure for Ayurveda and the health industry in general. It can make the treatment personalized for everyone as furnished by the elaborate range of herbs in Ayurveda. Furthermore, AI can help improve patient care by prompting effective communication between patients and physicians and supporting physicians to provide better care.

Various researches have been conducted for Ayurveda-based disease diagnosis using machine learning. One such example is Nadi-pariksha or pulse diagnosis.Applications frequently use artificial intelligence models, such as analyzing herbal components, patient clinical data, and forecasting prescriptions and path ways. Chemical standardization like biomarker and metabolite profiling has unfolded a diverse chemical space of safe and therapeutically relevant molecules. On-going research on Ayugenomics is adding evidence regarding the genomic correlates of Vata, Pitta and Kapha the three dynamic principle bio factors termed as Tridosha. (**GENOMIC VALIDITY OF TRIDOSHA**).

Exploring molecular and network pharmacology of intelligent traditional formulations to elucidate and validate safety, toxicity, pharmacokinetics, metabolic stability, drug-herb interactions etc. are gaining importance. Focus on the validation of clinical traditions and practices like Panchakarma, Marmachikitsa, Agnikarma, Parpatichikitsa, Sarpavishachikitsa, Rasayana needs in depth scientific exploration.

In India, Ayurveda is considered a formal medical care system equivalent to conventional Western medicine. It’s estimated that 80% of India’s population of 1.2 billion people use some form of traditional Ayurvedic medicine.

**DIGITAL HEALTH-AN AYURVEDA PERSPECTIVE** will bring the world to India to seek its knowledge in Ayurveda, a world Ayurveda Congress and Arogya Expo will be held in Dehradun Uttarakhand from 12-15$^{th}$ of December 2024. In this congress hundreds of Healthcare professionals and experts in traditional medicines participated from 50 countries and will gained first-hand knowledge of Ayurveda.

I am grateful to the UJPAH board members to make this issue a memorable for science fraternity of the Uttarakhand and to all those scientists, research scholars, students and teachers who contributed for bringing out this issue and the people of science at large. Before concluding, I express my special gratitude to our chief guests, special and other guest present and the management who have provided all the guidance. May almighty bless you.

**Dr. S. Farooq**
Chief Editor

# Contents

# Phytochemical Screening and Antimicrobial Activity of fruit extract of *Prunus cerasoides* Dehradun from Garhwal Himalaya

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**Abstract-**Phytochemical screening of the petroleum ether, chloroform, methanol, and aqueous extract of fruits of *Prunus cerasoides* and their in vitro antibacterial activity by agar disc diffusion method. All the extracts were found to contain alkaloids, flavonoids, glycosides, saponins except for the absence of tannins and steroids. The plant extracts were studied using *Staphylococcus aureus, Escherichia coli, Listeria Monocytogenes, Serrtia marccen-scens, Aspergillus flavus* for their antimicrobial activity.

**Keywords:** Phytochemical screening, methanolic extract, *Prunus cerasoides, Staphylococcus aureus, Serrtia marccen-scens.*

## Introduction

*Prunus cerasoides* (syn. Prunus puddum) is native of Garhwal Himalayan and also wildly growing in sub-Himalayan tracts to montane zone 2400 metre high, Sikkim, Nepal, Bhutan, Myanmar, West China and North-East Verma[1]. It belong to the family Rosaceae, and locally known as Payu, Padam, Padmakha, and Himalayan cherry. Deciduous tree to 10 m high; bark reddish-brown exfoliating in thin circular strips. Leaves conduplicate in bud, elliptic or ovate-lanceolate, 3.5-8.5×2.5-3.5 cm, apex acuminate. In traditional medicine, the plant is used in Leprosy, Asthma and shown antipyretic activity[2]. According to the earlier investigation, much work has been carried out on stem bark, sapwood, seed of

the plant due to the presence of high concentration of flavonoids and flavonoid glycosides [3-7] but fruits are scantly studied. This prompted us to carry out the phytochemical screening and their antibacterial efficacy against various bacterial strains.

**Figure-1** Ripe and dried fruits of *Prunus cerasoides*

## Material and Methods

### Plant materials

The fruits of the plants were collected from Dhanolti, located at North part of Garhwal Himalaya. Plant sample was authenticated by Department of Botany, HNB Garhwal University Campus Badshahi Thaul, Tehri, and voucher specimens were deposited in the Department. Shade dried leaves were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (Soxhlet). The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (60-80$^0$C), chloroform, methanol and water. Each time, the mare was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotator flash evaporator. The dried extracts were dissolved in Dimethyl Sulphoxide (DMSO) and subjected to antimicrobial activity.

### Phytochemical screening

Chemical tests were carried out on the methnolic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973)[8, 9, 10].

**Test for tannins:** About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

**Test for saponin:** About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered.

10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**Teat for flavonoids:** Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973)[8, 10]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2SO_4$. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

**Test for steroids:** Two ml of acetic anhydride was added to 0.5 g Methanolic extract with 2 ml $H_2SO_4$. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

**Test for cardiac glycosides (Keller-Killani test):** Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

## Microorganism and Media

**Gram positive bacteria:** *Staphylococcus aureus* (ATCC29213), *Listeria monocytogens* (ATCC 19115), Gram negative bacteria: *Escherichia coli* (ATCC 25922), *Serrtia marcescens* (ATCC 21075); and fungus: *Aspergillus flavus* (ATCC 32612), were obtained from the Department of microbiology G.B. Pant Agriculture University Hill Campus, Ranichauri, Tehri Garhwal for the study. The bacterial and fungal stock cultures were maintained on Muller Hinton agar and Saboured- Dextrose agar slant, respectively, which were stored at $4^0$ C.

## Antimicrobial screening

The extracts were screened for their antimicrobial activity in vitro by disc diffusion method[11] using *S. aureus*, *L. monocytogens*, *E. coli*, *S. marcescens* and *A. flavus* at test organism. Agar cultures of the test microorganism were prepared.

Three to five similar colonies were selected and transferred to 5ml broth with a loop and the broth culture were incubated for 24 h at $37^0$ C and suspension was checked to provide approximately $10^{10}$ colonies forming units per ml. 0.1 ml of organism's suspension were spread evenly on the agar plates. For screening, sterile 3mm dimeter disc (Whatman filter paper No 1) were impregnated with 0.2 ml of 1000 µg/ml of the various extracts of both the drugs, dried and then placed in inoculated plates of Muller Hinton agar and Saboured- Dextrose agar medium. DMSO solvent was used as negative control. The plates were inoculated at $37^0$ C for 24 h and room temperature for 48 h for bacteria and fungi, respectively. After incubation for 24 and 48h, the results were recorded by measuring the zone of inhibition surrounding the disc. *Penicillin* (10 µg/disc) and *Gentamycin* (20 µg/disc) were used as reference slandered for bacteria and fungi respectively. Each experiment was done in triplicate.

## Results and Discussion

Petroleum ether, methanol and aqueous extracts of *P. cerasoides* showed significance activity against E. coli and moderate activity against other organism except *L. monocytogens* and only the aqueous extract showed significant activity against *A. flavus* **(Table -1).**

Phytochemical screening of different extracts of *P. cerasoides* showed the presence of alkaloids, saponins, flavonoides and coumarins **(Table-2)**. Petroleum ether extracts of *Prunus cerasoides* were found to be more effective against *E. coli* and *S. marcescens* and *A. flavus*. When compared to other extracts of the plant. Phytochemical screening of the petroleum ether extract of *P. cerasoides* revealed the presence of alkaloids which suggest that these phytoconstituents may be responsible for their antimicrobial activity. Further studies are needed to isolate and characterize the bioactive principles to develop new natural drugs.

**Table-1 Antimicrobial activity of *Prunus cerasoides* fruits extracts**

| Organism | ZONE OF INHIBITION OF EXTRACTS IN mm | | | | |
|---|---|---|---|---|---|
| | PE | CE | ME | AE | STD |
| ***S. aureus*** | 6 | 11 | 12 | 10 | 18 |
| ***L. monocytogenes*** | 9 | NI | 6 | NI | 18 |
| ***E.coli*** | 21 | NI | 15 | 17 | 20 |
| ***S. marccenscens*** | 8 | 7 | 9 | 8 | 18 |
| ***A. flavus*** | NI | 8 | NI | 19 | *20 |

NI= No Inhibition, PE= Petroleum Ether ext, CE= Chroloform extract, ME= Methanol extract, AE=Aqueous extract; STD=Standared (*Penicillin*, **Gentamycin*). Values are average of three determinations.

**Table-2 Phytochemical screening of different extracts of *Prunus cerasoides* fruits**

| **Phytoconstituents** | ***Prunus cerasoides*** | | | |
|---|---|---|---|---|
| | PE | CE | ME | AE |
| **Alkaloids** | + | - | + | + |
| **Glycosides** | + | + | + | + |
| **Saponins** | + | - | - | - |
| **Tannins** | - | - | - | - |
| **Flavonoids** | + | + | + | + |
| **Steroids** | - | - | - | - |

PE= Petroleum ether ext, CE= Chloroform extract, ME= Methanol extract, AE=Aqueous extract; + = Present, - = Absent.

## Conclusion

The results obtained in the present study phytochemical screening assays confirmed the use of the plant and their species for further investigation potential natural bioactive compounds. It is the first report about antimicrobial effects of different extracts of *Prunus cerasoides* fruits against *S. marccenscens* and aqueous extract against *A. flavus*.

## Acknowledgements

Authors are thankful to Dr. J. Kumar, Microbiology division, G.B. Pant University, Hill Campus Ranichauri, Pantnagar, Tehri Garhwal, India for antimicrobial activity studies.

## Disclaimer Statement

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

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# Physiochemical analysis of agriculture soil of majra region

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**Abstract-** The status of soil structure, porosity, and water holding capacity greatly affects plant growth and health. Soil water relationship and soil plant relationship is affected by soil physical parameters. Soil is the main resource of the natural and agricultural ecosystem. An increase in the content of heavy metals in soils is an environmental threat to agriculture, as it is associated with the accumulation of toxicants in plants, which negatively affects the quality of crop production and poses a danger to human health. Each region has its own specifics crop yield, but due to rapid urbanization and increase in different types of pollutions; there is change in nature, quality and quantity of crop. The article presents the analysis data of soil of Majra, Dehradun region.

## Introduction

Soil quality is an indicator of environment quality (National research council 1993) and food security (Lal 1999) Soil quality refers to the status of soil as a result of

management (Karlen et al 2003) soil quality parameter is divided into physical chemical and biological parameters i.e, water holding capacity, relative field capacity to water saturation, macro porosity, bulk density, cation exchange capacity, contamination presence, pH, exchangeable, sodium etc (Reynolds et al. 2009, Zaid et.al. 2017). Degradation of soil in arid and semi-arid regions is due to lack of knowledge about soil conditions for farmers and lack of proper equipment. Also in such Circumstances soil was found to be with poor soil quality, high temperature, poor soil fertility, low available water holding capacity, soil organic carbon and high concentration of salt and pH (Zaid et al. 2017). The status of soil structure, porosity,

and water holding capacity greatly affects plant growth and health. Soil water relationship and soil plant relationship is affected by soil physical parameters. Soil is the main resource of the natural and agricultural ecosystem. Only 22% (3.26 billion ha) of the total area of the planet is suitable for agriculture and only 3%. (450 million ha) has high production capacity (Lal 1993).The diversity of contaminants is constantly evolving due to agrochemical and industrial developments.

This diversity and the transformation of organic compounds in soils by biological activity into diverse metabolites, makes soil surveys to identify the contaminants both difficult and expensive. The effects of soil contamination also depend on soil properties since these controls the mobility, bioavailability, and residence time of contaminants (FAO and ITPS, 2015).

Soil pollution is an alarming issue. It has been identified as the third most important threat to soil functions in Europe and Eurasia, fourth in North Africa, fifth in Asia, seventh in the Northwest Pacific, eighth in North America, and ninth in sub-Saharan Africa and Latin America. The presence of certain pollutants may also produce nutrient imbalances and soil acidification, two major issues in many parts of the world, as identified in the Status of the World's Soil Resources Report (FAO and ITPS, 2015).

The unique global estimate of soil pollution was done in the 1990s by the International Soil Reference and Information Centre (ISRIC) and the United Nations Environment Programme (UNEP), which estimated that 22 million hectares had been affected by soil pollution (Oldeman, 1991). The Revised World Soil Charter (FAO, 2015b) recommends that national governments implement regulations on soil pollution and limit the accumulation of contaminants beyond established levels in order to guarantee human health and well-being.

Recently, the United Nations Environmental Assembly (UNEA-3) adopted a resolution calling for accelerated actions and collaboration to address and manage soil pollution in the framework of Sustainable Development. This consensus achieved by more than 170 countries is a clear sign of the global relevance of pollution and of the willingness of these countries to develop concrete solutions to address pollution problems (UNEP, 2018).

**Physical properties of soil:** Soil texture and soil structure are the two important physical properties of soils. Some of the other important physical properties are

moisture, colour, density, porosity and temperature (Brady and Weil, 2001).

**Soil Texture:** Solid phase of the mineral soil mainly consists of discrete mineral particles as the amount of amorphous material including organic matter is usually small. Mineral particles are not exactly spherical but vary widely in their shape, therefore, these particles are usually classified into three conveniently separable groups according to certain size range based on their equivalent diameter (diameter of a sphere that has a velocity of fall in a liquid medium equal to that of the specific particle). The groups of different size range of mineral particles are known as soil separates, primary particles or textural fractions, namely: sand, silt and clay. Soil texture refers to the prominent size range of mineral particles, and is defined both qualitatively and quantitatively. Qualitatively, it refers to the feel of soil whether coarse and gritty or fine and smooth when rubbed between thumb and forefinger.

Quantitatively, soil texture is the relative proportion of sand, silt and clay content on weight basis. The term soil texture is often used interchangeably with mechanical composition of soil. It is more or less a static property affecting almost all others soil properties. For instance, a soil containing 40 percent sand, 40 percent silt, and 20 percent clay is called loam soil. (Jackson, 1962) Soil texture describes the distribution of these different size particles in a soil. There are twelve soils based on the distribution of sand, silt and clay.

**Soil structure:** Soil structure describes the arrangement and organization of soil particles in the soil, and the tendency of individual soil particles to bind together in aggregates. Aggregation affects water and air transport, which affects the movement of solutes and pollutants and effects biologic activity, including plant growth. The development of soil structure is influenced by the amount and type of clay, as well as the exchangeable ion on the clay.

**Soil density:** Soil density is related to the mineral and organic composition of a soil and to soil structure. The standard measure of soil density is bulk density, defined as the proportion of the weight of a soil relative to its volume. It is expressed as a unit of weight per volume, and is commonly measured in units of grams per cubic centimeter (g/cm3). Bulk density is an indicator of the amount of pore space available within individual soil horizons, as it is inversely proportional to pore space. Soils with high density may also impede root growth. Even at sites where selective grading is employed, compaction occurs as a result of construction equipment,

stockpiling and vehicle traffic (Randrup, 1998; Lichter and Lindsay, 1994)

**Soil porosity:** Although porosity is related to density, pore size is an important factor affecting soil processes. Soils with similar porosity may have different distributions of pore sizes. The smallest pores (>0.1 μm diameter) hold water too tightly for use by plant roots. Plant-available water is held in pores 0.1–75 μm in diameter. Macropores (>75 μm diameter) are generally air-filled when the soil is at field capacity, but they can rapidly transport water and solutes to deeper depths in the soil. The pore size distribution affects the ability of plants and other organisms to access water and oxygen; large, continuous pores allow rapid transmission of air, water and dissolved nutrients through soil, and small pores store water between rainfall or irrigation.

## Material and Methods

### Sample Collection

The soil sample was collected from different location of Majra, Dehradun. This region was once famous for basmati rice variety and Litchi. Soil samples were collected in plastic sample bags. Plant residues and stone pieces were removed by hand.

**Figure-1 Soil sample**

Soil sample were air dried and passed through a 2 mm brass sieve. Samples were stored at 30°C temperature in oven until use. Then 100g soil sample was transferred to sample bag. Sampling date, location of the sampling and sampling number were marked on the bags and soil samples were brought to the laboratory. Samples study carried out which based on wide range of physio-chemical properties and chemical properties.

The soil samples were brought to laboratory for further analysis. The soil reaction (pH) was determined by using pH meter. Temperature of a soil is measured with help of a routine mercury thermometer in a metal cone by penetrating it into the soil up to 2 cm depth. Water holding capacity of soil usually refers to amount of maximum water which can be held by saturated soil. Specific gravity is determined by using pycnometer. Grain analysis test is done by using sieve of different size.

Proper pH in the field is essential for plant productivity, and either too high or too low pH will adversely affect crop growth. The development of strongly acidic soils (< 5.5

pH) can result in poor plant growth as a result of one or more of the following factors:

- aluminium toxicity
- manganese toxicity
- calcium deficiency
- magnesium deficiency
- low levels of essential plant nutrients such as phosphorus and molybdenum.

Alkaline soils may have problems with deficiencies of nutrients such as zinc, copper, boron and manganese. Soils with an extremely alkaline pH (greater than 9) are likely to have high levels of sodium.

The correct balance is where the soil pH is between 5.5 and 7.5, so every effort should be taken to check soil pH levels regularly. Early identification of soil pH problems is important as it can be both costly and difficult to correct long-term nutrient deficiencies.

### Testing pH of soil

One calculates its hydrogen ions. pH values may range from 0 to 14. Grain size analysis is essential in determining the permeability, porosity, compressibility, and shear strength, which helps in making informed decisions for various engineering and environmental applications.

## Experimental Investigation

The overall testing program was conducted in two phases. In the first phase, the geotechnical characteristics of soil were studied by conducting laboratory test. In the second phase soil mixed with geo grid with different height of mould was subjected to various test.

## Following tests were conducted:

### Specific Gravity Test

This test is very important to study any type of soil which is defines as a ratio of mass of a given volume of solids to the mass of an equal volume of water. The specific gravity of solids for most natural soils which falls in the general range of 2.65 to 2.80, the smaller values are for the coarse-grained soils. It may be mentioned that specific gravity of different particles in a soil mass may be not same. Whenever the specific gravity of soil mass is indicated, it is the average value of all the solid particles present in the soil mass.

Specific gravity of the solid is an important parameter. It is used for the determination of void ratio and particle size. In the present study of soil, specific gravity of solid particles was found to be 2.70 and for this test pycnometer method is used which consists of 1000 ml capacity. The specific gravity of the soil particles is 2.2gm. Soils containing organic matter and porous particles may have specific gravity value

below 2.0. Soils having heavy substances may have values above 3.0.

### Grain Size Analyses

For grain size distribution, divide the soil in different category based on grain size. There are two categories that is coarse grain size and fine grain size. If the size of soil particle is more than 4.75 mm which is called coarse grain aggregate and if the soil particle is less than 4.75 mm which is called fine grain aggregate in geo technical terms. Fine aggregate grain size distribution is done by two methods, sieve analyses and hydrometer test. In the present study we adopted wet sieve analyses test. In this method 1 kg of oven dried soil was taken in 0.075 mm sieve through which water was flowed for a period until transparent water was flowing from the sieve. This sieve with wetted retained soil was kept in oven for someday to get oven dried soil. The oven dried soil was sieved through set of sieves which are generally made up of brass and sieve cloth. The set of sieves placed one above other in descending sequence like 4.75 mm, 2mm, 1mm, 600-micron, 425-micron, 300-micron, 150-micron, 75 microns, and pan for both retain.

### Soil Acidity Test (pH)

Knowing the pH level of your soil is essential to ensure an optimal plant growth. pH test results will guide in the decision whether and how much the soil needs supplements, like fertilizers and soil pH adjusters.

Collected samples of soil from the test area. (The soil should be taken from the same depth below the surfaces each time you test.) Took more samples around the plant and mixed them to get a homogenous sample.

Weighed out 1 unit soil (100 g is recommended). Added 2 unit (200 ml) distilled water to it. Stirred the sample for 30 seconds. Let it sit for 15 minutes. Filtered liquid into clean cup. Stirred it again, then look the measurement with Adwa pH meter. Held the electrode in the sample min. 1 minute before the reading.

### 4. Water Content Test

To find the moisture content of soil consists of the following steps: Cleaned and dried the container and weighed it (W1). Took a specimen of the sample in the container and weigh it (W2). Place the container in the hot air oven, arranged temperature to $110^{o}C \pm 5^{o}C$ and allow it to dry for a period varying with the type of soil (usually 24 hours). Recorded the final constant weight (W3) of the container with dried soil sample.

## Result and Discussion

Each test was repeated thrice and the average mean value was reported as result. The results of different physical parameters

of soil sample tests were represented in Table 1.

**Table 1: Different Physical Test carried out on Soil Sample**

| S.No | Test | Result |
|---|---|---|
| 1 | pH | 7.44 |
| 2 | Water content | 8.1 % |
| 3 | T.D. S | 2.0 gm |
| 4 | Temperature | 75 °F |
| 5 | Density | 1.3 g/cm³ |

## Physical parameters of soil

The soil sample was collected from different locations of majra, Dehradun. This region was once famous for basmati rice variety and Litchii. The results found were related to pH, temperature, specific gravity, grain analyses, moisture content and density of soil. The results are shown in (Table 1).

The acidity of the soil can be studied by soil reaction or pH. The pH is very important property of soil as it determines the holding capacity. The pH value of the soil is acidic then it is acidic; 6.5 if it is normal 6.5 – 7.8, alkaline 7.8 – 8.5, alkali >8.5. This one of the most important soil properties which effect drop growth. With the water holding capacity range from 0.14 ml/gm to 0.42 ml/gm and the moisture content range from 7.4% to 5.4%. Soil type was found to be silty and derbies. The optimum temperature for growth of plant ranges between 68°F and 86°F. High temperature even for short period, affects crop growth especially in temperate in case of wheat. Specific gravity of soil sample ranged from 2.17 – 2.32. this value it tells how much Heavier/lighter the material is in water. The grain analyses test is performed to determine the percentage of each size of grain within a soil sample.

## Water content

The optimal range of soil moisture content for crops depends on the specific plant species, but the range for most crops was found between 20% and 60%.

TDS levels below 700 mg/L and SAR below 4 are considered safe; TDS levels between 700 and 1,750 mg/L and SAR levels between 4 and 9 are considered possibly safe, while levels above these are considered hazardous to any crop. For irrigation, the TDS has been classified as TDS < 450 mg/l and is preferred for irrigation and TDS > 450–2000 mg/l is slight to moderate and TDS > 2000 mg/l is

unsuitable for agricultural purpose (FAO 2006)

The optimal and critical limits of soil BD are dependent on soil texture, particle size, management practices, and organic matter content (Reichert et al., 2009). A BD of less than or equal to 1.3 g $cm^{-3}$ is good, between 1.3 and 1.55 g $cm^{-3}$ is fair, and greater than 1.8 g $cm^{-3}$ is considered extremely bad.

Considering this concept (Eqs. 17.2 and 17.3), the bulk or dry density is defined as the ratio of mass of solid to the total volume of soil. Typical values of the dry or bulk density of most soil vary within the range of 1.1–1.6 g/$cm^3$. An average value would be 1.3 g/$cm^3$ for loamy soil.

## Conclusion

The results of different physical chemical test of soil sample showed that soil was still having good agricultural value but due to rapid conversion to urbanization, soil lost its agricultural value and fertility.

## Disclaimer Statement

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

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# In -Vitro Antioxidant Activity of *Capsicum Chinense* plant Leaves by Hydro-Methanolic Extraction

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**Abstract-**Generation of the reactive oxygen species (ROS) or intracellular abilities to elimination of ROS are out of balance, which leads to oxidative stress. It causes excessive damage to all biomolecules, including lipids, proteins, DNA, and RNA. The antioxidant activity of plants is caused by these phytochemicals, which interact with the antioxidants substances that prevents oxidation and oxidatives injury caused by free radical. As a result, it can potentially neutralize reactive oxygen species or free radicals. Capsicum Chinense, a member of the Solanaceae family, is a rumbustious, easily-growing species that is found from the Tezpur DefenseResearch Laboratory, in Indian state of Assam, that the king chilli is the hottest chilli worldwide.This species is utilized as hepatoprotective, antitumor, diuretic, anti-inflammatory, and bactericidal medicines due to its antioxidant activity. Numerous researches carried out in the last few years have shown that many types of chilli consist of phenolic chemicals, carotenoids, and capsaicinoids—all among which have been shown to have biological activity. In this study we identify the anti-oxidant impact of hydro-methanolic extract of leaves parts of Capsicum Chinense or to estimate the Anti-oxidant effects of hydro-methanolic extract and compare to it with Silymarin by DPPH Radical Scavenging Assay methods because there is no scientific data presented to the anti-oxidant activities of this plant. The current investigation also showed that Capsicum Chinense leaves portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species have enormous possibility for used in the treat for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant

potential as well, supportive the old-style uses of this plant with scientific evidence.

**Key Words: -** oxidative stress, anti-oxidants, Capsicum Chinense, DPPH, Silymarin

## Introduction

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

One of the few horticultural products that is as easily obtainable as chilli peppers is this particular variety. This fruit is used in the cooking of many different cuisines worldwide. The chili pepper is a member of the genera Capsicum, which is a part of the Solanaceae family of plants. This fruit contains a wide range of essential nutrients, such as proteins, fats, carbs, and fiber. There is a higher concentration of chemicals in chilli peppers that might impact biological processes. Numerous research carried out in the last few years have shown that many types of chilli consist of phenolic chemicals, carotenoids, and capsaicinoids—all among which have been shown to have biological activity. Regarding several bioactive substances, polyphenols are the ones that have undergone the most research. Some of the most important bioactivities associated with each one of these substances include the ability of polyphenols to lower blood pressure, lower blood sugar levels, and fight inflammation. This study offers a thorough examination of the both vivo as well as in-vitro bioactivities linked to capsaicinoids and polyphenols present in a range of chilli products. These specifics are useful when preparing meals or food ingredients that have many purposes. The

leaves of the capsicum Chinense plant contains the occurrence of alkaloid, glycosides, saponin, tannin, or other phenolic compound which possess antioxidants activity.

Widely identified by its popular name, King Chilli, sometimes called "Bhut Jolokia," is a highly hot kind of chilli that comes from India's northeast. It has been found from the Tezpur Defense Research Laboratory, in Indian state of Assam, that the king chilli is the hottest chilli worldwide[6].

**Figure-1 Leaves, fruits and flowers of Capsicum Chinense**

## Material and Method

### Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Methanol, were bought by Shaila enterprise. Analytical grade reagents were utilized in all other cases.

### Plant material collection and authentication

**Identification and Collection of the Plant:** The leaves part of the *Capsicum Chinense* plant has been collected from the local area of Mazbat, Assam and were air dried in the shade.

**Authentication of the Plant:** The herbal plant that is *Capsicum Chinense* is used in the study was authenticated by Botanical Survey of India, Noida, U.P.

**Authentication no-** BSI/BGIR-8(3)2021/88

### Preparation of plant extract

### Plant extraction

Dried leaves of Capsicum Chinese were collected and then grinded into coarse powder via clean mortar or pestle and are store in an air-tight container to protect from moisture. Hydro Methanolic method was used in the study for the extraction of the plants. Plant extracts was ready by the maceration extraction process where

100gm crude drug powder was soaked in 75% methanol in beaker for 72 hrs. at room temperature with occasional stirring. After 72 hrs the liquid phase stained, filtered using filter paper and evaporated to dryness in hot air oven as a result the extract was obtained and weighed[7].

## Extraction Method

### Maceration Method

Because of its ease of usage, maceration is a particularly often used technique for extracting polysaccharides. This method involves placing the medication, in powder form, in a container with the solvent and letting it sit at room temperature for three days while stirring constantly[8].

The combination is strained, the residual solid (marc) is pressed, and the collected liquids are filtered after that. This extraction method's primary goals are to remove unwanted components and retrieve desirable compounds with therapeutic relevance. The general maceration mechanism is leaching [9].

## In-vitro Antioxidant activities

### Antioxidant Assay

The antioxidant activities of the plants extraction were determined by 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay methods**.** Entirely the assays were carry out in triplicate or average value were considering.

### DPPH Radical Scavenging Assay

The free radical scavenging capacity of the hydro-methanolic extract of Capsicum Chinense leaves parts, determine by using DPPH. DPPH solutions (0.004% w/v) was prepare in 95% ethanol. Methanolic extracts given plants were mix with the ninety five percent ethanol and water respectively to prepared stock solutions (10 milligram /100ml). From this stock solution 1ml, 2ml, and 3ml, of solutions were taken in 3 test tube respectively and by sequential dilutions with the similar solvents, the final volume of each test tube was made up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml, 30 µg/ml respectively for all extracts. Fresh prepared of DPPH solutions (0.004% w/v) was add in each of these test tube or later 10 min, absorbance was taken at 517 nm using in a spectrophotometer (Double beam UV–visible spectrophotometer). Silymarin were uses as a reference standard drug or dissolve in a distill water to make the stocks solution with the similar concentrations. A control samples was preparing contains the same volumes without any extraction or references standard. % scavenging of the DPPH free radicals was measure uses the following equations [328-330].

**% DPPH radicals-scavenging = (Absorbance of control - Absorbance of test Sample) X 100**
**(Absorbance of control)**

## Results

**Table-I Result of phytochemical test Preliminary phytochemical investigation of Capsicum Chinense**

| TESTS | HAE |
|---|---|
| **Tests for Alkaloid** | |
| 1. Mayer's Tests | + |
| 2. Wagner's Tests | + |
| 3. Hager's Tests | + |
| 4. Dragendroff's tests | + |
| **Tests for Saponins** | |
| 1. Foam test | ++ |
| **Test for Flavonoids** | |
| 1. Alkaline reagent tests | + |
| 2. Lead Acetate tests | + |
| **Tests for Tannins** | |
| 1. Gelatine + extract | ++ |
| **Tests for Phenolic compound** | |
| 1. Ferric chloride solution | ++ |
| **Tests for Terpenoids** | |
| 1. Salkowski test | ++ |

+ represents presence; ++ represents present in more concentrations; - represents absence.

The phytochemical testing of Hydro-Methanolic extraction of leave of Capsicum Chinese shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

### Phytochemical screening

Preliminary phytochemicals screening of the *Capsicum Chinense* leaves extraction shows that the plants is riches in various actives ingredient ($2^{nd}$ry plant metabolite). The results of the phytochemicals screening revealed strongest to moderate presence of alkaloid, flavonoid, saponins and carbohydrates (Table-1).

## *In-Vitro* Antioxidant Activity

### DPPH radical scavenging activity

DPPH radical scavenging potential of Capsicum Chinense leaves extract at different concentrations investigated in the present study was determined together with standard antioxidant (Silymarin) at the same concentrations. Capsicum Chinense leaves extracts (hydro-methanolic extracts) showed significant scavenging effect on DPPH free radical in concentration dependent manner. When compared with standard antioxidants used in the experiment, the extract showed relatively lower DPPH free radical scavenging potentials.

**Table-2 Antioxidant activities of hydro-methanolic extracts of leaves parts of Capsicum Chinense by DPPH method.**

| S.NO. | Hydro methanolic Extraction of Capsicum Chinense | | Silymarin | |
|---|---|---|---|---|
| | Conc. (µg/mL) | % Inhibition | Conc. (µg/mL) | % Inhibition |
| **01** | 10 | 13.61 | 10 | 21.45 |
| **02** | 20 | 21.12 | 20 | 23.83 |
| **03** | 30 | 26.44 | 30 | 36.87 |

**Graphical representation I shows the % inhibition of DPPH radicals by extraction of Capsicum Chinense**

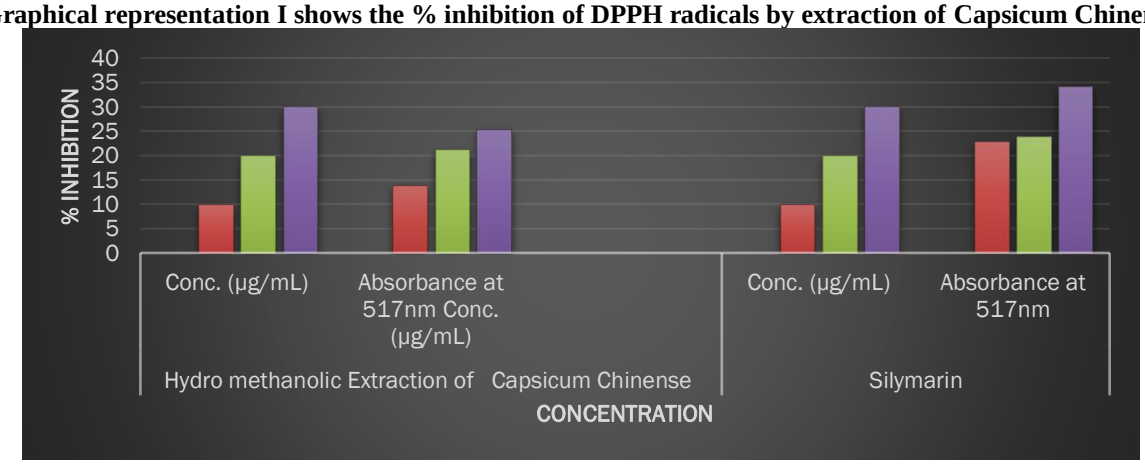
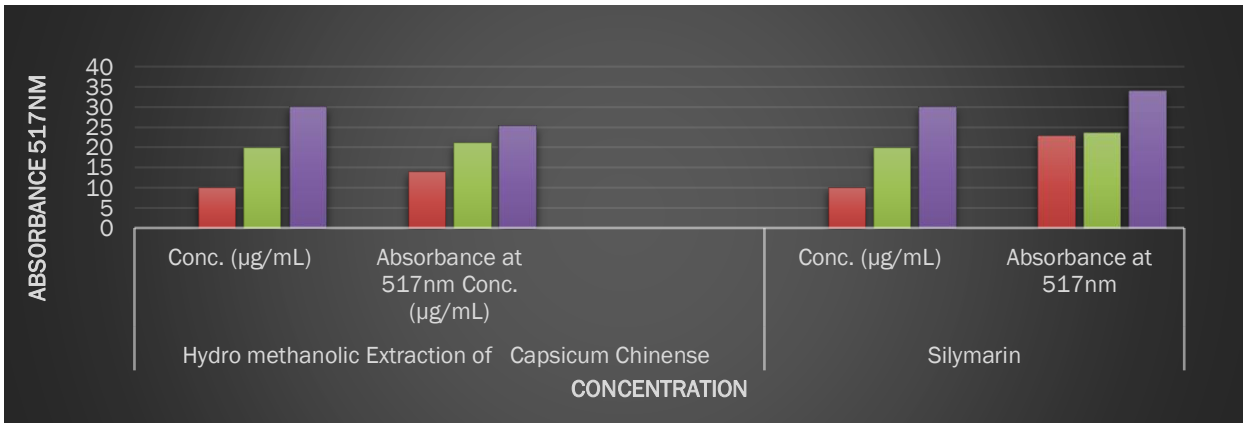



**Table-3 Antioxidant activities of hydro methanolic extraction of leaves part of Capsicum Chinense by DPPH method**

| S.NO. | Hydro-methanolic Extraction of Capsicum Chinense | | Silymarin | |
|---|---|---|---|---|
| | Conc. (µg/mL) | Absorbance at 517nm | Conc. (µg/mL) | Absorbance at 517nm |
| **01** | 10 | 13.87 | 10 | 22.89 |
| **02** | 20 | 21.12 | 20 | 23.73 |
| **03** | 30 | 25.34 | 30 | 34.21 |

**Graphical representation II showing the absorbance of DPPH radicals by extracts of Capsicum Chinense**



## Discussion

At normal temperature, DPPH is a purple stable free radical with a distinctive absorbance at 517 nm. An antioxidant called 1,1-diphenyl-2-picrylhydrazine readily stifles nitrogen free radical of DPPH. Purple color decolorization is stoichiometric and depends on the number of electrons acquired[10]. Leaves extracts from Capsicum Chinense demonstrated a substantial, concentration-dependent scavenging activity on the free radical DPPH. In contrast to the conventional antioxidants employed in the study, the extract exhibited comparatively reduced ability to scavenge free radical's DPPH. As a result, in vulnerable biological and food systems, Capsicum Chinense leaf extracts may be able to stop reactive radical species from causing damage to biomolecules like DNA, proteins, polyunsaturated fatty acids (PUFA), and carbohydrates. The high reactive species recognized as hydroxyl radical (HO•) is produced in biologicals system and targets DNA nucleotides, breaking DNA strands and causing cancer and mutagenesis. By removes the hydrogen atom from membrane lipids' polyunsaturated fatty acid, it starts the lipid peroxidation process. It has the ability to harm practically all of the molecules in living cell[11]. The leaves parts extract of Capsicum Chinense demonstrated the capacity to neutralize free radicals produced, and it also demonstrated concentration-dependent hydroxyl radical scavenging that was equivalent to that of the reference standard (silymarin) at the same dosages[12].

## Conclusion

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

## Disclaimer Statement

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

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# Extraction of eco-friendly natural dye from *Hibiscus acetosella* flowers for dyeing cotton fabric using natural mordant and its effect on pH-values

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**Abstract-**Current study revealed about the dyeing properties of *Hibiscus acetosella* on cotton fabrics using natural mordant. The plant *Hibiscus acetosella,* often known as African Rose mallow or Cranberry Hibiscus, is a member of the Malvaceae family. *Hibiscus acetosella* typically produces crimson or pinkish-red flowers. Dyes are extracted from *Hibiscus acetosella* flowers, producing a natural colorant. The dyeing pigments present in *Hibiscus acetosella* were extracted in this work by use of the aqueous extraction method, which required a 15-minute immersion in water and extracted dye was used to dyeing cotton fabrics without a natural mordant, and three fractions extracted were taken to observe the impact of three distinct mordanting techniques: pre, post, and simultaneous mordanting. The cotton cloth was soaked in a bath of boiling water with the coloring solution for five minutes. Following dyeing, the pH of the remaining sample was once more measured with a pH meter, and pH fluctuations were documented.

**Keywords:** *Hibiscus acetosella*, Natural mordant, Cotton fabrics, Natural dye.

## Introduction

Since ancient times, coloured clothes have been used. In order to colour these clothes, natural dyes were used. Even synthetic dyes were also available to colour them but natural dyes were always been better to use and to dispose as well. Natural dyes are environment-friendly and do not produce toxic substances because they are all made up of roots, leaves or flowers. Although clothes have been coloured with natural dyes since ages, synthetic dyes have already taken their place. Synthetic dyes have many disadvantages such as disposal of waste, but

still they are used in the industrial and textile sectors.

Although synthetic dyes provide diversity in colours, it expressed worries due to their environmental and health effects. They also pollute water as heavy metals or chemical can leach into water bodies. During the production of synthetic dyes if humans are exposed to harmful chemicals and dyes it leads to respiratory issues, skin irritation, allergic reactions etc. Both biodegradability and sustainability are missing in synthetic dyes. Because of this circumstance, natural dyes are selected as a suitable substitute, even though we are aware of their lack of economic success. Natural dyes[1,2] are beneficial as they are biodegradable[3] and are less harmful to nature, do not release toxic substances in water bodies, depends on renewable resources lowering reliance on fossil fuels and lessening the damage that resources extraction causes to environment, and require less energy and reduce the amount of greenhouse gases emissions.

After the industrialisation era, there is a major trend in the increase of pollution in the world. A major cause for the increase of pollution could be the use of synthetic chemicals and products. Therefore, there emerges the need for using alternative to these synthetic products that would cause lower or no harm to the environment. As awareness is increasing about the negative consequences of these synthetic products, the market for natural goods has expanded. Natural dyes can be used in place of multiple artificial colours used in textile manufacturing. This modification will encourage the sustainability[4,5,6] and ecological substitute for synthetic colours and hence providing the advantage for human health as well as environment. This will also encourage the future generation towards the use of natural resources[7,8] and encouraging healthy planet. Nowadays, the trend for the natural dyes has certainly grown up in India[9] because the people are becoming more and more conscious about the harmful effects of synthetic chemicals. The synthetic dyes are really harmful since they contain toxic substances and heavy metals which make them very difficult to dispose. Sustainable livelihoods are additionally promoted via promoting the use of natural dyes. But we know that the synthetic dyes are much better in terms of stability and colour fastness, hence the research is conducted continuously in order to satisfy customer expectations and their standards. It's not like the natural dyes only used for garments, they are used for a variety of purposes like household textiles, industrial uses and a lot more.

A process called "mordanting" is used to add mordants to fabric, which serves as a

binding agent for attaching the dyes to the cloth. Three different forms of mordanting processes exist: Pre-mordanting, Post-mordanting and Simultaneous mordanting. The process of Pre - mordanting involves treating the cloth with mordant before dyeing it. After that fabric is dried after being dipped in the dyeing solution. In Simultaneous mordanting the dye- bath receives a direct addition of mordant. In Post-mordanting before the fabric gets soaked in the mordant solution, it is dyed by dipping in a dyeing solution. Mordanting is needed in dyeing process for the fixation of dyes to ensure that they do not easily wash out or fade over time, to improve colourfastness means colour remain bright and oppose fading when exposed to light, washing etc.

## Botanical Description of *Hibiscus acetosella*

*Hibiscus acetosella*[10] is a member of family Malvaceae. The petals of the *Hibiscus Acetosella* flower are either purple or crimson in color. It grows to height of 3-6 feet. *Hibiscus Acetosella* develops a fruit that mimics a capsule and is filled with number of seeds.

## Chemical composition of *Hibiscus acetosella*

It contain anthocyanins which is responsible for its red or purple colour, flavonoids, phenolic compounds[11,12] such as Quercetin, Myricetin, Kaempferol etc. (Figure. 1). which contributes to dyeing properties on fabrics and antioxidant properties, vitamins such as Vitamin-C, minerals including calcium, iron, magnesium and potassium, organic acids such as citric and malic acid

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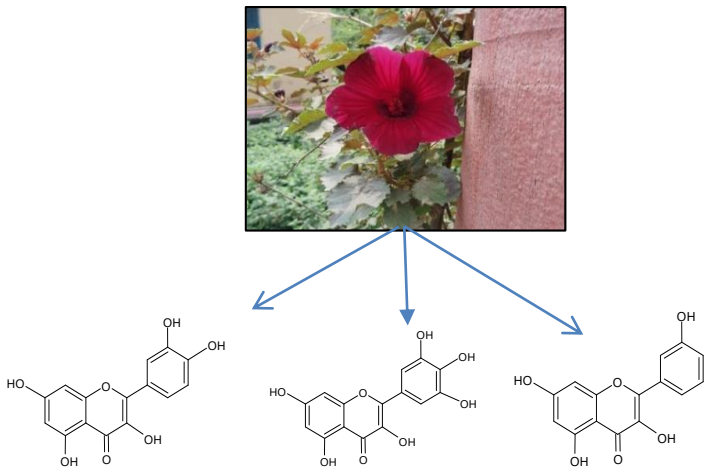


Quercetin Myricetin Kaempferol

Figure-1 A flower of *Hibiscus acetosella* and three major colour constituents

### Uses of *Hibiscus acetosella*

Plants of *Hibiscus acetosella* are leafy vegetables with leaves and young roots as their edible parts and can be used for various gastrointestinal problems as it contains anti-inflammatory properties due to the presence of flavonoids and phenolic acids. This species can also be used to lower down blood pressure and possesses antioxidant properties to reduce oxidative stress.

### Importance of *Hibiscus acetosella* as a natural dye bearing plant

Synthetic dyes are not at all environment friendly because they produce a number of toxic substances which are harmful to the environment both at production and disposal level. Since *Hibiscus acetosella*'s dye is a natural dye, it can be safely disposed and is easily biodegradable. In cultural practices it was used to dye clothes or to paint. Because *Hibiscus acetosella* natural dyes are non-toxic, they degrade quickly in the environment. They do not cause allergies or skin rashes and are safe for the skin. Colours obtained from *Hibiscus acetosella* come in number of varieties and textures. Different colour can be made based on the pH and mordant used. The antioxidant properties and bioactive compounds of alternative edible flowers of *Hibiscus acetosella*. The flower's extracts have phenolic composition and shows scavenging activity of free radicals. The flowers contain moisture content, carbohydrates, lipids, ashes, not detectable protein and have pH of 2.8. Because bioactive compounds have no cytotoxicity and have nutraceutical potential, they are relevant in healthy diet. Studies have indicated that flower extract of *Hibiscus acetosella* may be an important natural bioactive ingredient for usage in food supplements and beauty products. For the stability of bioactive compounds from *Hibiscus acetosella*, the low temperature during freeze-drying resulting in better retention of active constituents. Previously it has been reported that this species was responsible to the treatment of anaemia and has anti-anaemic properties. *Hibiscus acetosella* is a dye bearing plant with high colour potential to dyeing the fabrics including with or without mordanting methods and mordants.

## Material and Methods

### Material

- Flowers of *Hibiscus acetosella* were collected from college campus.
- Cotton fabrics for dyeing
- Lemon juice as natural mordant
- A pH meter

### Methods

Cotton fabric dyeing is done in five steps:

1. Dye extraction from flowers
2. Scouring
3. Without mordanting
4. Mordanting and Dyeing
5. Drying

First fresh flowers of *Hibiscus acetosella* were collected (Figure-2) and then petals are dried under the shade (Figure. 3). After drying, petals are crushed to get the powder form (Figure-4)

**Figure-2**

**Figure-3**

**Figure-4**

## Natural dye Extraction

**Aqueous extraction method-** 10gm of powdered form was soaked in 250ml distilled water and then kept it on a water bath and boiled it for 15 minutes. Then allowed it to cooled, finally filtered. The 200ml of the extracted material that was obtained after filtering is used for colouring. Divided the 200ml solution in four equal parts i.e., 50 ml each for Pre, Post, Simultaneous mordanting and without mordanting (Figure-5).


**Figure-5** Four equal parts of 200ml solution for pre, post and simultaneous mordanting with natural mordant and without mordantin

**Scouring of Cotton fabric**- Cotton fabric was boiled in water to remove the impurities and washed with cold distilled water (Figure-6)

**Figure-6** Clean cotton scouring fabric

## Mordanting

Mordanting is a process of adding mordants to fabric. Mordant acts as a binding agent for fixing dye to the fabric. There are three different methods of mordanting:

**(a) Pre – Mordanting:** In Pre – Mordanting first cloth is dipped in a mordant solution then mordant containing cloth is transferred in a dyeing solution.

**(b) Post – Mordanting:** In Post – Mordanting first cloth is dyed by dipping in a dyeing solution and then treated with mordant solution.

**(c) Simultaneous Mordanting:** In Simultaneous Mordanting mordant solution is added to dyeing solution.

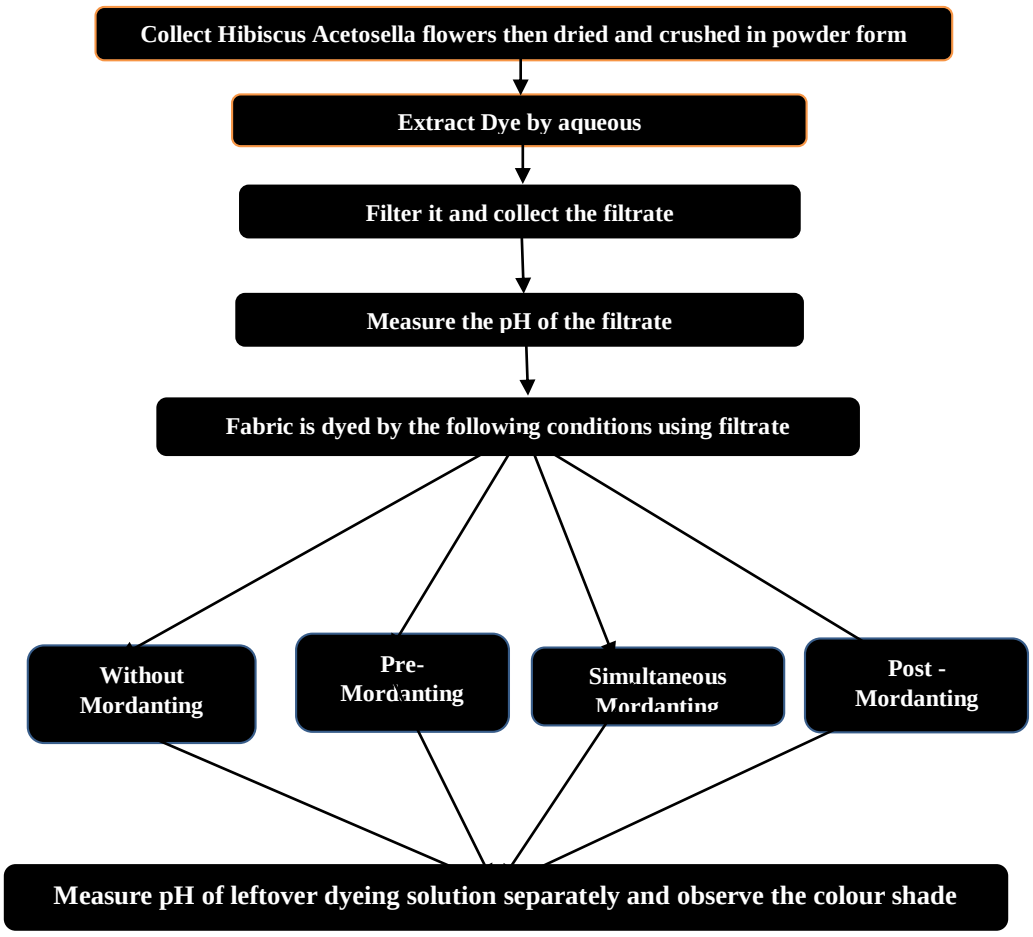


**Figure-7** A scheme for extraction, dyeing and mordanting

## Results and Discussion

The observed results are as follows:

- **Without Mordanting-** The Cotton Fabric is soaked in 50 ml dyeing solution and kept on boiling water bath for 5 minutes. Then cotton fabric is removed and pH is noted of leftover dyeing solution.
- **Mordanting with a natural mordant and Dyeing-** Mordant acts as binding agents i.e., used for fixing the dye on the fabric. 2ml Lemon juice was mixed with 10 ml water for Pre - Mordanting, and same mixture is prepared for other two methods also. This is a mordant solution.
  - **(a) Pre-Mordanting:** First clean cotton scouring fabric is soaked in a mordant solution and then transfer the mordant containing cotton fabric in another dyeing solution of 50 ml. Keep the dyeing solution on boiling water bath for 5 minutes. Then the pH was noted of leftover dyeing solution.

**(b) Post-Mordanting-** First clean cotton scouring fabric is soaked in a dyeing solution of another 50 ml and kept the dyeing solution containing fabric on boiling water bath for 5 minutes. After that, the cloth is taken out of the dyeing solution and dipped in a mordant solution and pH of the remaining dyeing solution was measured.

**(c) Simultaneous Mordanting:** Mordant was added to another 50 ml dyeing solution and then the solution is divided into two parts. The pH of one part is noted i.e., before the dyeing. The Cotton fabric is soaked in other part of solution and solution is kept on a boiling water bath for 5 minutes. Then fabric is removed from dyeing-mordant solution and pH of leftover dyeing-mordant solution is noted i.e., after dyeing.

**Figure: 8-** Leftover dyeing solution after dyeing in each case

- **Drying-** The dyed cotton fabric is dried at room temperature.
- Variations in pH with respect to before dyeing and after dyeing with few mordanting conditions:

**Table 1.** Variations in pH values w.r.t. before and after dyeing the fabrics

| S. No. | Conditions | pH before dyeing | pH after dyeing |
|---|---|---|---|
| 1 | Without mordant/After dye extraction | 4.65 | 4.90 |
| 2 | Pre-Mordanting | | 4.47 |
| 3 | Post-Mordanting | | 4.93 |
| 4 | Simultaneous Mordanting | 4.02 | 3.98 |

The different shades of color were obtained from Pre- mordanting, Post-mordanting, and Simultaneous mordanting or without any Mordant. The variations in color in each case are given below:

**Table 2.** Variations in colour shades with and without natural mordant under Pre-mordanting, Post- mordanting, and Simultaneous mordanting

| S. No. | Mordanting conditions | Dyed fabric without natural mordant | Dyed fabric with natural mordant | Colour Shades obtained |
|---|---|---|---|---|
| 1. | Pre-Mordanting |  (Dark purple in colour) |  | Bleached cedar |
| 2. | Post-Mordanting | |  | Wine berry |
| 3. | Simultaneous Mordanting | |  | Old Mauve |

## Conclusion

From the current study, it was concluded that the cotton fabric can be dyed with extract from *Hibiscus acetosella* flowers to achieve a variety of colour tones, either with or without the use of a natural mordant like lemon juice. pH variations are seen when various mordanting techniques, such as simultaneous, post-, and pre-mordanting, were used. When cotton cloth is dyed without the use of a mordant, the pH value varies as well. People are becoming more conscious of the negative consequences of synthetic colours and are increasingly worried about both the environment and their own health. This will incentivize the

next generation to preserve the environment and public health by using natural dyes rather than synthetic ones. As a result, more plants of this species will be planted in order to provide natural dyes that are good for both the environment and people.

## Acknowledgement

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## Disclaimer Statement

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

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# Fenugreek: Phytochemical study, nutritional value and uses

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**Abstract-** The literature on fenugreek's botanical characteristics, productivity, properties, and uses is reviewed in this work. This species originated from seeds and leaves. The agronomic and environmental elements have an impact on the significant production of fenugreek. This species' distinctive characteristics are also influenced by habitat conditions, agricultural techniques, and diversity. The crop fenugreek (Trigonella foenum graecum L.) is one of these species. Fenugreek is an eco-friendly plant that is a member of the legume family. The genotype, climate, environmental factors, cultivation methods, fertiliser use, and irrigation all affect plant yields. The seeds had lower levels of zinc, manganese, and copper, but they were higher in biogenic elements like phosphorus, sulphur, magnesium, and calcium. The biologically active compounds (protein, amino acids, biogenic elements, lipids, and fatty acids) found in fenugreek seeds and leaves are utilised in traditional medicine, as functional food items, and in the cosmetics industry. Fenugreek is used in traditional medicine to make muscle-building supplements, tinctures, meads, water and alcohol extracts, infusions, and tonics with antidepressant and psychotonic effects. Fenugreek is a component of premium dairy cattle feed that enhances the animals' overall health. This review paper's conclusions will be helpful to consumers who want to incorporate healthy biogenic components and fatty acids into their meals in order to improve their health.

**Key words:** Trigonella foenum-graecum, chemical compounds, benefits.

## Introduction

Fenugreek (Trigonella foenum-graecum L.) is a nutritious food that offers both health benefits and functional properties at an affordable cost. This plant is primarily cultivated in countries such as China, India, Turkey, Canada, Australia, as well as various regions in northern and southern Africa, and southern Europe[1]. The seeds of

fenugreek (Trigonella foenum graecum) are frequently utilized as a spice in Indian households. These seeds are said to possess nutritional benefits and to promote digestive activity[2].The seeds of fenugreek, a condiment that is frequently utilised in Indian homes, are an essential source of saponins and fibre (50%) particularly gel fibre (20%). These seeds were found to have hypoglycemic effects in a short-term study[3]. Greek hay is another name of fenugreek. Its seeds taste a little harsh and smell strongly. The M editerranean region, Western Asia, and Southern Europe are the native habitats of fenugreek[4]. Fenugreek has been utilized for the treatment of colic, flatulence, dysentery, diarrhea, dyspepsia accompanied by loss of appetite, chronic coughing, dropsy, enlargement of the liver and spleen, rickets, gout, and diabetes. Additionally, it is employed for its gastroprotective properties, antiurolithic effects, diuretic function, antidandruff capabilities, anti-inflammatory properties, and antioxidant effects[5]. Additionally, it has been noted that fenugreek seeds exhibit significant activity in scavenging free radicals[6]. beneficial source of Fenugreek are carbohydrates, dietary fiber, protein, and fats etc[7]. In Egypt and nations in the Middle East. the seeds are serve as a flavor enhancer[8]. It contain a high fiber content (48%)[9].

**Preparation of fenugreek seed extract with ethyl alcohol:**

The powdered seeds were finely ground. A total of 10 grams of the seed powder was measured, and 50 milliliters of ethyl alcohol was added to this amount. The mixture was stirred continuously for 30 minutes and then allowed to kept at room temperature for 24 hours before being filtered. The resulting filtered solution was further refined using Whatman filter paper number 3 and was subsequently stored at 4˚C for future use[10].

## Preparation of Solutions

a) **Fehling's solution:** firstly take a beaker then mix it with same volume of copper sulphate, sodium potassium tartarate and sodium hydroxide.
b) **Wagner's reagent:** 2 gm of iodide is taken and mix with 6 gm of potassium iodide in water of 100 ml and mix it properly.

## Tests

a) **Phytochemical screening:** ethyl alcohol is most preferable and mostly used as standard for identifying various residues of the extract in every phytochemical analysis.
b) **Procedure for alkaloids:** for identifying the presence of alkaloid in the extract, firstly we need to take 2ml of extract and in that Wagner's reagent

(2 ml) is added. A brownish color of precipitate formation was seen which indicates the presence of alkaloids.

c) **Cardiac glycosides:** for testing the presence of glycosides, 2ml of extract is firstly mixed with 2ml of chloroform after that carefully add concentrated sulphuric acid for forming a layer. Deep reddish brown colour at the interface of steroid ring shows the presence of cardiac glycosides.

d) **Flavonoids:** To know if flavonoid is present in the seeds, 2ml of extract is taken along with 2ml of 10% lead acetate. Yellowish green colour shows the presence of flavonoids.

e) **Saponins:** For saponin testing, 2ml of extract is mixed with Benedict's reagent (2 ml). Blue black precipitate shows the presence of saponins.

f) **Tanins:** for knowing the presence of tannins, firstly 2ml of extract is treated with 0.1% of Ferric chloride. Brownish green layers indicate the presence of tannins.

g) **Terpenoids: (salkowski test):** for identifying the presence of terpenoids, 2ml of extract is treated with 2ml of chloroform along with concentrated sulphuric acid to form a layer. A reddish brown colour is seen which shows the presence of terpenoids.

h) **Anthraquinones:** To check whether anthraquinones is present or not in fenugreek seed extract, 1ml of extract is firstly boiled with 10% HCL for few minutes in water bath. Then it is filtered and allowed to cool. Same volume of CHCL3 is added to the filtrate obtained and few drops of 10% Ammonia is added to the mixture and then it is heated. A rose pink colour is found that indicates the presence of anthraquinones.

i) **Reducing sugars**: The extract was first shaken with distilled water and then filtered. The filtrate is boiled with Fehling's solution A and B for some time, an orange red precipitates indicate the presence of reduced sugars.

j) **Glycosides:** for identifying this, extract is hydrolyzed with HCL solution and neutralized with NAOH solution. Few drops of Fehling's solution A and B are then added, Red color indicates the glycosides presence.

k) **Phlobatanins**: The test is for checking the presence of Phlobatanins, the extract is dissolved in distilled water and filtered. The filtrate is boiled with 2% HCL solution. Red precipitate shows the presence of phlobatanins.

## Results and Discussion

**Results of phytochemical analysis:** By this analysis we can conclude that fenugreek seeds consists of Tanins, anthraquinones, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, reducing sugars, phlobatanins, steroids, aminoacids, phenolic and proteins[11].

**Table-1** Phyto Chemical Analysis of *Trigonella foenumgracum* (Methi seeds**)**

| Sl. No | Phytochemicals | Distilled Water | Methanol | Acetone | Ethanol |
|---|---|---|---|---|---|
| 1 | Tanins | Positive | Positive | Positive | Positive |
| 2 | Anthraquinones | Negative | Positive | Positive | Positive |
| 3 | Flavanoides | Positive | Positive | Positive | Positive |
| 4 | Alkaloides | Positive | Positive | Positive | Positive |
| 5 | Terpenoids | Positive | Positive | Positive | Positive |
| 6 | Saponins | Positive | Positive | Positive | Positive |
| 7 | Cardiac glycosides | Positive | Positive | Positive | Positive |
| 8 | Glycosides | Positive | Negative | Positive | Positive |
| 9 | Reducing Sugars | Positive | Positive | Positive | Positive |
| 10 | Phlobatanins | Positive | Positive | Positive | Positive |
| 11 | Steroids | Positive | Positive | Positive | Positive |
| 12 | Phenolic | Positive | Positive | Positive | Positive |
| 13 | Aminoacids | Positive | Positive | Positive | Positive |
| 14 | Proteins | Positive | Positive | Positive | Positive |
| 15 | Quinones | Positive | Positive | Positive | Positive |

## Disclaimer Statement

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

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# *Datura stramonium* leaves: A Potential Element of Anti –Microbial Activity

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**Abstract-** Plant have benefited as an alternative medicine in treatment and prevention of diseases. Medicinal plants like *Datura stramonium* are assessed for phytochemical components and anti-microbial activity. Plants have important medicinal components like tropane, alkaloids, amino acids, tannins, carbohydrate. The components phyto-chemicals are used to cure different human diseases like skin disorder, ear pain, cough, fever, burns and asthma. In the present study the experiments were performed on phytochemicals and antimicrobial activity on leaves of *Datura stramonium* using various specific extracts like petroleum ether, chloroform, ethanol and water, crude extract to indicate the presence of flavonoids, terpenoids, glycosides, etc. Mostly antimicrobial activity was studied by Disc diffusion method. In antimicrobial activity, extracts were found active against pathogens like Bacillus azotoformans, Staphylococcus aureus, Bacillus pasteurii and Pseudomonas aeruginosa

**Keywords:** *Datura stramonium*, Phyto-constituents, Phytochemicals, antimicrobial activity

## Introduction

Plants have always played a major role in the treatment of human traumas and diseases worldwide. The demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural product. Herbal medicine is an important part of both traditional and modern system of medicines[1]. *Datura stramonium* is a widespread annual plant from the Solanaceae family. It is one of the widely well-known folklore medicinal herbs. It is a

wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH3) and include the anti-cholinergic drugs atropine, and scopolamine. From ancient civilization it was traditionally used for religious visionary purposes throughout the world and used by witchcraft in medieval Europe. The God lord Shiva was known to smoke Cannabis and Datura. People still provide the small thorn apple during festivals and special days as offerings in Shiva icons at temples. An extract made from the leaves is taken orally for the treatment of asthma and sinus infections, and stripped hark are applied externally to treat swellings, burns and ulcers. The incidence of *D. stramonium* poisoning is sporadic with a cluster of poisoning cases in the 1990s and 2000s, the United States media reported some cases occurring mostly among adolescents and young adults dying or becoming seriously ill from ingesting. Some medicinal uses of the plant are its anti-inflammatory property, stimulation of the central nervous system, respiratory decongestion and treatment of dental and skin infections, alopecia and in the treatment of toothache. It is a hallucinogenic plant that causes serious poisoning. Consumption of any part of the plant may result in a severe anti cholinergic reaction that may lead to toxicity and occasionally cause diagnostic difficulties. Cases of poisoning have been reported after eating the berries. Death may occur from heart failure after ingesting its seeds, because the seeds contain the highest concentration and has a rapid onset of action, thus may be potentially useful as an alternative to atropine for the treatment of the muscarinic symptoms of organo-plasphate toxicity and some of central anti cholinergic effects. The wide distribution, the strong toxicity and the potential for occurrence in foodstuffs are responsible for the numerous incidents in humans[2]. Datura genus distributes over tropical and warm temperate regions of the world. About ten species of Datura are found, of which Datura anoxia and *D. stramonium* are most important drug plants. Datura has long been known as a medicinal plant and as a plant hallucinogen all over the world. Pre-historic use of Datura in medicinal and ceremonial rituals could be observed in aboriginal in Indian sub-continent[3]. The therapeutic activities of most plants are due to the presence of one or more of such components like alkaloids, tannins, saponins and cardiac glycosides. The phytochemical screening revealed the presence of saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides[4]. Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants. All parts of the

plant are toxic, but the highest amount of alkaloids is contained in the ripe seeds[5]. Many cases of accidental poisoning by *D. stramonium* have been reported when these plants were eaten accidentally[6]. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide. The main objective of this research work is to analyze the various solvent extracts obtained from the leaves samples of *Datura stramonium* and to qualitatively screen them for phytochemicals using standard tests. Successful extraction, determination and isolation of biologically active components from plant material are largely dependent on the type of solvent[7] used.

## Material and Methods

### Collection

*Datura stramonium* plant samples were collected from Shamsergadh, Balawala, Dehradun, Uttarakhand, India.

### Authentication

Plant has been authenticated from Botanical Research Institute, Dehradun, India.

### Preparation of Plant Extracts

The fresh plant leaves samples were collected, washed individually under running tap water and dried in an oven at 50 °C for 3 days. The dried leaves material was ground into powder using an electrical blender. About 100 grams of dry powdered plant leaves material was taken and subjected to extraction by soxhlation method using various solvents like petroleum ether, chloroform, ethanol and water. Extracts were then concentrated by air distillation and the concentrated residual extracts were stored at 4 °C in a dry airtight container until further use[8].

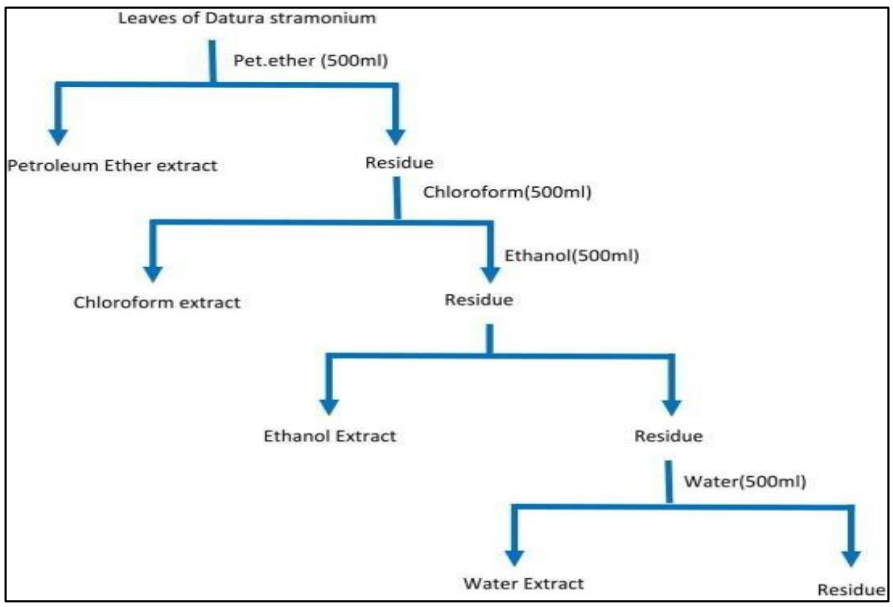


**Figure- 1 Extraction Flow Chart**

**Figure- 2 Soxhlet apparatus**

**Figure-3 Air distillation assembly**

## Phytochemical Screening[9-18]

Crude plant leaves extracts were subjected to preliminary screening for the presence of active secondary metabolites. Each plant extract was tested individually with specific chemical reagents according to standard procedures. Visible color change or precipitate formation was taken into consideration for the presence (+) or absence (–) of particular active constituents.

**The following tests were carried out to identify the various phytochemical constituents:**

The petroleum ether, chloroform extract, ethanol extract and water extract were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as alkaloids, steroids, terpenoids, flavonoids, carbohydrates proteins, and amino acids.

### Determination of Saponins

Each of the plant leaves extracts (0.4g) was separately stirred in a test tube, foaming which persisted on warming was taken as an evidence for the presence of saponins.

### Determination of Tannins

Extract of each sample (0.4g) was separately stirred with 10ml of distilled water and then filtered. To the filtrate was added two drops of 4% Iron (III) Chloride ($FeCl_3$) reagent. Blue – black or blue – green coloration or precipitate was taken as an indication of the presence of tannins.

### Determination of alkaloids

Each sample (0.4g) was separately stirred with 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drop of Mayer's reagent. The two solutions were mixed and made up to 100ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extracts.

### Determination of glycosides

Coarsely powered plant leaf material (1g) was introduced into two different beakers. To one of the beakers was added sulphuric acid (4ml) while water (4ml) was added to the other beaker. The two beakers were heated for 3 minutes and the contents filtered into labelled test tubes. The filtrate was made alkaline with sodium hydroxide (0.4ml) and allowed to stand for three minutes. The presence of reddish brown precipitate in the filtrate was taken as positive for glycosides.

### Determination of flavonoids

To the extract of each piece of test plant leaf extract was added a small piece of magnesium ribbon, this was followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones.

### Carbohydrates

To 1 ml of the filtrate, 4 ml of Benedict's reagent were added. The mixture was heated. Appearance of red precipitate indicated the presence of reducing sugar.

### Amino acids

Added 4 drops of millon's reagent to I ml of test solution and heated on a water bath for 10 min, cooled and added 1% sodium nitrite solution. Appearance of red colour confirmed the test.

### Proteins

To 2 ml of the test solution added 4 drops of 1% copper sulphate solution and 2 ml of 10% NaOH. Mixed thoroughly. Formation

of purple or violet colour confirmed proteins.

**Phytosterols**

Extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid as added. A brown ring formation at the junction and the turning of the upper layer to dark green colour confirmed the test for the presence of phytosterols.

**Triterpenoids**

Approximately 2 mg of dry extract was taken in a test tube and shaken with 1 ml of chloroform and a few drops of concentrated sulphuric acid were added. A red brown color formed at the interface indicated the test as positive for triterpenoids.

## Antibacterial Study of different extracts of *Datura stramonium* leaf

The following bacterial cultures were used for antimicrobial activity.

a) *Bacillus azotoformans*
b) *Bacillus pasteurii*
c) *Pseudomonas aeruginosa*
d) *Staphylococcus aureus*

The cultures were obtained from the standard cultures maintained in the microbiology department of Sardar Bhagwan Singh University. These cultures were maintained on nutrient agar slants at first being incubated at 37° C for about 18-24 hrs and thenstored at 4 °C as stock cultures for further antibacterial activity, Fresh cultures were obtained by transferring loop-full cultures into nutrient broth and then incubated at 37°C, over night. To test antibacterial activity, the disc diffusion method was used. After confirming the identity of the isolated bacteria by biochemical tests, tested for susceptibility resistant to number of extracts by disc diffusion method using Muller Hinton Agar assay medium. The organisms were inoculated into Saline water. Broth culture was then spread on Petri plate on Muller Hinton Agar and after 10-14 min discs of different extract to be tested were placed on the surface of seeded Petri plates. Plates were observed for zone inhibition. After 24 hrs of theincubation at 37°C, Zones of inhibition were measured in terms of Inhibition Zone Diameter Scale in mm.

**Preparation of culture media:** The medium used for anti-bacterial was nutrient agar that was prepared and sterilizedat 121 °C and 14 lbs for 14-30 min.

**Nutrient Agar Media**

| Ingredients | Composition (g/ml) |
|---|---|
| Beef Extract | 3.0 gm |
| Peptone | 4.0 gm |
| Sodium Chloride (NaCl)) | 4.0 gm |
| Distilled Water | 1000ml |
| Agar | 18 gm |
| pH | 7.2± 0.229 |

**Figure 3.1 Nutrient Agar Media**

**Figure 3.2 Different slants of Nutrient Agar Media**

**Nutrient Broth**

| Ingredients | Composition (g/ml) |
|---|---|
| Beef Extract | 3.0 gm |
| Peptone | 4.0 gm |
| Sodium Chloride (NaCl) | 4.0 gm |
| Distilled Water | 1000ml |
| pH | 7.2 ± 0.2 |

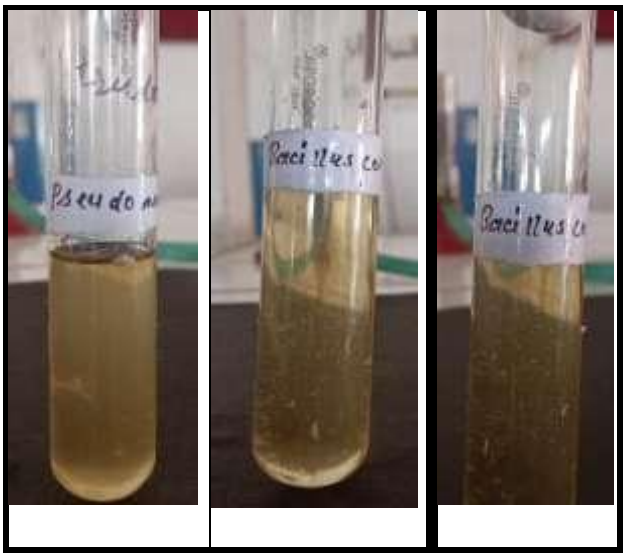

**Figure 3.3 Nutrient Broth**

## Plate preparation

20-24 ml of autoclaved nutrient agar medium was poured into 90 mm diameter pre sterilized petriplates under aseptic conditions and was allowed to solidify in the presence of UV light. Then the prepared plates are pre incubated for 24 hr.

## Preparation of dilution

Four different dilution of all the four extract were being prepared of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml these dilution had been used for anti-microbial activity along with the standard antibiotic (ciprofloxacin) 30mg/ml solubilised in distilled water.

**Figure 3.4 Dilution of petroleum ether extract**

**Figure 3.5 Dilution of chloroform extract**


**Figure 3.6 Dilution of water extract**


**Figure 3.7 Dilution of ethanol extract**


**Figure 3.8 Dilution of standard (ciprofloxacin) in water**

## Determination of antimicrobial activity

Determination of antimicrobial activity was done according to the standards recommended by CLSI (Clinical and Laboratory Standards Institute), namely at first Agar well diffusion method. The pre incubated plates were taken and bacteria were spreaded over those plates with the

help of a spreader. After the bacteria had dried the well were bored into agar plates with help of metallic hollow road. Then 100µl of extracts and 30µl of standard antibiotic (ciprofloxacine) were dropped onto discs understerile conditions and were incubated at +3710.1°C for 24 hr. After incubation, the diameters of inhibition zones were measured in millimeters on all plates. All experiments were repeated three times.

## Measurements of zone of inhibition

After incubation of the plates, the antibacterial spectrum of the extracts was determined in the terms of zones of inhibition around the wells. The diameters of zone of inhibition produced by the plant extract were compared with those produced by antibiotics. The experiments were performed and average zone diameter was recorded.

The results of cache plate were observed and recorded after 24-26 hrs of incubation at 37˚C by measuring zone diameter (mm), caused by *Datura stramonium* leaves extracts of different solvents.

## Result and Discussion

### Phytochemical screening[19, 20]

The phytochemical screening test was conducted using four different solvents such as petroleum ether, chloroform, ethanol and water, crude extract of *D. stramonium* leaves were summarized in Table-1. The results obtained from this study pointed that the presences of tannins, alkaloids, triterpenodis, flavonoids, saponins, carbohydrates, proteins, amino acids and phytosterols in the plant extract. According to the previous study, a qualitative phytochemical screening test of water and ethanol extract of *D. stramonium* extract also showed the presence of different class of chemical constituents such as tannins, alkaloids, triterpenodis, flavonoids, saponins, carbohydrates, proteins, amino acids and phytosterols.

**Table-1 Phytochemical screenings of crude extracts of *D. stramonium* leaves**

| Solvents | Petroleum ether | Chloroform | Ethanol | Water |
|---|---|---|---|---|
| Tannins | - | + | ++ | -- |
| Alkaloids | - | + | ++++ | ++++ |
| Triterpenodis | - | + | + | - |
| Flavonoids | + | + | + | - |
| Saponins | + | + | + | + |
| Carbohydrates | + | + | + | + |
| Proteins | + | + | + | - |
| Amino acids | - | - | - | - |
| Phytosterols | + | + | + | - |

+ = the presence and - = the absence of chemical constituents


Figure 3.9


Figure 3.10


Figure 3.11


Figure 3.12


Figure 3.13


Figure 3.14


Figure 3.15


Figure 3.16


Figure 3.17


Figure 3.18


Figure 3.19


Figure 3.20


Figure 3.21


Figure 3.22


Figure 3.23


Figure 3.24


Figure 3.25


Figure 3.26

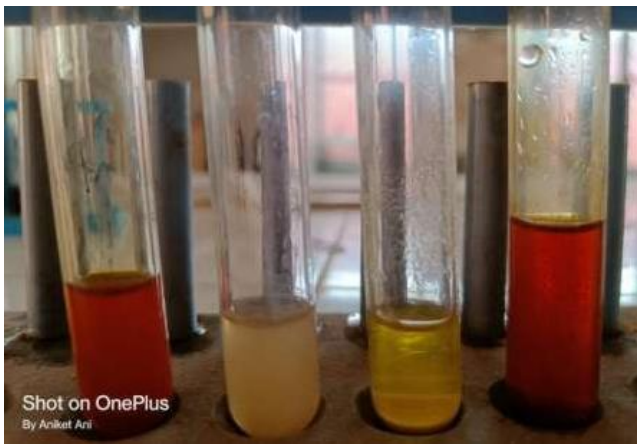

Figure 3.27

**Figure- Results of phytochemical test performed**

## Antibacterial

The antibacterial activity of crude extracts of *D. stramonium* was tested by disc diffusion method. The extract of plant leaves has been found to be potent against Pseudomonas, B. cereus and S. aureus. The antibacterial activity of *D. stramonium* leaves extract of different solvents are summarised in Table 2. Chloroforme extract shown the maximum inhibition zone against S.aureus (9.4mm) in 200mg/ml concentration and minimum against B. azotoformans (4.4mm) in 50mg/ml and Ethanol extract shown the maximum inhibition zone against Pseudomonas (10.4mm) in 200mg/ml concentration and minimum against B. azotoformans (4mm) in 50mg/ml. Petroleum ether and water extract has not shown any anti-microbial activity in all the four concentration against all the four bacteria[21, 22].

**Table- 2 Antibacterial activities of *D. stramonium* leave crude extracts**

| Bacteria | Concentration | Petroleumether | Chloroform | Ethanol | Water |
|---|---|---|---|---|---|
| **Pseudomonas aeruginosa** | 50mg | - | 7.4mm | 4.4 mm | - |
| | 100mg | - | 7mm | 4.4 mm | - |
| | 150mg | - | 7mm | 4 mm | - |
| | 200mg | - | 7.4 mm | 10.4 mm | - |
| | +ve | 20 mm | 21 mm | 19 mm | 18 mm |
| **Staphylococcus aureus** | 50mg | - | 7 mm | 4.4 mm | - |
| | 100mg | - | 8 mm | 6 mm | - |
| | 150mg | - | 8.4 mm | 7.4 mm | - |
| | 200mg | - | 9.4 mm | 8 mm | - |
| | +ve | 22 mm | 20 mm | 21 mm | 19 mm |
| **Bacillus azotoformans** | 50mg | - | 4.4mm | 4 mm | - |
| | 100mg | - | 6mm | 6 mm | - |
| | 150mg | - | 6mm | 6.4 mm | - |
| | 200mg | - | 7.4mm | 8.4 mm | - |
| | +ve | 22.4 mm | 20 mm | 19 mm | 20.4 mm |
| **Bacilluspasteurii** | 50mg | - | 7mm | 9 mm | - |
| | 100mg | - | 7.4mm | 9.24 mm | - |
| | 150mg | - | 7.4mm | 9.4 mm | - |
| | 200mg | - | 8.4mm | 10 mm | - |
| | +ve | 20 mm | 20.4 mm | 22 mm | 21 mm |

+ve = Cprofloxcin, - = no result

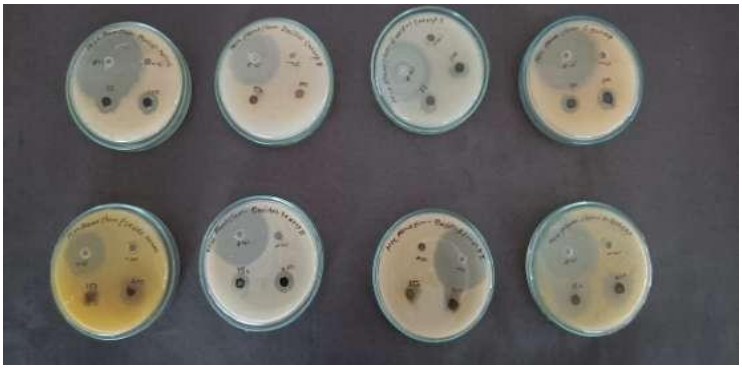

**Figure 3.28 Inhibition of zones of chloroform extract on different bacteria.**

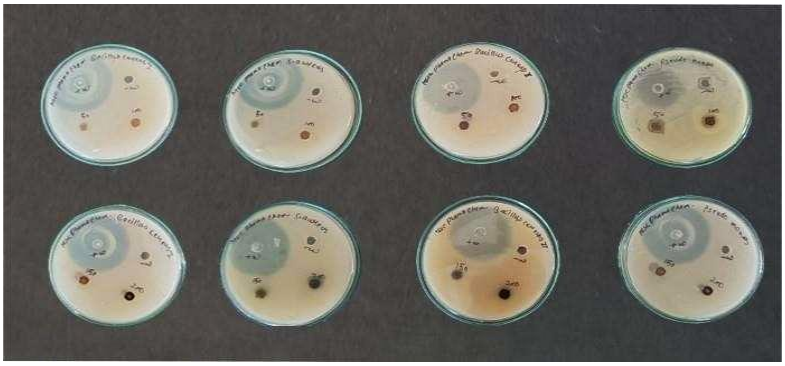

**Figure 3.29 Inhibition of zones of petroleum ether extract on different bacteria.**

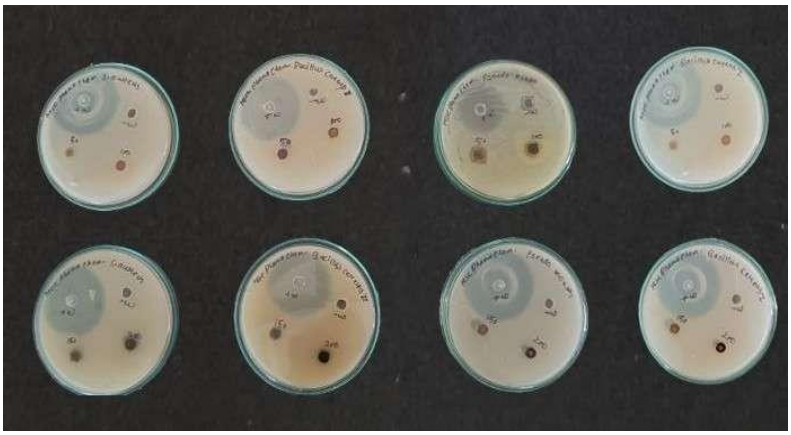

**Figure 3.30 Inhibition of zones of ethanol extract on different bacteria.**

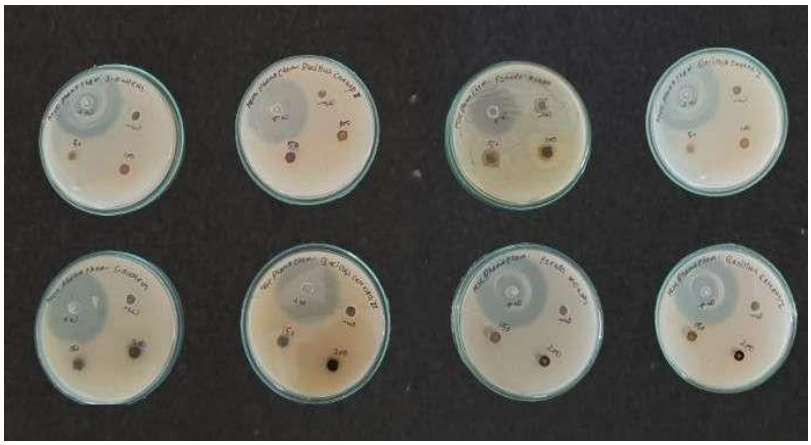

**Figure 3.31 Inhibition of zones of water extract on different bacteria.**

## Conclusion

From the above study, it can be concluded that the leaf extract of *D. stramonium* have phytochemicals that could contribute in antibacterial activities. Possible antibacterial substance in extract of different solvent includes as tannins, alkaloids, triterpenodis, flavonoids, saponins, carbohydrates, proteins, amino acids and phytosterols. The antibacterial characteristics of the plant can be further tested to use in treatment of bacterial infection. The crude extract of *D. stramonium* can be used against some selective microorganisms. Crude extract of

*D. stramonium* can be better alternative to the conventional antibacterial additives in food industry. Also traditionally the anti-bacterial potency of crude extract of *D. stramonium* leaves has been justified.

## Disclaimer Statement

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

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# Histopathological effect of carica papaya seeds on reproductive organs of female

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**Abstract**-Carica Papaya (Papita) is a fruit eating plant but unripe fruit and seeds are said to impair the reproductive function of male and female animals including human beings,. To assess the above activity the dried seed powder of this plant was administered orally as aqueous suspension at doses of 10, 20 and 30 mg /kg / rat to adult female albino rats for 30 days. All the rats were killed, weighed and their reproductive organs- ovary and uterus were taken out. These organs were processed for pathological examination. The maximum dose (30mg/kg) caused a significant reduction in genital organs weight. There was no effect on body weight. Cellular organization of ovary and uterus was affected leading to total degeneration. Atrophic changes were found in both ovary and uterus in treated female rats. It appears that the seed powder of Carica papaya have contraceptive properties.

**Keywords:** Carica Papaya, Phytotherapy, Herbal drugs, Reproductive organs, Contraception.

## Introduction

**Carica Papaya (Papita)** is a popular edible fruit plant. Various parts of this plant has been used as therapeutic agents in Ayurvedic medicine. It is said that consumption of raw (unripe) fruit with latex (milky juice) causes abortion in pregnant women. Recently, various parts of this plant such as latex of green fruit (Rosen and Milman, 1955), unripe fruit pulp (Garg and Garg, 1970, 1971) oil from unripe fruit pulp (Garg, 1974) and seeds (Sareen et.al., 1961, Bodhankar et al, 1974) had reported for control of fertility of female rats. Dar et al (1965) reported ant helmintic property in seeds. Das (1980) reported anti spermatogenic effect of seeds in male albino rats. Singh and Singh (1992) studied

anti-implantation effect of seeds in female albino rats. Singh (2008) also reported anti spermatogenic effect of seeds in male House sparrow (Passer domesticus Linn). From the above studies, it appears that seeds of Carica papaya has strong antifertility activity. No research work on its histopathological effects on female reproductive organs has been reported therefore, present study was under taken and the results are presented in this paper.

## Phytochemistry

Active chemical substance from seeds of Carica papaya was isolated and named as carpasemine. Its melting point is $165^0$C and molecular formula is $C_8H_{10}N_2S$. Chemical properties of this compound together with its degradetion products have been studied and some new derivatives have been prepared from it. A carpasemine have been identified as Benzlythiourea or Banzylthiocarbamide by mixed melting point with the synthetically prepared Benzylthiourea. This research was performed by Panse and Paranjape, 1943). Nineteen different carotenoids were identified in the fruit and a major being cryptoxanthene. Oxycarotenoids were higher in preportion as compared to carboxhydrocarbons. The percentage of cryptoflavin and Beta Carotene were 13 and 29.5 respectively (Subbarayan and Cama, 1964).

The mass spectrum of Pseudocarpain isolated from plant showed that it was dimeric. The fragmentation pattern observed for pseudocarpain was identical with that of carpaine but for minor difference in the relative intensities of the ions. The two alkaloids had the same gross structure. Pseudocarpain on acid hydrolyses yielded carpamic acid and pseudocarpamic acids (Govindachari et al, 1965).

## Material and Method

Adult, healthy with regular cyclicity, 20 in number weighing between 100 to 125 gms female albino rats (Rattus rattus norvegicus) were selected from our laboratory maintained animal house for present experimental study. Four groups of female rats, each group with five rats were made 1st group served as control and 2nd, 3rd and 4th group served as experimental or treated group with seed powder doses. The dried seed powder of Carica papaya was dissolved in distilled water (w/v) in such a way that 10mg powder corresponded to 01ml of water. Thus, 10mg/kg/ rat administered orally to 2nd group of rat with the help of soft catheter tube fitted into a syringe. Similarly, other doses 20 and 30 mg/kg/ rat were prepared and administered to 3rd and 4th group of female rats. The gum acacia powder was added at the rate of .01mg/dose in all the doses. The 01mg/kg/rat gum acacia powder dissolved

in distilled water was given at the rate of 01ml/rate as vehicle to the control female rats in the similar manner as given to powder treated group of rats, The doses were given daily for 30 days to all the rats, control and experimental rats. Before the start of experiment, the weight of each rat was recorded. The rats were maintained under uniform husbandry condition provided with proper feed (Hindustan Levers Ltd.) and drinking water.

On day 31st, the rats of all groups were weighed and sacrificed or killed with chloroform. The ovary and uterus from each rat were disscted out, freed form surrounded tissues, blotted on filter paper and weighed quickly on a semi micro balance for histological studies. The ovary and uterus of all rats, control and powder treated were fixed in Bouin.s fluid over night, washed in distilled water, dehydrated in xylene. Now the organs were transferred to the molted paraffin wax and embedded in paraffin blocks, Thus, the blocks were made. The thin sections of ovary and uterus blocks were cut at 6 micron using Rotary Microtom. Slides of ovary and uterus were stained with haemotoxylene and eosine dyes. Thus slides of orary and uterus prepared and studied for histopathological changes and photographed. The histological changes were described. The data were statistically analyzed by student 't' test. $P<0.05$ was considered as significent in comparison to control group of female rats. All the experiments were conducted and supervised as per guidelines of institutional, Animal Ethical Committee, appointed by the than Principal of College.

## Observations

### Effect on Body and Reproductive organ weight

Table 1 depicts the changes in body and ovarian and uterine weight. The control group of rats did not reflect any change in body weight and both the reproductive organ weight. It was maintained through out experimental period. No depletion in body weight was seen at any dose level of Carica papaya seed powder treatment the weight of ovary and uterus was decreased at the doses of 20 and 30mg/kg/rat. The significant reduction in weight was noticed at 30mg/kg/dose in both the reproductive organs.

**Table-1 Effect of Carica papaya seed powder on body (gms) and reproductive organs weight (Mgms) of female rats treated with 20 and 30 mg/kg doses for 30 days .05 rats were included in each group. Values are mean ± standard error**

| Doses (mg) | Body weight (gm) | | Reproductive organ weight (Mgms) | |
|---|---|---|---|---|
| | Initial | Final | Ovary | Uterus |
| Central | 100 ± 3.50 | 132.5 ± 3.91 | 50 ± 3.64 | 80 ± 5.65 |
| 10 mg | 110 ± 2.42 | 130 ± 2.66 | 45 ± 5.20 | 70 ± 3.50 |
| 20mg | 118 ± 3.35 | 126 ± 4.25 | 18.8 ± 1.10* | 30 ± 3.7* |
| 30mg | 125 ± 1.29 | 120 ± 2.50 | 16.2 ± 1.20* | 24 ± 1.60* |

*Significant p values < 0.05

## Effect on histology of reproductive organs

### Effect on ovary

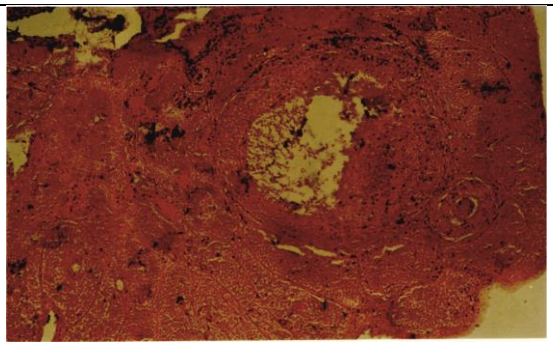
The histology of ovary of control female rats did not show any change in structural organization. The normal cellular structures revealed well developed corporalutea, developing follicles (Primary and secondary and mature orgravid follicles) with defined ovum (egg). The germinal epithelium and vascularity appeared normal in loose stroma **(figure-1)**. The dose 10mg/kg for 30 days of regular feedings of Carica papaya seed powder caused no atrophic changes in folliecles of ovary. The vascularity appeared normal in loose stroma. No change in structure of developing and mature follicles.

The doses 20 and 30mg /kg adminsitration for 30 days caused serious atrophic (degenerative) changes in the ovary. Maximum untoward (degenerative) effect was noted at 30mg/kg dose. It caused mass atrophy of developing and mature folliecles. Nuclear degeneration noted in all ova (Eggs) of mature follicles. No corpora lutea were observed. They also showed atrophic changes. Similarly, very less number of developing (Primary follicles) were seen towards germinal epithelium. Vascularity affected as no blood vessels observed in stroma **(figure-2)**

### Effect on Uterus

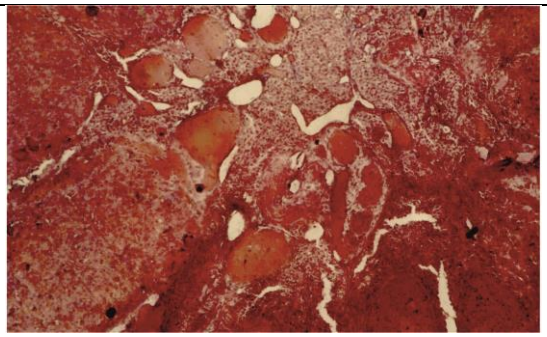
The uterus of control group of rats shows normal histo architectural features. The endometrium made up of columner epithelial cells. They shows mitotic nuclei and leucocytic infiltration. The uterine cavity or lumen was wide. The uterine glands were tortuous and irregular with distended lumen. The musculature appeared well developed. The stroma and vascularity seen quite normal **(figure-3)**

The dose 10mg/kg for 30 days of administration caused no changes in histology of uterine cellulor structured. No untoward effect was noted in endometrium, myometrium and luminal epithelium. The uterine cavity or lumen was fully distnded. The uterine glands were normal and tortuous. Musculature and vascularity appeared normal. It was comparable to uterus of centrol female rats. The dose 20 and 30mg/kg for 30 days appeared very effective to cause deleterious changes in uterine histoarchitecture. The 30mg/kg dose was found to maximally effective dose. This dose caused shrinkage and atrophy in uterine glands, reduction in uterine cavity (lumen) and height of endometrium. Musculature and vascularity were reduced **(figure-4)**



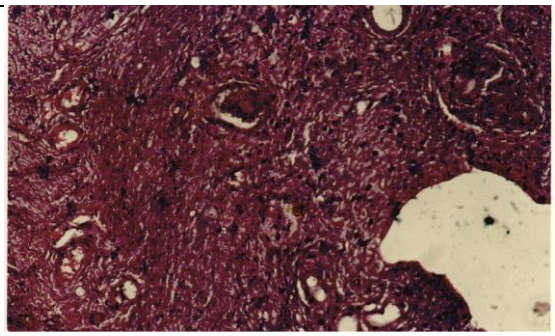
**(Figure-1)**

T.S. of ovary of female rat of control group (Vehicle treated) for 30 days shows normal celluler structure with organized germinal epithelium, all types of follicles (Primary, developing to mature follicles) and corpora lutea stroma and vascularity also appear normal x 150



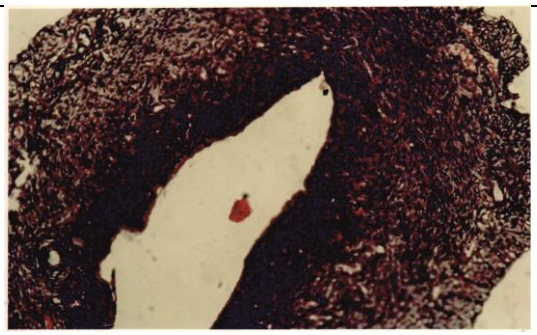
**(Figure-2)**

T.S. of ovary of female rat of treated group with seed powder of Carica papaya at doses of 20 and 30 mg/kg/day for 30 days shows small maturing follicles with degenerated ovum. Many atretic follicles and less corpora lutea. Poor vascularity and compact stroma x 150



**(Figure-3)**

T.S. of uterus of female rat of control group (Vehicle treated) for 30 days shows normal histological structure with organized myometrium and endometrium. well developed uterine glands (Tortuous) in stroma. wide uterine lumen (Cavity) with epithelium and vascularity x 150.



**(Figure-4)**

T.S. of uterus of female rat of treated group with seed powder of Carica papaya at doses of 20 and 30mg /kg/day for 30 days shows atrophic changes in uterine histoarchetectures, shrinkage of musculature (Myometrium) with less developed uterine glands. Poorly developed endometrium and narrowed uterine lumen (cavity). Reduced vascularity and epithelial cell height x 150

## Discussion

In the present study no reduction in body weight was noted after 30 days of administration of three doses of Carica papaya seed powder as a drug. The ovarian and uterine weight was reduced significantly with increasing doses i.e. 20 and 30mg / kg/rat for 30 days ($P<0.05$). The present study also revealed histopathological changes in female reproductive organs i.e. ovary and uterus. The response of a drug depends upon the dose and duration of treatment. The changes in female reproductive organs weight is controlled by ovarian hormones – Estrogen and Progesteron. Formation of follicles with normal ova (Egg), ovulation, fertilization, implantation and maintenance of estrus cycle / menstrual cycle are also controlled by these hormones as described above through Pituitary gland (Lerner,

1969). The petroleum ether extract of pulp of Carica Papaya showed significant antifertility activity in female rat (Garg and Garg, 1971). Latex of Green fruit have been reported to possess Oxytocic activity. The seeds decreased fertility of albino female mice (Sareen et, al 1961). Various compounds isolated from seeds of this plant, only Benzylthiourea was found most potent and found to be responsible for anthelmintic and antifertility activity. It did not cause toxic symptoms even at doses of 30mg/kg in rats (Dar, et. al., 1965)

The results noted in the present study on histopatholigical changes in ovary and uterus due to oral administration of Carica papaya seed powder as aquous solution at doses of 20 and 30mg/kg/day for 30 days are comparable of the study done by chakraborti et.al., 1968) in which the female rats were fed with green leaves of Aristolochia odoratissimus. Follicular atresia and other degenerative changes were observed in this study. Similar results were reported by Kholkute and Udupa (1974) following the administration of flowers of Hibiscus rosa sinensis Linn. Both ponderal (organ weight) and histological changes in ovary and uterus respectively were reported (Prakash, 1979) through administration of Embelia ribes seeds in female albino rats. Dixit (1977) reported effect of chronically administered Malva Viscus conzattii flower extract in female genital tract of female Indian gerbel (Meriones-hurriani, Jerdon) which is a rodent like albino rat. The dose 25mg/kg was given for 20 days. it caused degenerative changes in ovary. Corpora lutea were also affected in this study. Carica papaya seed also caused similar effects in ovary and uterus of female albino rats as described above. Chinoy et al (1995) revealed that when an aqueous extract of Carica papaya seeds were administered in female albino rats, it was found to cause contraction of rats uterus stripes. Singh et al (2000) reported degenerative effects in female genital organs of albino rats with the administration of seeds of Randia dumetorum. Singh (1917) also reported anti fertility and histapathological changes in female reproductive organs with the administration of leaves powder of stevia rebaudiana.

From the present investigation, it may be concluded that the Carica papaya seeds has potentiality to impair fertility of female mammals by cauing histo pathological changes in female reproductive organs and can be used in family planning programme

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## Disclaimer Statement

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

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# Antimicrobial Activities of Seed Extracts of Mango (*Mangifera indica L.);* **Jamun** *(Syzygium cumini)*, **or black plum; Karela** **(***Momordica charantia***)*or bitter gourd*** **and Neem** *(Azadirachta indica)* **or Margosa**

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**Abstract-** The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, micro-biologists, and natural-products chemists are searching for new phytochemical entities which could be developed for treatment of infectious diseases. Traditional healers have long used plants to prevent or cure infectious conditions.

The present study aimed to investigate the *in vitro* antimicrobial activities of methanol and ethanol extracts of mango, Jamun, Neem and Karela seeds against gram positive, gram negative bacteria and *Candida albicans*. Seed were extracted by Soxhlet using methanol and ethanol as solvents. The extracts were tested against the microorganisms using disc diffusion method at different concentrations: 5 mg/mL, 4 mg/mL, 3 mg/mL, 2 mg/mL, 1 mg/mL and 0.5 mg/mL). *In vitro* antibacterial activities of methanol and ethanol extracts of all seeds showed inhibitions to tested organisms with variable inhibition zones. Resistance among the tested strains was shown in low concentration of the extract. The mean zone of inhibition produced ranged between 8 mm and 18 mm. *Staph aureus* showed the highest zone of inhibition (18 mm) followed by *Candida albicans* (16 mm). The methanol followed by ethanol extracts of Jamun seeds showed good inhibitory effects against almost all tested strains. The inhibition zones produced by seeds extract were less than those produced by standard positive control drug. This could be due to low diffusion rate of seeds extract in agar medium. These plant products can be a

potential new and promising antimicrobial therapy in infectious diseases.

**Keywords:** Antimicrobial Activities, Mango (*Mangifera indica* L.), Jamun *(Syzygium cumini)*, Karela (*Momordica charantia)*, Neem *(Azadirachta indica)*

## Introduction

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties.

Due to report of increasing developments of drug resistance in human pathogen as well as undesirable side effects of certain antimicrobial agents, it is necessary to search for new agents that are better, cheaper and without side effect for treating infectious diseases especially in developing countries. A wide variety of plant/natural products are used in the treatment of infections.

Phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests[1]. There have been several reports on the pharmacological effects and suitability of medicinal plants as phytotherapies for diseases. Seeds extracts has been reported to possess anti-inflammatory, analgesic and immunoprotective effects[2,3].

Methanol and ethanol extract of different plant seeds was done against 4 bacterial strains. The presence of phytoconstituents in the seeds extracts may be responsible for the antibacterial activity of the plant[1]. No research has been carried out on antimicrobial activity of seeds extract of mango, jamun, karela and Neem In this study, the in vitro antimicrobial activity of methanol and ethanol extracts of the seeds of mango, jamun, karela and Neem was investigated.

## Material and Methods

### Plant seeds Collection and Identification

Fresh seeds of all plants were collected from the HWC Dehradun unit Uttarakhand, India and were identified and authenticated in the Department of Pharmacognosy. The collected seeds were kept at plastic bags at room temperature till use.

### Extraction of Seeds

1000 grams of air dried and coarsely powdered of clean seeds were extracted in Soxhlet apparatus to obtain methanolic and ethanolic extracts. The extracts were filtered, and the filtrates were vaporized to dryness, and weighed in order to determine the % yield of the extracts, following the formula: % yield = (weight of extract/weight of ground plant material) × 100. The stock solutions of the crude methanolic and ethanolic extracts were prepared by dilution the dried extracts with 50% methanol and 50% ethanol to obtain the desired final concentrations of: 5 mg/mL, 4 mg/mL, 3mg/mL, 2 mg/mL, 1mg/mL and 0.5 mg/mL. These concentrations were used to impregnate filter paper disks (5.5 mm diameters). Disk impregnated into 50% methanol and 50% ethanol was used as control, while standard antimicrobial discs of ciprofloxacin was used as positive control.

### Preparation of Extracts

The extraction was carried out using methanol and ethanol (separately). 400 gm each of dry seeds were extracted with 80% methanol till the color of the solvent returned colorless.

Solvent was evaporated under reduced pressure using Rotary evaporator apparatus (BUCHI Rotavapor R-200/ 20). Extracts were finally allowed to dry at air at room temperature till complete dryness. Extraction using ethanol followed the above procedures.

### Assay for Antimicrobial Activity

The original extracts were subject to serial dilution was made as follows: 5 mg/mL, 4 mg/mL, 3mg/mL, 2 mg/mL, 1mg/mL and 0.5 mg/mL. Filter discs (5.5/mm) were made and impregnated into each of the above dilutions. The discs were dried at 37˚C for one hour. The dried discs were therefore having the following concentration: 5 mg/mL, 4 mg/mL, 3mg/mL, 2 mg/mL, 1mg/mL and 0.5 mg/mL**.**

### Bacterial and Fungal Strains

Bacterial strains and *Candida albicans* recovered from frozen stocks at Department of Microbiology HWC Dehradun unit. One to three loopful of 24 h old cultures from each test strains were used to prepare 0.5 McFarland standard suspensions. Mueller Hinton's agar (Hi Media) plates were used the in vitro antimicrobial testing as recommended by clinical and Laboratory Standards Institute[13]. Then impregnated dried discs plus positive and negative control discs.

## Results and Discussion

### Antimicrobial Activity

The antimicrobial activity of all seeds extract against the test organism is shown in Table 1-4. The jamun seeds extract showed potent antibacterial and

anticandidal activity against all strains tested[4]. *Staphylococcus aureus* was the most susceptible strain amongst test bacteria followed by *Candida albicans* showed the diameter of zones of inhibition 18mm and 16 mm respectively (Table-2). After gram positive *Staph aureus E.coli* was the most susceptible test strain showed the zone size of 16 mm. Methanol was most strong solvent than ethanol. It is revealed from this study that *Syzygium cumini seed extract* possess remarkable antibacterial and anticandidal activity.

**Table-1 Antimicrobial activity of Seeds extracts of *Mangifera indica***

| Test organisms | ATCC No. | Extract concentrations | | | | | | - ive control | + tive control |
|---|---|---|---|---|---|---|---|---|---|
| | | 5mg/ml | 4 mg/ml | 3mg/ml | 2 mg/ml | 1mg/ml | 0.5mg/ml | | |
| | | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | | |
| *Staph aureus* | | 14/12 | 13/11 | 12/10 | 10/8 | 8/R | R | R | 25 |
| *E.coli* | | 12/10 | 11/10 | 10/8 | 8/R | 8/R | R | R | 23 |
| *Pseudomonas aeruginosa* | | 13/12 | 12/10 | 10/R | 8/R | R/R | R | R | 23 |
| *Salmonella sp.* | | 10/8 | 10/R | 10/R | 8/R | R/R | R | R | 23 |
| *Candida albicans* | | 13/11 | 12/11 | 10/10 | 10/8 | 10/8 | R | R | 26 |

***ME- Methanol, EE- Ethanol and R- Resistant**

**Table-2 Antimicrobial activity of Seeds extracts of *Syzygium cumini***

| Test organisms | ATCC No. | Extract concentrations | | | | | | - ive control | + tive control |
|---|---|---|---|---|---|---|---|---|---|
| | | 5mg/ml | 4 mg/ml | 3mg/ml | 2 mg/ml | 1mg/ml | 0.5mg/ml | | |
| | | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | | |
| *Staph aureus* | | 18/14 | 16/12 | 14/11 | 12/10 | 11/8 | 8/8 | R | 25 |
| *E.coli* | | 16/14 | 15/13 | 13/13 | 11/8 | 10/R | R | R | 23 |
| *Pseudomonas aeruginosa* | | 15/12 | 14/10 | 12/8 | 11/8 | 10/R | R | R | 23 |
| *Salmonella sp.* | | 13/10 | 12/10 | 11/8 | 10/8 | 8/R | R | R | 23 |
| *Candida albicans* | | 16/14 | 14/12 | 13/10 | 13/8 | 12/8 | 10/R | R | 26 |

***ME- Methanol, EE- Ethanol and R- Resistant**

**Table-3: Antimicrobial activity of Seeds extracts of *Momordicha charantia***

| Test organisms | ATCC No. | Extract concentrations | | | | | | - ive control | + tive control |
|---|---|---|---|---|---|---|---|---|---|
| | | 5mg/ml | 4 mg/ml | 3mg/ml | 2 mg/ml | 1mg/ml | 0.5mg/ml | | |
| | | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | | |
| *Staph aureus* | | 12/11 | 10/8 | R/R | R/R | R/R | R/R | R | 25 |
| *E.coli* | | 10/8 | 8/R | R/R | R/R | R/R | R/R | R | 23 |
| *Pseudomonas aeruginosa* | | 8/R | 8/R | R/R | R/R | R/R | R/R | R | 23 |
| *Salmonella sp.* | | 10/8 | 8/8 | R/R | R/R | R/R | R/R | R | 23 |
| *Candida albicans* | | 11/8 | 10/8 | R/R | R/R | R/R | R/R | R | 26 |

***ME- Methanol, EE- Ethanol and R- Resistant**

**Table-4: Antimicrobial activity of Seeds extracts of *Azadirachta indica***

***ME- Methanol, EE- Ethanol and R- Resistant**

| Test organisms | ATCC No. | Extract concentrations | | | | | | - ive control | + tive control |
|---|---|---|---|---|---|---|---|---|---|
| | | 5mg/ml | 4 mg/ml | 3mg/ml | 2 mg/ml | 1mg/ml | 0.5mg/ml | | |
| | | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | ME/EE | | |
| *Staph aureus* | | 14/13 | 12/12 | 12/10 | 10/8 | 8/8 | R/R | R | 25 |
| *E.coli* | | 12/10 | 11/9 | 11/8 | 10/R | 8/R | R/R | R | 23 |
| *Pseudomonas aeruginosa* | | 12/11 | 11/10 | 10/8 | 10/R | 8/R | R/R | R | 23 |
| *Salmonella sp.* | | 10/8 | 8/R | R/R | R/R | R/R | R/R | R | 23 |
| *Candida albicans* | | 14/12 | 12/10 | 12/8 | 10/8 | 8/R | R/R | R | 26 |

Many sections in the rural population rely on medicinal plants and folklore healing methods for primary health care[1,8]. While 25% to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials.

Traditional healers have long used plants to prevent or cure infectious conditions[7]. Medicinal plants constitute an effective source of both traditional and modern medicines, but assessment of antimicrobial potential of these sources is essential. The present study found a very promising and readily available source for treating infections caused by bacteria and fungi. This is particularly significant because drug resistance to human pathogens has been increasing not only in the developing countries but throughout the world due to indiscriminate use of antibiotics[8]. The drug resistance bacterial and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients[9]. In the present scenario due to emergence of multiple drug resistance to human pathogenic bacteria and fungi, especially the antibiotic penicillins, cephalosporins and chloromphenicol types involve the enzymic inactivation of the antibiotic by hydrolysis or by the formation of an active derivative. This has opened a new vista for the search of new antimicrobial substance.

The results of the present study revealed wide ranges of inhibition zones from as low as 8 mm up to 18 mm. Both methanol and ethanol extract of the seeds extract showed zones of inhibitions in most strains tested. These levels of activities is promising. More application on these products will ensure its wide applicability and their bacteriostatic or bactericidal, fungistatic or fungicidal actions. It is clear that further research will be needed to cover a wide range of bacteria including multidrug (MDR) resistance ones. In this study *Staphylococcus aureus* was inhibited by these extracts, this could open way for testing MDR organisms and to determine

the MIC concentration of these extracts.

In addition in the present study we tested the methanolic extract of seeds and it

showed potent antimicrobial activities. Ethanol extract is more likely to be selected for further pharmaceutical experimentation for human and animal use. This is because methanol is risky due to its high toxicity and not applicable for usage. It is noticed in this study, as expected, that inhibition of microorganisms is directly proportional with the concentration of the extract. The use of Mueller Hinton's agar along with a unified inoculums size (0.5 McFarland) follows the standard methods[6]. But is it expected that antimicrobial activity and inhibition zone diameter might have been affected by factor related to its diffusion in agars such as the Mueller Hinton's.

In the present study both ethanolic and methanolic were investigated. Both solvents are low molecular weight alcohols, polar compound and shows very little difference in their extractive abilities. But ethanol extraction is more convenient and non toxic for the biological purpose. Ethanol extraction shows little increase in the yield percentage, zone of inhibition of ethanol extract shows little increase than methanol extract when treated with different microorganisms and Methanol extraction which we have done for comparative studies for our knowledge.

## Conclusion

In the present study encouraging results have been produced with both ethanolic and methanolic extracts.From the present screening, it could be concluded that the seed of *Syzygium cumini* are more potent antimicrobial agent than other extracts and could be compared to the known antibiotics. Further, the detailed phytochemical research is required to identify the active principal responsible for aforementioned activities and testing wider range of organisms would be encouraged.

## Disclaimer Statement

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

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# Comparative study of active components in *Spinacia oleracea* grown Hydroponic (without soil) and indigenous (soil) cultivation system

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**Abstract-** Green leafy vegetables (GLV) play a substantial role in human nutrition and are essential for a healthy life. This study was undertaken to determine the active component of *Spinacia oleracea* grown in Hydroponic system and in soil system. As *Spinacia oleracea* consumed in India and all over the world especially for its iron source. The spectrophotometric as well as gravimetric methods were used to determine the concentration of iron, tannin, flavonoids, and total alkaloids. The result shown that iron and other active component were higher in hydroponic growing system as compare to soil system. This possibility is due to iron content present in micro nutrients. As in hydroponic system the micronutrients always in contact with the plants, hence the possibility of iron absortion is more here as compare to soil system. The reason of higher concentration of other component may be due to the compatible growing conditions available in hydroponic system like light source for photosynthesis, temperature and pressure those were controlled automatically. Surprisingly we have seen that the growing of *Spinacia oleracea* is fast in hydroponic system as compare to soil system. In hydroponic system the spinach mature in about 20 days as compare to 30 days in soil system.

**Key words**: *Spinacia oleracea*, Hydroponic system, soil system iron, tannin, flavonoids, total bitter, and total alkaloids.

## Introduction

Green leafy vegetables play a significant role in human nutrition and are essential for a healthy life[1, 2]. These vegetables provide an adequate amount of dietary fibers, minerals, vitamins, and other nutrients require by human body to prevent several diseases[3, 4]. Spinach contains various minerals that play a significant role in growth and metabolism. Elements such as

sodium, potassium, iron, and calcium provide an alkalizing effect to the acidity produced by other foods[5]. Iron is one of essential transition metal in the living system which carries oxygen to the tissues and is responsible for the appropriate protection against microbes. The total iron present in an average adult is about forty grams which is mostly stored in the body organs like spleen, liver, and bone marrow[6,7,8]. The deficiency of iron is the most common and widespread nutritional deficiency globally, and it has been estimated that 30 to 40 percent of the world's population is iron deficient specially children and womens[9]. Iron is a component of hemoglobin, present in the ubiquitous RBC in the body that conveys oxygen throughout it[10]. Hydroponics can be briefly defined as cultivation of plants without soil[11]. Actually hydroponics is a Greek word where "hydro" means water and "ponos" means "labour". It is a technique of growing plants in soil-less condition in which their roots immersed in nutrient solution[12]. Professor William Gericke coined the word hydroponics in the early 1930s describe the growing of plants where their roots suspended in water having mineral nutrients. Mostly Europe is the biggest market for hydroponics vegetables in which France, the Netherlands, Spain and United States of America. Recent report shows that it is expected to reach a world growth of 18.8% from 2017 to 2023, corresponding to a global hydroponic market USD 490.50 Million by 2023[13]. Continuous production of vegetables is possible only through hydroponic systems so that vegetable of any season will be available for round the year. This technique require less space, and plants can be growing anywhere with a controlled conditions like temperature, pH, Light for photosynthesis, flow rate of water, concentration of nutrients[14]. Hydroponics system mostly allows them to have higher productivities and yields without any constrains of climate and weather conditions[15]. The quality of hydroponic crop is superior because it uses a highly controlled environment and enables a more homogeneous production without any loss of water and nutrients. Moreover, it is not dependent on seasonality[16].

## Material and Methods[17-20]

### Plant sample Collection

*Spinacia oleracea* leaves was collected from the hydroponic and field of Himalaya Wellness Company Faridabad, Haryana. These are thoroughly washed in running tap water and air dried at room temperature in the shade for 7 days, then powdered using a mixer grinder and stored in an air tight container at 4°C for further use.

### Iron concentration

5 g of samples powders were place in silica

crucible and place in a muffle furnace at 500 ºC for one hour. The obtained powders were moistened with 5 drops of sulphuric acid and taken with distilled water in small portions, filtered and passed into 50 ml graduated flasks and brought to the mark with distilled water (Stock Solution) 10 ml of Stock sample solution were taken into a 25 ml volumetric The following reagents (same reagents as for the standard scale) are added to the samples:

- 5 ml 25% sodium acetate
- 1 ml 10% hydroxylamine chlorhydrat,
- 1 ml 0.5 % ethanol alcoholic solution of oo'-phenanthroline.

The added solutions were stirred well, distilled water was added to the mark and then the obtained mixture was leave to stand for 1 hour.

The blank solution was prepared from:

- 5 ml 25% sodium acetate
- 1 ml 10% hydroxylamine chlorhydrat,
- 1 ml 0.5 % ethanol alcoholic solution of oo'-phenanthroline.

The added solutions were stirred well, distilled water was added to the mark and then the obtained mixture was leave to stand for 1 hour.

The standard solutions (standard scale), consisting in 10 Fe (II) solutions with concentrations between 1-5 mg/L are prepared as follows:

In 25 ml graduated flasks were added:

- 5 ml 25% sodium acetate
- 1 ml 10% hydroxylamine chlorhydrat,
- 0.5 % ethanol alcoholic solution of o-o'-phenanthroline.
- 1,2,3,4 and respectively 5 ml Fe (II) etalon solution (25 mg/L)

The added solutions were stirred well, distilled water was added to the mark and then the obtained mixture was leave to stand for 1 hour. All the spectrophotometric determination was performed at 510 nm.

## Determination of total tannins

Total tannins content was determined by Folin-Ciocalteu Phenol reagent with some modification. Five microliter of the sample extract was added into 2.5 ml of Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The mixture was shaken well, kept at 40 °C temperature for 30 min and absorbance was measured at 700 nm with the UV/Visible spectrophotometer. Blank was prepared with reagent instead of the sample. Total tannin content was determined from calibration curve made with standard tannic acid.

## Determination of Flavonoids

The flavonoid content was determined by aluminum chloride method using Quercetin

as standard. Extracts and Quercetin were prepared in (10 mg/ mL). 0.1 mL of extract was mixed with 0.9 mL of distilled water in test tubes, followed by addition of 75 µL of 5% sodium nitrite solution. After 6 minutes, 150 µL of 10%aluminium chloride solution was added and the mixture was allowed to stand for further 5minutes after which 0.5 mL of 1M Sodium hydroxide was added to the reaction mixture. Then add 2.5 ml of distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. A calibration curve was generate during various concentrations of Quercetin (20-100µg). Blank consist of all the reagents, exceptfor the extract or Quercitin is substituted with 0.1ml of Results were expressed as the Quercetin equivalence (QE) of the sample was expressed in mg/g of the extract.

### Determination of Alkaloids

To 5 g of plant powder in a 250 ml beaker, 200 ml of 20% acetic acid in ethanol was added. This was covered and allowed to stand for 4 h. The solution was then filtered, and the extract was allowed to become concentrated in a water bath until it reached a 1/4th volume of the original volume. To this concentrate, ammonium hydroxide was added until the precipitation was completed. The whole solution was left to settle down, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The precipitated residue was dried and weighed.

## Result and Discussion

**Table-1** The result obtained from the quantitative estimation of *Spinacia oleracea* leaves.

| Active component | In hydroponic system Mg/100gm | In indigenous (soil) system Mg/100gm |
|---|---|---|
| Iron | 10.35 ± 0.82 | 08.74 ± 0.77 |
| Tannins | 92.15 ± 12.38 | 82.54 ± 14.14 |
| Flavonoids | 11.05 ± 0.21 | 9.82 ± 0.72 |
| Alkaloids | 4820 ± 22 | 4572 ± 18 |

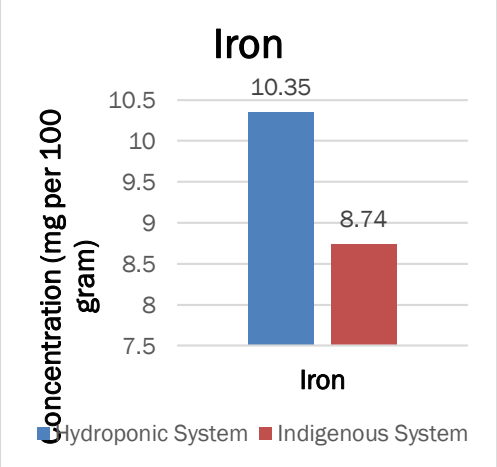


Figure-1 Concentration of Iron

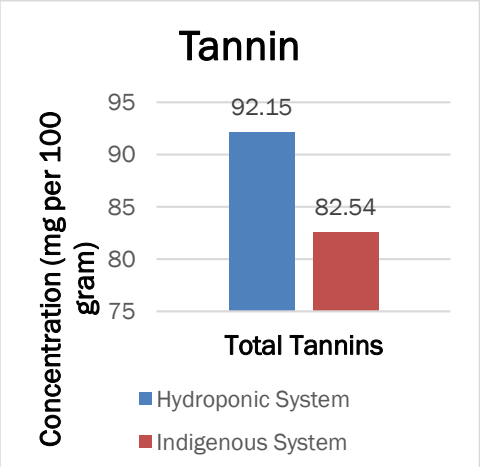


Figure-2 Concentration of Tannin

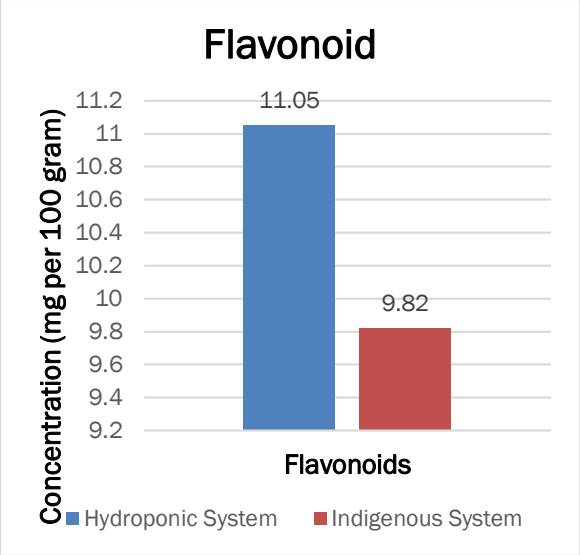


Figure-3 Concentration of Flavonoid

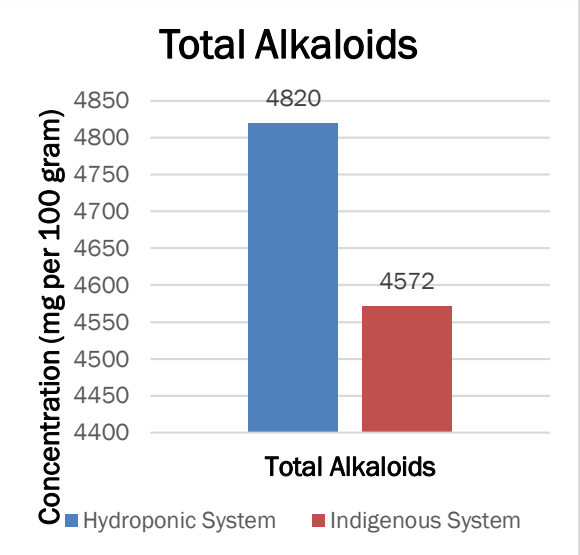


Figure-4 Concentration of Total Alkaloids

From the above table we find that the spinach leaves grow in hydroponic system (Hydroponic Leaves) has more iron content and other phyto-constituents are also high in the same leaves. Iron is determined by using colorimetric method of spectroscopy. The result shows the iron content in hydroponic leaves is 10.35 ± 0.82 mg as compared to 8.74 ± 0.77 mg in leaves that grown in soil (soil leaves). Tannins are determines by taking the absorbance of sample against the tannic acid as standard. The tannin content is 92.15 ± 12.38 mg in hydroponic leaves whereas soil leaves contains 82.54 ± 14.14 mg. The flavonoid is determined by UV-Spectrophotometer by using Quercetin as standard and found that hydroponic leaves contain 11.05 ± 0.21 mg as compare to 9.82 ± 0.72 mg in soil leaves. The total alkaloid was determined by gravimetric analysis and the result are higher in hydroponic leaves with 4820 ± 22 mg while soil leaves contains 4572 ± 18 mg.

## Conclusion

The above study reveals that Spinach *(S. oleracea)* contains phytochemicals such as Iron, Tannins, Flavonoids and Total alkaloids. From the result we can say that adaptation of hydroponic system for growing of spinach as well as other leafy vegetables is good choice because the leaves grown in this system have more quantity of phytochemicals as compared to the indigenous growing system that is soil.

## Disclaimer Statement

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

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# Antimicrobial activity of different tea extracts

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**Abstract**-Tea is a popular beverage known for its unique taste and vast health benefits. The main components in tea change greatly during different processing methods, which makes teas capable of having different biological activities.

Due to the presence of various bioactive compounds including catechin, polyphenols and saponins etc, tea exhibits antimicrobial activity against microorganisms. Catechins may be considered as the antimicrobial components of the tea extracts. Reports showed that the catechins damaged the cell membrane and increased cell membrane permeability, leading to changes in the relative electrical conductivity and the release of certain components into the cytoplasm.

This study was designed to investigate the antimicrobial activity of five tea extracts, including green, blue, black, hibiscus and chamomile tea extracts against standard strains of *Staph. aureus* (ATCC 6538) and *E. coli* (ATCC 8739). Solvent extraction method was used to prepare the extracts and antimicrobial activity was determined by Agar well diffusion method.

**Keywords**: tea extracts; antibacterial activity; catechins; cell membrane; Solvent extraction method

## Introduction

Tea is one of the most popular non-alcoholic beverages, consumed by over two-thirds of the world's population because of its refreshing, mild stimulant and medicinal properties. It is the second-most drank and refreshing beverage after water since the time immemorial. Tea could be categorised as true tea and herbal tea based on the type of plant and part of plants used.

All “true” tea comes from the plant, called the *Camellia sinensis*. Any leaf, root, fruit or flower that comes from a different plant is considered an herbal tea. For example, chamomile flowers and peppermint leaves

are considered herbal teas because they do not come from the traditional tea plant. It is important to distinguish between real tea and herbal tea since the flavour, health benefits and nutritional characteristics vary from plant to plant

## True tea or Tea

A beverage made from the leaves of *Camellia sinensis*, originated in China and has become increasingly popular worldwide in recent year[(11)]. Indeed, tea, which is considered the most widely consumed beverage in the world after water, has been the subject of extensive research in recent years due to its chemical composition. Tea harbours more than 700 bioactive compounds viz, different classes of polyphenols, unique amino acid L-Theanine, alkaloids (Caffeine, Theobromine), and Volatile Flavor Compounds (VFC). In line with literature data, the most commonly consumed type of tea in Turkey is black tea, followed by green tea, yellow tea, and oolong tea, especially in the western regions (18)

## Green tea

Green tea is rich in EGCG (Epigallocatechin gallate; one of nature's most potent antioxidants) and is gaining widespread popularity due to its natural ability to promote weight-loss and good health. As a result of minimal processing, green tea retains its natural appearance and vibrant colour as well as high levels of the plant's healthy properties. Green tea varies dramatically in flavour from grassy and sweet, to floral and fresh, to nutty and roasted. Like fine wine, green tea's flavour depends on the plant varietal, season of harvest, soil, elevation, weather, cultivation and origin. Each region has its own distinct flavour and aroma.

## Herbal Tea

**H**erbal teas are made from dried fruits, flowers, spices or herbs that comes from a plant other than the tea plant is considered as a herbal tea. For example, chamomile flowers, peppermint leaves, spice blends, and rooibos (which is often called red tea) are considered herbal teas because they do not come from the traditional tea plant. Most herbal teas are naturally 100% caffeine-free, making them an excellent choice for evening or for people with caffeine-sensitivities. Herbal teas like chamomile, hibiscus, blue tea offer several health-promoting properties and could help improve heart health, digestion, sleep quality and more.

Here are most popular herbal tea categories-

**Chamomile tea**- Chamomile is an aromatic perennial flower, producing feathery leaves and white, daisy-like flower heads with

yellow centers. Chamomile has a bright, golden-colored infusion and a fragrance reminiscent of honey, fruit blossoms and apples. It is often taken with honey and lemon.

**THERAPEUTIC EFFECTS-**Chamomile has been prized for thousands of years for its therapeutic effects. In fact, chamomile was dedicated to the ancient Egyptian gods for its ability to calm the mind and comfort the senses. Today, chamomile is used as a nurturing herbal tea ideal for countering PMS, easing stress, relieving headaches and enhancing a peaceful night's sleep. Chamomile is also known to have anti-inflammatory and anti-allergic properties as well as acting as a digestive stimulant, muscle relaxant and mild sedative. Chamomile may be a beneficial treatment for arthritis and is even used in lotions and cosmetics for its soothing, antiallergic properties on the skin

**Hibiscus tea -** Hibiscus tea has been extensively studied for its potential antihypertensive effects. Several randomized controlled trials have shown that regular consumption of hibiscus tea can lead to significant reductions in systolic and diastolic blood pressure (Herrera-Arellano et al., 2004; Mozaffari-Khosravi et al., 2009)[1,2]. The hypotensive properties of hibiscus tea are believed to be attributed to its rich content of bioactive compounds, including anthocyanins and polyphenols, which possess vasodilatory and diuretic effects (Hopkins et al., 2013)[3]. Recent studies have highlighted the potent antioxidant activity of hibiscus tea, attributed to its phenolic compounds, such as flavonoids and anthocyanins (Ali et al., 2015)[4]. Additionally, hibiscus tea has demonstrated anti-inflammatory properties by inhibiting inflammatory mediators and enzymes (Tseng et al., 2013)[5]. Research has shown that regular consumption of hibiscus tea can lead to reductions in total cholesterol, LDL cholesterol and triglyceride levels, while increasing HDL cholesterol (Mozaffari-Khosravi et al., 2009; Serban et al., 2015)[2,6]. These lipid-lowering effects are attributed to the presence of flavonoids and other bioactive compounds in hibiscus tea, which modulate lipid metabolism and inhibit cholesterol synthesis (MozaffariKhosravi et al., 2009)[2]. Several animal and human studies have reported that hibiscus tea can reduce fasting blood glucose levels and improve insulin resistance (Herrera-Arellano et al., 2004; Mozaffari-Khosravi et al., 2009; Serban et al., 2015)[7,2,6]. The hypoglycemic effects of hibiscus tea are attributed to its ability to enhance insulin secretion, inhibit carbohydrate absorption, and enhance glucose utilization (Hopkins et al., 2013)[3] Hibiscus Blood pressure regulation and antioxidant effects McKay et al., 2006[8].

**Blue tea-** In Indian traditional medicine it is termed as Aparajit (Hindi), Kakkattan (Tamil) and Aparajita (Bengali)[14] . It is perennial twinning herbaceous plant and is widely distributed in tropical regions of India, Malaysia, Sri Lanka, Philippines islands and Burma[12,13]

It's an herbal infusion native to South Asia made by steeping butterfly pea flowers (*Clitoria ternatea L*) in hot water. It contains wide range of phytoconstituents such as triterpenoids, flavanol glycosides, tannins, alkaloids, amino acids, proteins, ternatins (poly acylated anthocyanins) and carbohydrates etc. Its high anthocyanin (delphinidin) content gives the tea its characteristic bright blue colour and medicinal properties.

**THERAPEUTIC EFFECTS**: Blue tea is well known for its potential health advantages, it possess antioxidant, antimicrobial, antidiabetic, hepato-protective, nootropic, central cholinergic, antidepressant, antianxiety, analgesic, antipyretic, wound healing, , improved heart and brain health, anti-diabetic, cancer fighting properties and many other medicinal propertiesAdditionally essential to preserving general health and wellbeing are antioxidants. Due to its propensity to increase collagen, blue tea is thought to have anti-inflammatory characteristics and is linked to maintaining healthy hair and skin.

## Material and Methods

### Plant collection

Tea samples were collected from super market and authenticated by Pharmacognosy department HWC, Dehradun Unit.

### Extract preparation

10g of each of the ground leaves were extracted by soaking for 2 days using 100ml of solvent in a 250ml sterile conical flask followed by intermitent shaking. Distilled water and methanol was used as solvent to prepare the extracts. Extracts were filtered using Whatman filter paper No 1. The filtrates were then concentrated by using water bath 60°-80°c and stored in screw-capped tubes in refrigerated prior to use.

## Determination of anti-microbial activity

### Well-Diffusion method

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

## HPLC Analysis

**Sample preparation (5mg/ml):** Weight accurately about 0.5g of sample in a 100ml beaker.Add 50 ml of methanol and soncate for 5-10 mins. Allow the residu to settle and decate the dissolved extract into 100ml volumetric flask. Repeat the same process with 15 ml methanol eacg, until methanol extract becomes colourless. Make the volume upto the mark with methanol. Filter through 0.45µ syringe filter.

**Hplc conditions:**

Coloumn : C18 Phenomenex Luna (250 x 4.6mm 5µ)

Mobile Phase : Ortho Phosphoric acid 0.1% in water (solvent-A)

: Acetonitrile (solvent- B)

Flow rate : 1ml/minute

Wavelength : 210 nm

Injection volume : 20 µl

Temperature : 35°c

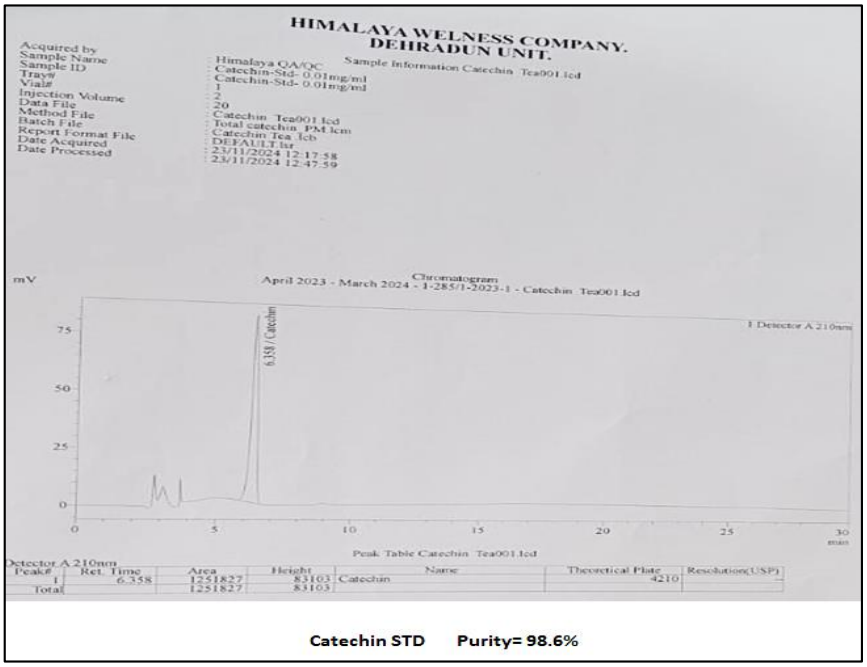
**Standard catechins:** weight 10mg of standard catechin into a volumetric flask add 7-8 ml methanol dissolve by sonication and make the volume up to the mark with methanol. Take 1 ml of above solution and dissolve in to 1oo ml of methanol .

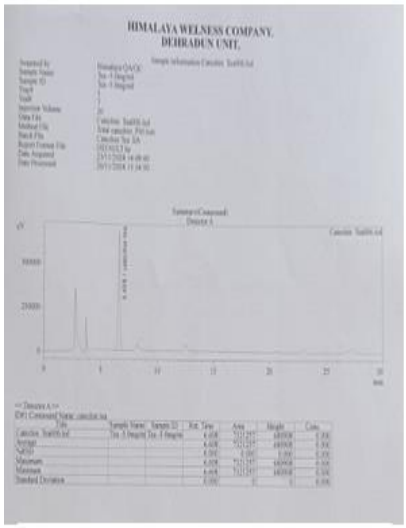
## Calculation

$$\text{Catechin \%} = \frac{\text{Area of sample peak}}{\text{Area of standard peak}} \times \frac{\text{conentration of standard (mg/ml)}}{\text{conentration of sample (mg/ml)}} \times \text{\% purity (std)}$$

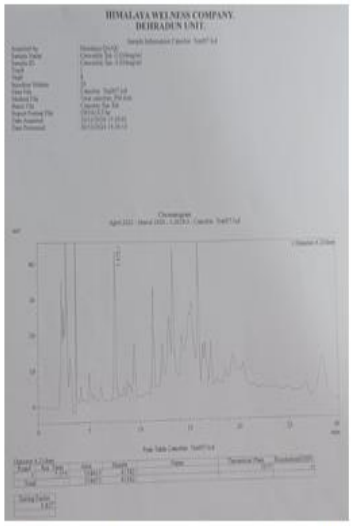
## Results and Discussion

**HPLC (High Performance Liquid Chromatography)** is a powerful analytical technique used to separate, identify and quantify catechins in various Tea samples in different chromatogram. Table- 1 shows the catechin content in different tea samples. The table and chromatogram provided in the image summarize the catechin content in different tea samples.Antimicrobial activity of tea extracts was depicted in **Table-2.**

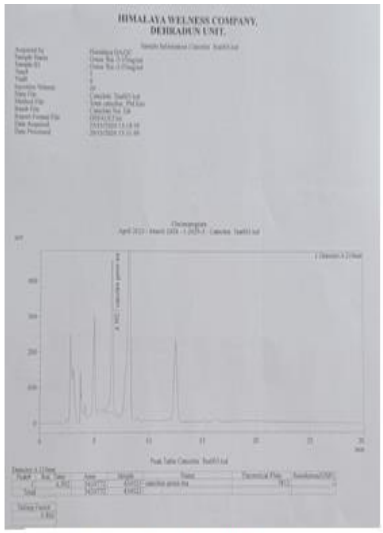




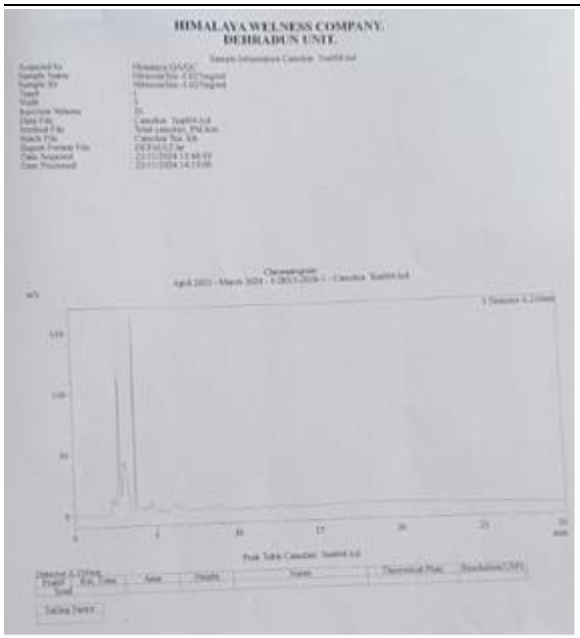

TEA(*Camellia sinensis*)



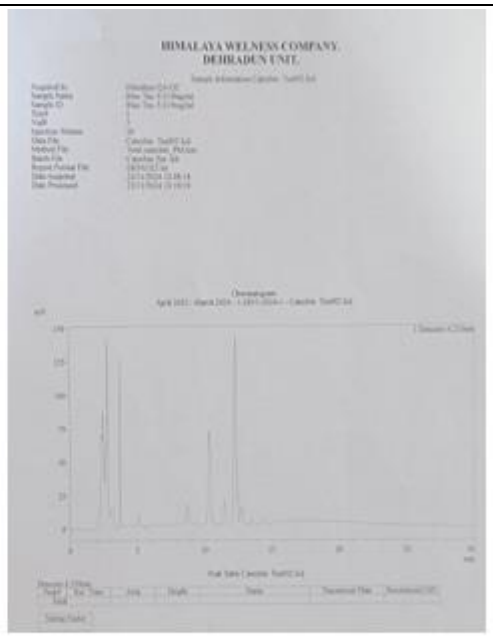

Camomile Tea (*Matricaria chamomilla*




Green Tea (*Camellia sinensis* )




Hibiscus Tea (*Hibiscus rosa-sinensis*)




Blue Tea (*Clitoria ternatea*)

**Table-1 Catechin content in Different Tea extract through HPLC**

| Sample | Catechin % |
|---|---|
| Tea | 1.14 |
| Green tea | 0.84 |
| Chamomile tea | 0.087 |
| Hibiscus tea | Absent |
| Blue tea | Absent |

Catechin content is highest in Tea *(Camellia sinensis)*, followed by green tea (*Camellia sinensis*). Chamomile tea (*Matricaria chamomilla)* has the lowest catechin content among the listed samples, with hibiscus (*Hibiscus rosa-sinesis*) and blue tea (*Clitoria ternatea*) showing no detectable levels.

**Table-2 Antimicrobial activity of Different Tea extract.**

| Sample | solvent | S.aureus | E.coli |
|---|---|---|---|
| **TEA (*Camellia sinensis*)** | Aqueous | 20 mm | 16 mm |
| | Methanol | 24 mm | 21 mm |
| **GREEN TEA (Camellia sinensis)** | Aqueous | 23 mm | 22 mm |
| | Methanol | 27 mm | 18 mm |
| **BLUE TEA (*Clitoria ternatea*)** | Aqueous | 22 mm | NA |
| | Methanol | 20 mm | NA |
| **CHAMOMILE TEA (*Matricaria chamomilla)*** | Aqueous | NA | NA |
| | Methanol | 14 mm | 13 mm |
| **HIBISCUS TEA (*Hibiscus rosa-sinesis*)** | Aqueous | 25 mm | 20 mm |
| | Methanol | 28 mm | 26 mm |
| **Positive control** | | 32 mm | 30mm |

The table-2 shows the antimicrobial activity of different tea extracts against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli.* As its clear from the data that almost all the tea extracts found to be remarkably active against the tested pathogens except chamomile aqueous extract but its methanolic extract was active for both bacteria. Above all the tea extracts mentioned Hibiscus tea extract have highest antimicrobial activity followed by Green tea and Black tea.

## Conclusion

Our results revealed that all the tea extracts were found active against *Staphylococcus aureus* and *E.coli*. Hibiscus tea had the highest activity for both bacteria in comparison to our true tea extracts i.e *Camellia sinensis* and further investigation is needed to explore the active phytochemical constituents responsible for antimicrobial activity of Hibiscus against *test pathogens*

## Disclaimer Statement

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

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# Invitable clinical significance of Herbal Phytochemicals

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**Abstract-** The most important and promising source of phytochemicals are traditional plants for herbal medicines preparation. Now a days the pharma industry has been forced by unwanted side effects, drug resistance and rise in population to speed up the development and processing of phytochemicals from plants used as herbal medicines to address current health scenario.Medicinal herbal plants not only serve as complements or substitutes for conventional treatments, which are often inadequately available but also enhance the health and security of indigenous population. Thus, these herbal plants play indispensable roles in daily life and are deeply connected to diverse socio-cultural, and economic events associated with life, aging, illness, and death.It has been evident from previous so many researches that the herbal plants have the potentiality of its medicinal value because it is a rich sources of phytochemical ingredients. Present review is an attempt to explore the clinical vitality of phytoconstituent of traditional herbal plants and their potentiality in protecting against different types of diseases.

**Keywords:** Phytochemicals, Herbal medicines, Traditional herbal plants

## Introduction

Phytochemicals are nutritional/non-nutritional bioactive compounds present in different parts of plants, fruits, vegetables and cereals.They may have health advantages in addition to basic nutrition, such as protecting/lowering the risk of major chronic diseases. The major phytochemicals present in plants are carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and

polysaccharides etc.These phytochemicals shows strong antioxidant activities and exhibit antimicrobial, antidiarrheal, antihelmintic, antiallergic, antispasmodic, and antiviral activities, help to regulate gene transcription, enhance gap junction communication, improve immunity, and provide protection against lung and prostate cancers[1–6].

As per WHO, the primary health care of most population of developing countries depend on traditional medicines and mostly natural plant products (Vines 2004). About 75–90% of the rural population in the world (excluding western countries) relies on traditional system of medicines as their only health care system and is not only because of poverty where people cannot afford to buy expensive modern drugs, but traditional systems are also more culturally acceptable and meet the psychological needs in a way conventional medicine does not (Fassil Kibebe 2001).

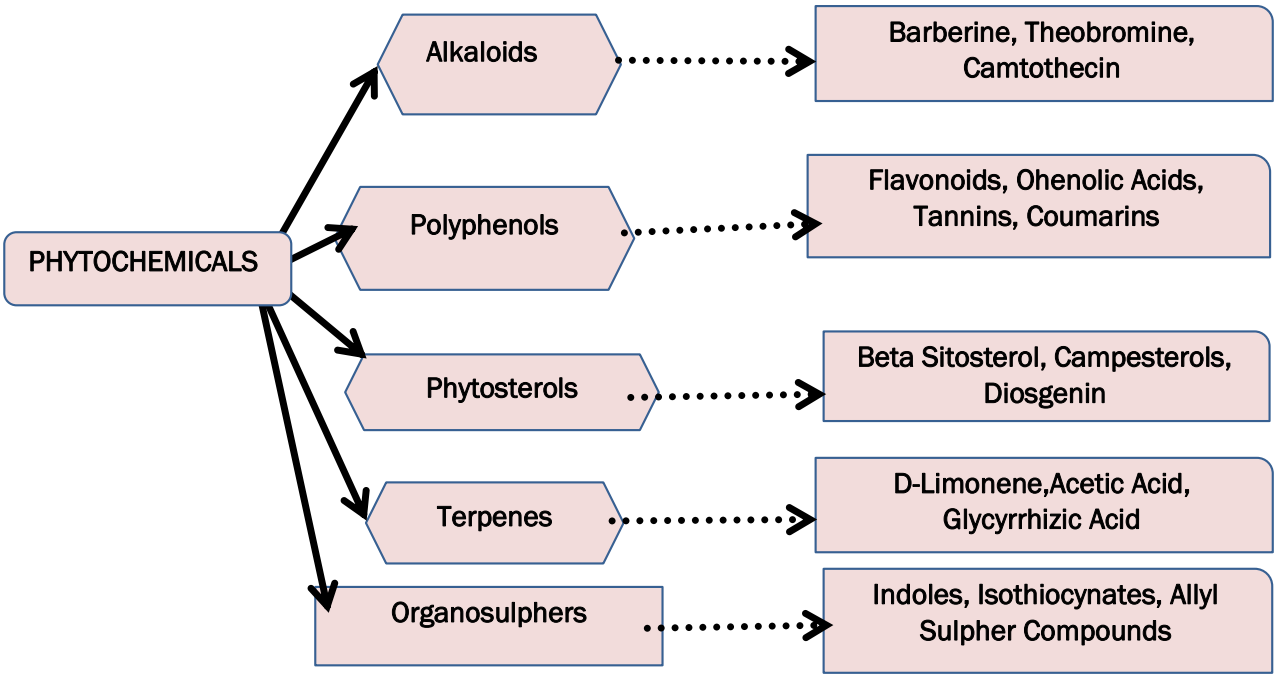


**Figure- 1 Different Variants of Phytochemicals**

Phytochemicals includes a variety of plant ingredients having different structures and are capable of health-promoting effects.These are natural substances but are not called nutrients in the traditional sense, since they are synthesized by plants neither in energy metabolism nor in anabolic or catabolic metabolism, but only in specific cell types. They occur only in low concentrations and usually have a pharmacological effect. Since antiquity, these effects have been used in naturopathy in the form of medicinal herbs, spices, teas, and foods. With the development of highly

sensitive analytical methods, a variety of these substances could be identified. These phytochemicals may have health benefits or adverse health effects, depending on the dosage[7].

## Aims and Objectives

1. To explore the concepts of Phytochemicals
2. To explore the relationship between Phytochemicals and health related benefits
3. To explore the therapeutic utility/ vitality of Phytochemicals

## Material and Methods

The articles were searched for in research engines such as Google Scholar, Research Gate, Science Direct, and Pub Med. Synonyms and alternative words were identified and used to obtain the current literature. The major search terms and key words used were phytochemicals, traditional herbs, phytosterols, major bioactive compounds in plants, carotenoids, isoprenoids, saponins, anthocyanins, flavonoids, dietary fiber, polysaccharides in plants, health benefits of phytochemicals.

### Why phytochemicals are important:

Phytochemicals are nothing but plant metabolites, biologically active compounds and are naturally present in plants. They provide health benefits for humans beyond those attributed to common nutrients.Their biological activities include antioxidant and antimicrobial activities, detoxification enzyme modulation, and immune system stimulation, as well as hormone metabolism modulation.These are not vital nutrients, and are not needed by the human body to sustain life, but they do have important properties to prevent or combat some common diseases (Acidri et al., 2020; Saxena et al 2013). In view of that, phytochemicals play significant role in protecting against cardiovascular diseases, cancer and other chronic degenerative diseases such as cataracts, macular degeneration, neurodegenerative diseases and diabetes mellitus (Farah & Donangelo, 2006; Patay et al., 2016; Saxena et al., 2013).Phytochemicals are an important component of the human body, particularly in their role as antioxidants. They serve as a protective shield for cells, defending cells against the injury (Oxidative stress) caused by free radicals. The antioxidant properties of some phytochemicals, such as carotenoids and polyphenols, are especially strong, enabling them to neutralize free radicals and reduce oxidative stress[8-10]. It has been shown in so many previous researches that higher levels of carotenoids- α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin, and polyphenols in the diet or plasma are associated with to lower frailty and a reduced risk of cardiovascular disease . In

addition, phytosterols, which can be found in nuts and unrefined pressed oils, have been linked to cholesterol reduction[11-15]. In recent times, phytochemical index (PI-Dietay index), has been developed to efficiently evaluate the health related effects of phytochemical-rich foods in large population-based epidemiological studies. PI serves as a convenient surrogate measure of phytochemical intake.

## Therapeutic benefits of important phytochemicals

Phytochemicals are low-molecular-weight (LMW) secondary metabolites that play a significant role in the normal cellular metabolic process and promote health and disease prevention[16]. Primary metabolites comprises of glucose, starch, poly-saccharide, protein, lipids and nucleic acid which are helpful for growth

and development of the human body. Plants produce secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils etc[17-18].

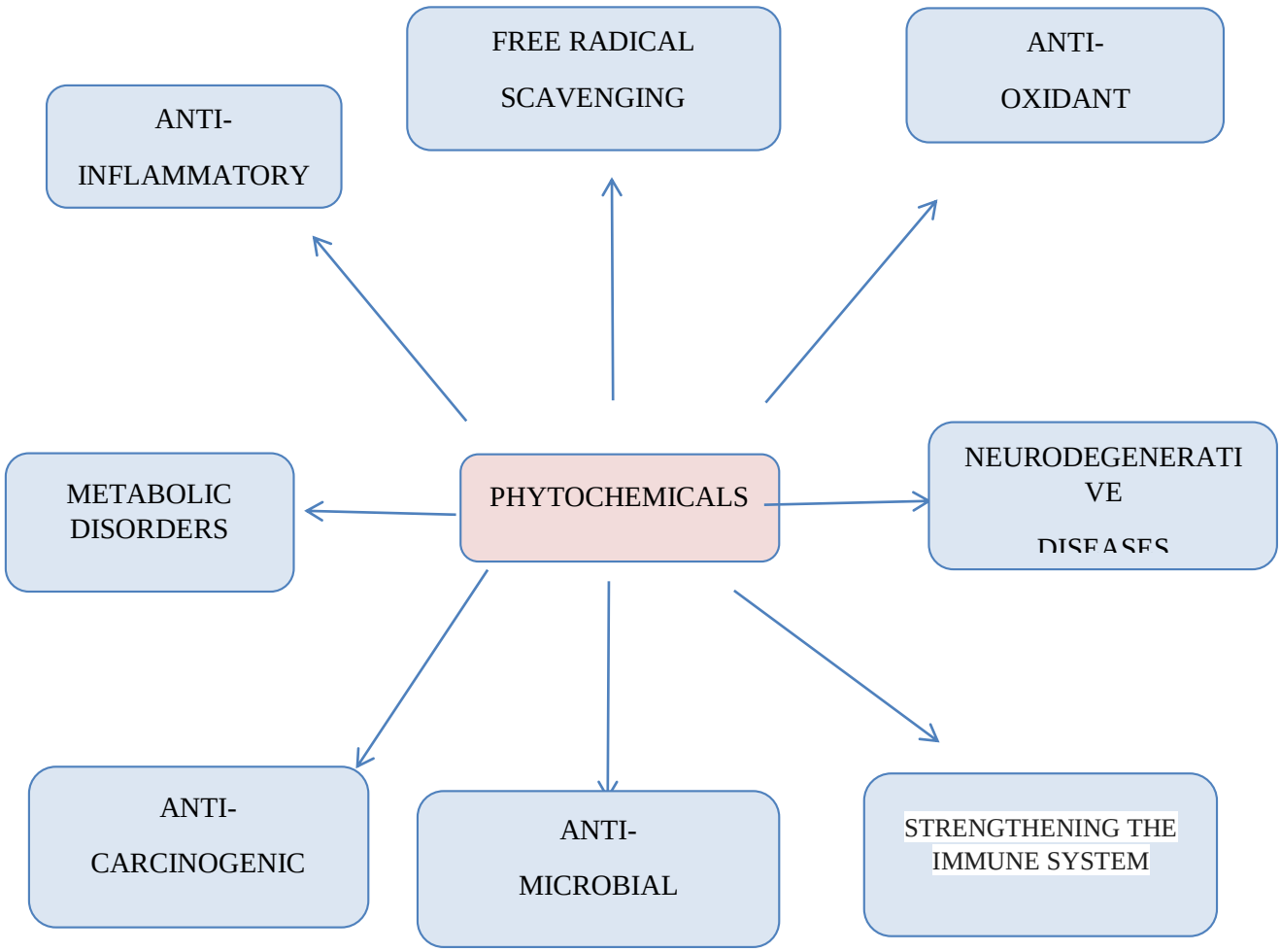


**Figure- 2 Different functions of Phytochemicals**

**Alkaloids**-having pharmacological importance like antihypertensive (many indole alkaloids) and antiarrhythmic (quinidine, spareien), antimalarial activity (quinine) and anticancer actions (dimeric indoles, vincristine, vinblastine).Some alkaloids contains caffeine, nicotine, and morphine etc possessing the stimulant property and used as the analgesic and quinine as the anti-malarial drug[19].

**Carotenoids**- are bright yellow, red, and orange-colored pigments found in plants, algae, and photosynthetic bacteria, abundantly found in carrots (Daucus carota L.), tomatoes (Solanum lycopersicum L.), parsley (Petroselinum crispum L.), orange (Citrus sinensis L.), cabbage (Brassica oleracea L.), spinach (Spinacia oleracea L.), fenugreek (Trigonella foenum-graecum L.), and green leafy vegetables,health benefits of carotenoids include gene transcription regulation by lutein, α-carotene, and β-carotene,enhancement of gap junction communication by β-carotene,improvement of immunity by β-carotene ,lutein; protection against lung and prostate cancers by α-carotene, β-carotene, lycopene, and zeaxanthin[20-23].

**Polyphenols**- has four major sub-classes, such as flavonoids, stilbenes, phenolic acids, and lignans. These are abundantly found in artichoke (Cynara cardunculus var. scolymus L.), spinach (Spinacia oleracea L.), broccoli (Brassica oleracea var. italica L.), chicory (Cichorium intybus L.), flax (Linum usitatissimum L.), onion (Allium cepa L.) etc. Health benefits of polyphenols include action against free radicals; protective effects against cardiovascular diseases, cancers, and other age-related diseases; and prevention of inflammation and allergies. Flavonoids have been also found to be useful in angina, cervical lesions, chronic venous insufficiency, dermatopathy, diabetes, gastrointestinal dosorders, lymphocytic leukemia, menopausal symptoms, rhinitis, traumatic cerebral infarction, etc.[24-26].

**Phytosterols-** collective name of plant sterols and stanols, abundant in olive oil ,oils of corn (Zea mays L.), sesame (Sesamum indicum L.), sunflower (Helianthus annuus L.), peanuts (Arachis hypogaea L.), beans (Phaseolus vulgaris L.), and almonds (Prunus dulcis L.),Health benefits in general, include support for prostate health, hair growth, reduction in LDL cholesterol, and high antioxidant activity[27-28].

**Saponins-** abundant in the legumes viz. black gram (Vigna mungo L.), garden pea, (Pisum sativum L.), pigeon pea (Cajanus cajan L.), and common bean (Phaseolus vulgaris L.).The general impact on health includes- acute impact injuries, erectile dysfunction, venous edema in chronic deep vein incompetence, and SLE[29].

**Polysaccharides and Dietary Fibers-** may be storehouses of energy such as starch and glycogen or non-digestible components such as cellulose, pectin, beta-glucan, hemicelluloses, resistant starch, lignin, etc., and are collectively known as dietary fiber. These components are not digested by human digestive system but are broken down by the gut microbiota in the large intestine, where they selectively support the growth of healthy microorganisms. All plant-based foods are rich sources of dietary fiber e.g. chicory, tamarind (Tamarindus indica L.), barley (Hordeum vulgare L.), corn, oats (Avena sativa L.), wheat (Triticum aestivum L.), and green beans (Phaseolus vulgaricus L.). The regular consumption of dietary fiber helps to prevent cancer, inflammation, hypertension, hyperlipidemia, hypercholesterolemia, obesity, and cardiovascular diseases, as well as improving insulin sensitivity and promoting healthy microbiota in the gut[30-31].

**Table-1 Some traditional herbs their phytochemicals and health related benefits:**

| HERBS | PHYTOCHEMICALS | BENEFITS |
|---|---|---|
| Aloe vera (Ghrit Kumari) | ß-sitosterol, compesterol, emodin and aloin | Anti-diabetic,healing properties,antiseptic effects, anti-viral and anti tumor, Helps to nourish skin and hairs **Mittal et.al 2014** |
| Curcuma longa (Haldi) | Flavonoid | Anti-inflammatory, anticancer,hepato-protective **Sharma et.al 2013** |
| Azadirachta Indica (Nimb) | Di and Tri terpenoids, limonoids | Blood purifier,anti-diabetic, inhibit colon cancer, anti-allergic **Gupta et.al 2014** |
| Phyllanthus emblica (Amla) | Emblicanin B, punigluconin and pedunculagin | Good for skin, eyes and hairs, antiviral,anticancer, antidiabetic, and hepatoprotective **Paarakh et.al 2010** |
| Allium sativum (Lasun) | Allicin | Anti-inflammatory, cardioprotective **Joshi et.al 2005** |
| Tinospora cordifolia (Giloy) | Tinosporin, isoquinoline alkaloids | Cardioprotective,anti-diabetic, Immuno-modulator, chemo prevention **Nisar et.al 2012** |
| Withania somnifera (Ashwagandha) | Withanolides, steroidal lactones , | In Alzheimer's and Parkinson's disorders helps to enhance memory Immuno-modulatory, anti-cancerous and chemo preventive **Rathinamoorthy et.al 2014** |

## Discussion and Conclusion

Today's lifestyle is a major issue related with human health.Conventional medicines often work effectively against the disease but may show extreme side effects in certain cases. Commonly manifested side are face swelling, rashes on the body, inflammation, and drug resistance etc. A safer alternative to treat diseases is indigenous herbs or plant derived medicines that have been used since the ancient period. The versatile and vast pharmacological effects of medicinal plants are completely dependent on their phytochemical constituents. The present review reveals secondary metabolites (terpenes, alkaloids, flavanoids, phenols etc) which shows a wide range of pharmacological activities like antihypertensive effects, antimalarial activity, anticancer actions, antioxidants, anti-inflammatory, antidiarrhoeal, cytotoxic, antibacterial activities etc. Based on many previous researches, indigenous medicinal plants of India would emerge to be a hopeful source of novel drugs and can be used as effectively of new pharmaceuticals.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## Ethical approval

No ethical approval is required as no animals or humans have been used in the study.

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# Medicinal potential of *Cascabela thevetia* flowers extract

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**Abstract**-The present study highlights the medicinal potential of *Cascabela thevetia* flowers extracts (aqueous, acetone, ethanol). Cardiac glycosides, tannin, phlobatannin, phenolic compound, alkaloids, phytosterols, terpenoids, flavonoids, anthocyanin and quinones were the phytoconstituents present in significant amount in plant. Phytochemicals are highly active in the presence of ethanol extract. Cardiac glycosides, phlobatannin, tannin also showed significant reaction in acetone extract of flower. This phytochemical screening is more prominent in ethanol and acetone extract as compared to aqueous extract

**Key Words**: *Cascabela thevetia*, flower extract, medicinal potential, phtochemicals.

## Introduction

Natural products played an essential role as a complementary cytotoxic agent avoiding complications of therapies. Herbal drugs of have attracted more attention for more bioavailability and less active dose. Phytoconstituents individually or in the combination, determine the therapeutic value of a medicinal plant. (Surana and Wagh, 2015). Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the important phytochemicals with diverse biological activities.

*Cascabela thevetia* is a very common plant on the premises of temples in India, A highly toxic plant, used medicinally, and also as a hedging plant. Generally found in

Himalayas region in India, Sri Lanka, Bangladesh, China (including Taiwan), West Africa, its flowers are used for worship and other rituals. The plant parts of this species are also used to cure many health problems. The qualitative phytochemical analysis of flowers using three extracts (aqueous, acetone and ethanol).

## Material and Methods

### Collection of plant material

The flowers of *C. thevetia* were collected from the Botanical Garden, Department of Botany, D.A.V(PG) College, Muzaffarnagar, UP, India.

### Preparation of plant extracts

Preparation of sample and extraction were carried out as described by Saha *et al (2004)* with slight modifications. The fresh flower samples were washed with distilled water and cut in to small pieces, shade- dried under for 1 week and followed by complete drying at 50 °C in oven. Then grinded to from powder. 15g of these dried sample from each variety was extracted separately with 150 mL of three different solvents: aqueous, ethanol and acetone for 24 h in a shaker at 100 rpm at room temperature. The extracts were filtered using Whatman filter paper and filtrates were used as an extract. Extracts were kept at 4 °C for the further analyses. Detection of different phytochemicals was carried out using standard methods (Hebbar *et al.*, 2024; Mahendru *et al,* 2024; Mety *et al,* 2024).

### Test of Alkaloids

(Wagner's test)A few drops of Wagner's reagent were added to 1 mL of extract. The formation of a creamy brown/ reddish precipitate was considered positive for the alkaloid.

### Test of Cardiac – Glycosides

(Keller- Killani Test): 1 mL of acetic acid and 2 - 3 drops of ferric chloride were added to 1 mL of extract, then 2 mL of concentrated sulfuric acid was added in this solution, the colour change was observed. Blue colour solution was observed in the acetic acid layer.

### Test of Tannin

(Braymer's Test): 3 mL of 10% of ferric chloride solution were added in I mL of extract. The blue green colour formation confirmed the presence of tannin.

### Test of Flavonoids (Alkaline reagent Test)

1 ml of extract was taken and added 2 ml of 2 % NaOH solution followed by few drops of HCl into it. The colour initially turned to an intense yellow colour with the addition of NaOH solution and later become colourless with addition of dilute acid. This change in colour confirmed the presence of flavonoids.

### Test of Terpenoids

About 2 ml of the filtrate of fruit extract was added with 6 drops of chloroform and place it in the water bath for few minutes. Then 6 drops of concentrated $H_2SO_4$ were added. The appearance of reddish-brown interface confirmed the presence of terpenoids.

### Test of Phenolic Compounds

(Ferric chloride test) 1 ml of extract was taken and few drops of 5% Ferric chloride solution were added into the extract. The appearance of bluish black colour provides the positive result of the phenolic compounds.

### Test of Phlobatannins

(Foam Test) When an aqueous extract was boiled with 1%aqueous HCl, red precipitate was deposited which was taken as evidence for the presence of phlobatannins.

### Test of Phytosterols

(Salkowski Test) 5 mL of extract mixed with 2 ml of chloroform then 2 mL of concentrated $H_2SO_4$ were added into it. Red colour was observed in lower layer of chloroform indicates the presence of phytosterols.

### Test of Quinones

2ml of extract were mixed with 3ml of concentrated HCl to form green colour indicates the presence of quinones.

### Test of Anthocyanins

2ml of aqueous extract is added to 2ml of 2NHCl and ammonia. The appearance of pink-red turns blue violet indicates the presence of anthocyanins.

## Results and discussion

Phytochemical screening was carried out and it was found that phytochemicals including tannin, saponin, flavonoids, terpenoids, phenolic compound, alkaloids, cardiac glycosides, phlobatannin, phytosterols and quinones are present in all the extract. (Table) In ethanol extract, above phytochemicals are abundantly present. Cardiac glycosides, phlobatannin, tannin showed significant amount in acetone extract. This phytochemical screening is more prominent in ethanol and acetone extract as compared to aqueous extract as phytochemicals are organic in nature and soluble in organic solvent. The detected compound might be responsible for the toxicity, antimicrobial, anti-inflammatory and antioxidant properties in plant. Presence of tannin in all extracts of flower might be responsible to treat fungal infections. It is also used in the treatment of external wounds, infected area, ring worms and tumours in traditional system of medicine (Bisso BN *et al*, 2022). The presence of classes of phytochemicals as such; flavonoid, alkaloid, tannin showed cytotoxic effect (Chowdhury *et al.* 2017).

**Table- Medicinal potential of *C. thevetia* flowers extract in different solvent**

| S. No. | Phytochemical | Test Name | Aqueous | Acetone | Ethanol |
|---|---|---|---|---|---|
| 1 | Alkaloids | Wagner's test | ++ | + | +++ |
| 2 | Cardiac Glycosides | Killer- Killani test | + | +++ | +++ |
| | | Baljet Test | + | +++ | +++ |
| 3 | Flavonoids | Alkaline reagent Test | ++ | ++ | ++ |
| 4 | Phenolic compound | Ferric chloride test | +++ | +++ | +++ |
| 5 | Tannin | Braymer's Test | ++ | +++ | +++ |
| 6 | phlobatannin | Foam Test | +++ | +++ | ++ |
| 7 | terpenoids | | ++ | ++ | +++ |
| 8 | phytosterols | Salkowski Test | +++ | + | ++ |
| 9 | quinones | Con HCl test | + | ++ | ++ |
| 10 | Anthocyanin | HCl test | ++ | ++ | +++ |

## Conclusion

Plants are a great source of phytochemicals that could be utilized in curing various types of diseases. The study was only based on qualitative analysis. Cardiac glycosides, tannin, phlobatannin, phenolic compound, alkaloids, phytosterols, terpenoids, flavonoids, anthocyanin and quinones were the phytoconstituents present in significant amount in plant. The majority of the biologically active phytochemicals were found present in ethanol, acetone as compared to aqueous extracts of flowers of *C. thevetia*. The study provided an important basis for further investigation into the isolation and quantitative determination of phytoconstituents from the selected plants for the development of drugs.

## Disclaimer Statement

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

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# Phytochemical screening and TLC-bioautography detection of antioxidant constituents of some spices of Apiaceae family

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**Abstract-** Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices have been used during the middle Ages for flavoring, food preservation and medicinal purposes. The present study was carried out on the three spices cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*) of apiaceae family to determine their phytochemical constituents and were proved to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The work was designed to study the TLC bio autography antioxidant activity and to identify the main active components of the spices. The results provide evidence that the spices used as a potential source of safe and effective natural antioxidant agents in pharmaceutical and food industries.

**Keywords:** Phytochemical screening, Antioxidant TLC-Bio autography, *Cuminum cyminum, Foeniculum vulgare and Carum carvi*

## Introduction

Spices are the dried parts of aromatic plants, generally used for flavoring, seasoning and imparting aroma in foods. They contain many classes of valuable compounds, which can also exert different biological activities, such as antioxidant and antimicrobial activity. It has been widely accepted that oxidative stress, induced by the over production of free radicals and reactive oxygen species in the human body, plays an important role in the disturbance of health and the pathogenesis of various diseases. Antioxidant compounds have the ability to scavenge these free radicals or reactive oxygen species (ROS) to improve human health, prevent, and even treat diseases. However, some synthetic antioxidants have been reported to elicit side effects. Various

methods have been developed and applied for the screening/evaluation of antioxidant activity *in vitro*, measuring the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenge capacity **(**Quah et al., 2020).Spices are any of several vegetable substances used to season or flavour food. They are usually dried for use and have distinct flavour and aroma. Common examples Include cumin, fennel and caraway, they stimulate appetite by increasing the flow of gastric juice and are used in most homes and restaurants all over the world (Nwinuka et al., 2005).

Cumin (*Cuminum cyminum*) is known as a small and herbaceous annual plant. It is commonly used as a spice and flavoring agent due to its distinctive aroma. Cumin seeds contain antioxidants and anti-inflammatory compounds that may help reduce inflammation and oxidative stress in the body, which can contribute to chronic diseases. Cumin is used in traditional and veterinary medicines as an astringent, carminative, and stimulant, and to treat diarrhea, flatulence, and indigestion (Al-Snafi , 2015) .

Carum genus has 25 species, which Carum carvi or caraway is the only annual and biennial economical one as spice, aperitif, and carminative in food and pharmaceutical industries. Caraway is widely used in food products due to its pleasant flavor and preservative properties. Caraway fruits are used as remedy to cure indigestion, pneumonia, and as carminative, appetizer, and galactagogue in different traditional systems[1, 2]. According to European Union herbal monograph, caraway is traditionally used for symptomatic relief of digestive disorders (bloating and flatulence). Caraway fruits are used as popular remedy to mask alcoholic breath, anemia, and as antidote agent against venomous beats. Caraway fruits are used for flavoring of rye bread and its infusion is a remedy for colic and digestive disorders, and to fight worms[3]. Caraway fruits possess stimulant, expec-torant and antispasmodic effects and is used for stomach aches, constipation, and nausea. It increases the secretion of gastric juice and promotes the discharge of bile, which increases the appetite and has digestive stimulatory effects[4] Caraway (*Carum carvi*) is the only annual and biennial economical one as spice, and carminative in food and pharmaceutical industries. Caraway is widely used in food products due to its pleasant flavor and preservative properties. Caraway fruits are used as remedy to cure indigestion, pneumonia, and as carminative, appetizer in different traditional systems (Malhotra S, 2006). Caraway fruits are used for flavoring of rye bread and its infusion is a remedy for colic and digestive disorders, and to fight worms ( Attokaran M, 2017). It increases the secretion of gastric juice and pro-motes the discharge of bile, which increases the

appetite and has digestive stimulatory effects (Peter K, 2006).

*Foeniculum Vulgare* is usually a perennial, aromatic plant belonging to Apiaceae family with many subspecies and varieties. Fennel (*Foeniculum vulgare*) is an essential aromatic plant with medicinal properties and has well-defined anti-inflammatory and antimicrobial activities (Faudale *et al*., 2008). It has traditionally been regarded as a spice and a medicinal herb. It is a highly valued medicinal crop that is used as an anti-inflammatory, anti-oxidant, intoxicant, gastrointestinal, mucolytic, and spasmolytic agent (Ruberto *et al.,* 2000).

This study was aimed to find out the phytochemicals and antioxidant activity of some spices by TLC guided bio autography. It was hypothesized, that complex compounds can be responsible for the antioxidant action of methanol extracts of cumin, caraway and fennel. A great number of TLC techniques have been developed and successfully applied for qualitative and quantitative analysis of antioxidants ( Zhao *et al*., 2010), and the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was often used as a derivatization reagent for this purpose (Olech M, *et al*., 2012). In the screening of antioxidants, the TLC bio autography assay is the method of choice due to several advantages that include flexibility, simplicity and high throughput (Badarinath, A.V, et al., 2010).

## Material and Methods

### Plant material and extraction

*Cuminum cyminum*, *Carum carvi* a*nd Foeniculum Vulgare* seeds were collected from Himalaya wellness company, Dehradun. The dried seeds of each spice were crushed separately using a mixer grinder, obtained powder was added with methanol (ie.50g of sample powder in 250ml of solvent) in an iodine flask and kept overnight for 24h. Samples were filtered dried, weighed and stored in refrigerator at $4^0$C till use. The dried samples were used with appropriate solvent before study (S Mpofu et al., 2014).

### Thin Layer Chromatography

HPTLC finger printing performed for standardization of the drug. CAMAG HPTLC system equipped with Linomat -5 for sample applicator for sample application, Reprostar3 for visualization under UV light at 254nm and 366nm respectively and winCATS software was used to analyse the methanol extract of different spices (Wagner H *et al*., 1984).

### TLC DPPH bio-autography for antioxidant activity

TLC Bio autography Assay Thin-layer chromatography (TLC) was used to separate the chemical constituents. The filtrate methanol extract were loaded on to the activated Silica gel G (Merck) plate. The antioxidant compounds were separated using two type of mobile phase of Toluene: Ethyl

acetate (75:25) and also in chloroform: methanol (90:10). Once the run is completed, plates were air dried for 15 min and the plates were sprayed by 0.002% DPPH solution in methanol using a spray gun for 5 sec. The image was observed under visible light at exactly 2 min after spraying using a white light illuminator. The bright yellow bands against the purple background confirm the antioxidant molecule (LI Mensor et al., 2001). The Rf value of the samples were calculated.

## Phytochemical Screening

The crude extract was tested for the presence of bioactive compounds by using following standard methods (Hamburger, M. *et al.,* 1991) and (Madhukar. C., 2013).

### Test for Carbohydrates

Molish's Tests (general test) To 2ml of the extract solution few drops of molish's reagent and conc. sulphuric acid was added in the test tube. Violet colored ring appears at the junction of two liquid.

### Test for Steroid and Triterpenoid

Salkowaski Test - To 2 ml of the extract solution 2 ml of chloroform and 2 ml of conc. H2SO4 was added and shake well. Chloroform layer appears red colour

### Test for Flavanoids

Shinoda Tests - To 2 ml of the extract solution, few magnesium turnings, 5 ml 95% ethanol and few drops of conc. HCl was added. Pink to red colour develops.

**Test for Alkaloids:** Evaporate the aqueous and alcoholic extract separately. To the residue add dil. HCl shake well and filter, perform the following tests.

1. **Dragendorff Test -** To 2 ml of the extract solution, Dragendroff reagent (potassium bismuth iodide solution) was added. Orange brown precipitate is formed.
2. **Mayers Test -** To 2 ml of the extract solution, Mayers reagent (potassium mercuric iodide solution) was added. Precipitate is observed.

### Test for Tannins

1. To 2 ml of the extract solutions add 5% ferric chloride solution, deep blue colour is observed.
2. To 2 ml of the extract solution add few drops of 10% lead acetate solution, white precipitate is observed.

### Tests for Phenol

Methanol Extract To 2mL of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

### Tests for Saponins

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

### Tests for Glycosides

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5 ml of concentrated sulphuric acid was carefully added by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of glycosides.

TLC profiling of Methanol extracts gave an impressive results that directing towards the presence of number of phytochemicals. Different phytochemicals gave different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts was achieved by analyzing the Rf values of compounds in different solvent system.

## Result and Discussion

In phytochemical screening of the present study carried out in the spices revealed the presence of medicinal active constituents

**Table-1 Results of phytochemical screening of methanol seed extract of three spices:**

| Phytochemical Test | *Cuminum cyminum* ( Methanol extract) | *Carum carvi* ( Methanol extract) | *Foeniculum Vulgare* ( Methanol extract) |
|---|---|---|---|
| Tannins | + | + | - |
| Flavanoids | + | + | - |
| Terpenoids | - | - | - |
| Saponins | + | - | - |
| Steroids | + | + | + |
| Carbohydrates | + | + | + |
| Glycosides | + | + | + |
| Alkaloids | + | + | + |
| Phenol | + | + | + |

**Key:** + indicates presence of the Phytoconstituents
- indicates absence of the Phytoconstituents

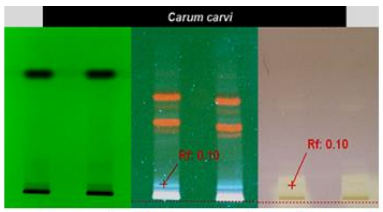


Fig 1: TLC Bio autography of *Carum carvi* in Toluene: ethyl acetate ( 75: 25)

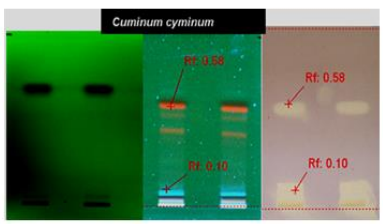


Fig 2: TLC Bio autography of *Cuminum cyminum* in Toluene: ethyl acetate ( 75: 25)

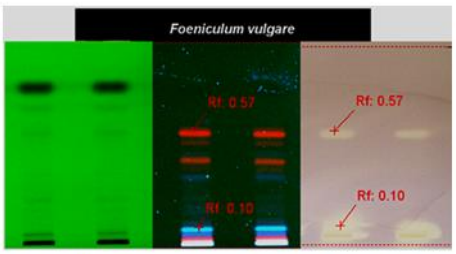


Fig 3: TLC Bio autography of *Foeniculum vulgare* in Toluene: ethyl acetate ( 75: 25)

## TLC-DPPH assay

TLC-DPPH test was applied to the plant extracts to identify radical scavenging activities of separated compounds. DPPH bioautograms of plant extracts were compared $UV_{254}$ and $UV_{366}$ visualization of TLC plate developed by using toluene: ethyl acetate: (75:25 v/v) mobile phase. TLC analysis was carried out for separation of more than six standard compounds including alkaloids, flavanoids, terpenoids, Phenol and steriods was detected in all samples. (**Figuer-1**) shows the DPPH activity *of Carum carvi* at Rf between 0.01 to 0.20. In (**Figure-2**) *Cuminum cyminum* shows strong activity at Rf 0.58 and also some constituent shows antioxidant activity between Rf 0.01-0.20. In (Fig -3) *Foeniculum vulgare* shows two distinct spots are visible, labelled with Rf values of 0.57 and 0.10.The spot with Rf 0.57 is likely a more polar compound, interacting more strongly with the stationary phase. The spot with Rf 0.10 is less polar and interacts more with the mobile phase.

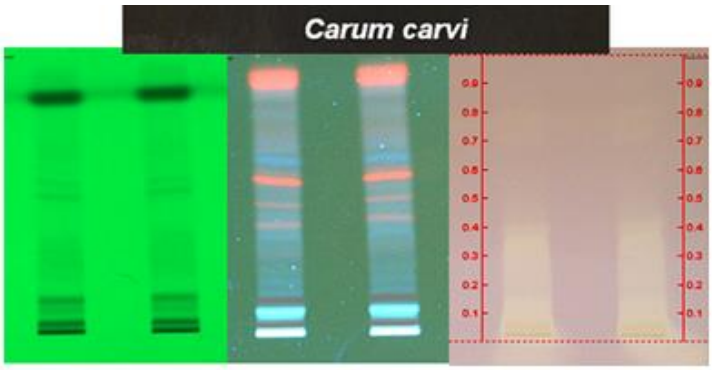


Fig 4: TLC Bio autography of *Carum carvi* in Chloroform :Methanol ( 90: 10)

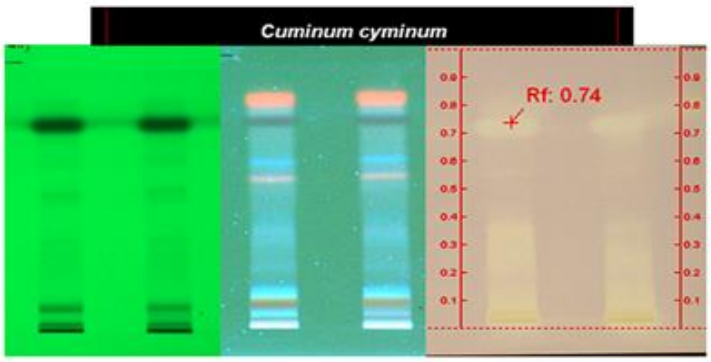


Fig 5: TLC Bio autography of *Cuminum cyminum* in Chloroform :Methanol ( 90: 10)

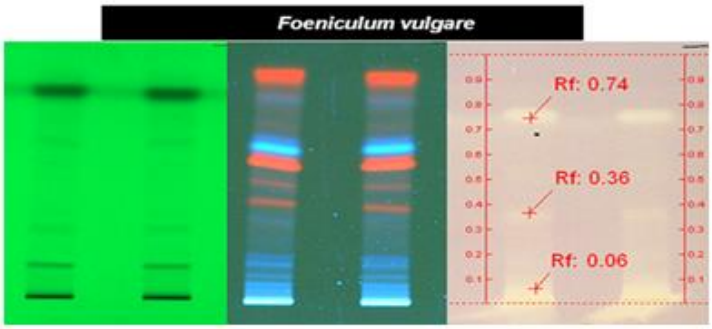


Fig 6: TLC Bio autography of *Foeniculum vulgare* in Chloroform :Methanol ( 90: 10)

## DPPH assay was used to determine the free radical-scav-

The plant extract was applied to a TLC plate and developed using mobile phase chloroform: methanol (90:10). The TLC plate shows several bands, suggesting the presence of multiple compounds of *Carum carvi*. It shows the strong antioxidant activity at Rf value (0.01 to- 0.40) (**fig-4**). In (**fig- 5**) *Cuminum cyminum* shows one distinct band is visible with an Rf value of 0.74. This suggests the presence of a compound with a specific polarity that may have antioxidant activity. In *Foeniculum vulgare* the extract shows multiple zones of inhibition with Rf values around 0.74, 0.36 and 0.06. This indicates the presence of multiple antioxidant compounds in the extract (**Fig-6**).

## Conclusion

Spices are indeed effective sources of antioxidants. Antioxidants are compounds that help protect cells from damage caused by harmful molecules called radicals. Free radicals can contribute to various health problems, including chronic diseases like heart disease, cancer, and neurodegenerative disorders. TLC bioautography provides a valuable tool for understanding the antioxidant profiles of cumin, fennel and caraway. By identifying and characterizing these bioactive compounds, we can connect the power of nature to promote human health and well-being. Considering the problems, we can say that bio-autographic detection technique would create a new era in separation science.

## Disclaimer Statement

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

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# Cultivation of Oyster Mushroom and a Comparative Analysis of its Bioactive Components

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**Abstract-**Mushrooms are one of the most loved foods not only for its exotic taste but also for its benefits for health. It is consumed in various forms like fresh, pickled, dried, powdered, canned etc. Its farming has picked up a fast pace among contemporary entrepreneurs owing to its nutritional and medicinal benefits and low cost. Mushrooms are a fleshy fungus (Basidiomycota & Agaricomycetes) having a stem, cap and gills underneath the cap. They can be edible, wild and some of them can be toxic too. It contains more than 90% water and less than 1% fat, loaded with Vitamin B copper and selenium and low in sodium. Usually vegetables, milk and other food products are fortified with Vitamin D by irradiation or direct addition but mushrooms are unique in this sense because they are naturally a rich source of Vitamin D which otherwise is procured from animals or poultry. The reason being that it contains copious amount of plant sterol "Ergosterol", it is a precursor of Vitamin which when stimulated by sunlight or artificial lightening source converts to Vitamin D.

**Key Words:** Mushroom, cultivation, spawn, substratum, harvesting, Polyphenols, Flavonoids

## Introduction

Mushrooms are a fleshy spore-bearing fruiting body of a fungus having a stem, cap and gills undeneath the cap. Mushrooms are natural source of foods and medicine. Approximately 15,000 species of mushrooms are known worldwide, about 2000 are used for human consumption and more than 700 have medicinal properties[1].

Mushroom or toadstools can be edible or toxic based on their aroma, taste, toxicity and physical appearance. Mushrooms can also be categorized based on their habitats such as temperate, tropical or sub-tropical mushroom. *Pleurotus* consists of about 40 species distributed in a wide range of tropical and temperate regions Twenty-six species, including *Pleurotus eryngii* (*PE*), *Pleurotus citrinopileatus* (*PC*), *Pleurotus flabellatu* (*PFL*), *Pleurotus ostreatus* (*PO*), *Pleurotu djamors* var. *roseus* (*PDR*), and *Pleurotus florida* (*PF*), have been reported to be cultivated using different types of lignocellulosic wastes[2]. *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide after Agaricus bisporus[3]**.** Mushroom *Pleurotus ostreatus* (Fr.) Kumm., used in cultivation for over 100 years, is now in the third place in the world in terms of production volume, after champignon mushrooms and Lentinula edodes (shiitake)[4]. The most cultivated mushroom worldwide is Agaricus bisporus, followed by Lentinus edodes, Pleurotus spp., and Flammulina velutipes[5]**.** Pleurotus species are widely cultivated throughout the world owing to its excellent flavor and low-cost production technology. It can be cultivated at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55–70% because of its ability to adapt a variety of factors[6]**.**

The production of oyster mushrooms and the research thereof, dates back to 1917 by Falck, who described the cultivation of Pleurotus on tree tumps and logs[7]**.** It is estimated that the first mushroom was cultivated around 600A.D. This was *Auricularia auricula*. Later, around 800-900 A.D Flammilina *velutipes* was also cultivated in China.*P. ostreatus* is considered as a healthy food because it is rich in a variety of bioactive compounds including polysaccharides, proteins, amino acids, polyphenols, vitamins and fatty acids[8]. *P. ostreatus* are well known for their medicinally beneficial activities such as anticancer activities, immune-modulatory effects, and antiviral, antibiotic anti-inflammatory and cholesterol lowering activities[9].

## Literature Review

### Global Scenario:

Oyster mushrooms are the third largest cultivated mushroom. China, the world leader in Oyster production, contributes nearly 85% of the total world production of about a million tonnes. The other countries producing oyster mushrooms include Korea, Japan, Italy, Taiwan, Thailand and Phillipines[10].

The global mushroom market size was USD 17.25 million tonnes in 2023 and is projected to grow from USD 18.39 million

tonnes in 2024 to USD 32.04 million tonnes in 2032 at a CAGR of 7.18% during the forecast period (2024-2032)[11]. Majorly six mushrooms dominate the global production and market viz, shiitake mushroom (26%), oyster mushroom (21%), black ear mushroom (21%), button (11%), Flammulina (7%), paddy straw mushroom (1%), and others mushrooms (13%).

### Indian Scenario:

India receives a lukewarm response in the production of mushroom though mushroom cultivation, both in east and west started many centuries ago. First systematic attempt in cultivating button mushroom was made in 1961, when a scheme entitles "Development of Mushroom Cultivation in Himachal Pradesh" was started at Solan by H.P. Government in collaboration with ICAR, New Delhi[12].

Oyster mushrooms were analysed using the HPLC-DAD technique by Palacios and co-workers (2011). Several polyphenols were identified in the samples, including p-coumaric acid (11.15 ± 0.85 mg/g), ferulic acid (20.16 ± 0.16 mg/g), gallic acid (290.34 ± 3.61 mg/g), gentisic acid (292. 62 ± 3.42 mg/g), p-hydroxibenzoic acid (4.69 ± 1.59 mg/g), homogentisic acid (629.86 ± 1.54 mg/g), myricetin (21.99 ± 0.89 mg/g) and protocatechuic acid (19.32 ± 0.84 mg/g)[13].

Phenolic compounds such as phenol, flavonoid, and tannins are known to exhibit a significant amount of antioxidant properties. Rahimah et al. conducted a study on white oyster mushroom to evaluate antioxidant properties; it reveals that nearly 5.45 to 8.03 mg GAE (gallic acid equivalent)/g of total phenol were shown in different concentrations[14].

In the study of Fatimah Buba at.al,. Protein content was 28.45±1.15 mg/g, the elemental content (1.56±1.14 µm/g) for calcium Ca2 (5.04±3.58 µm/g) potassium "K" and (0.55±0.53 µm/g) phosphorus "P". Mushroom appears to have a high content of potassium than the other element[15].

## Material and Methods

### Present Work

Cultivation of oyster mushroom (*Pleurotus ostreatus)* and analysis of its nutritional values. Oyster mushrooms are a rich source of nutraceutical and other bioactive components like phenolic components, flavonoids, alkaloids, tannins, lectins, laccase, vitamins and polysaccharides such as β-glucan as well as other components with high antioxidant activities and therapeutic properties. So the aim for this research project is to cultivate oyster mushroom and analyze nutritional values of oyster mushroom and button mushroom. In my experimental work I did analysis of

polyphenols, flavonoids and alkaloids in button and oyster mushroom among the bioactive components of oyster and button mushroom.

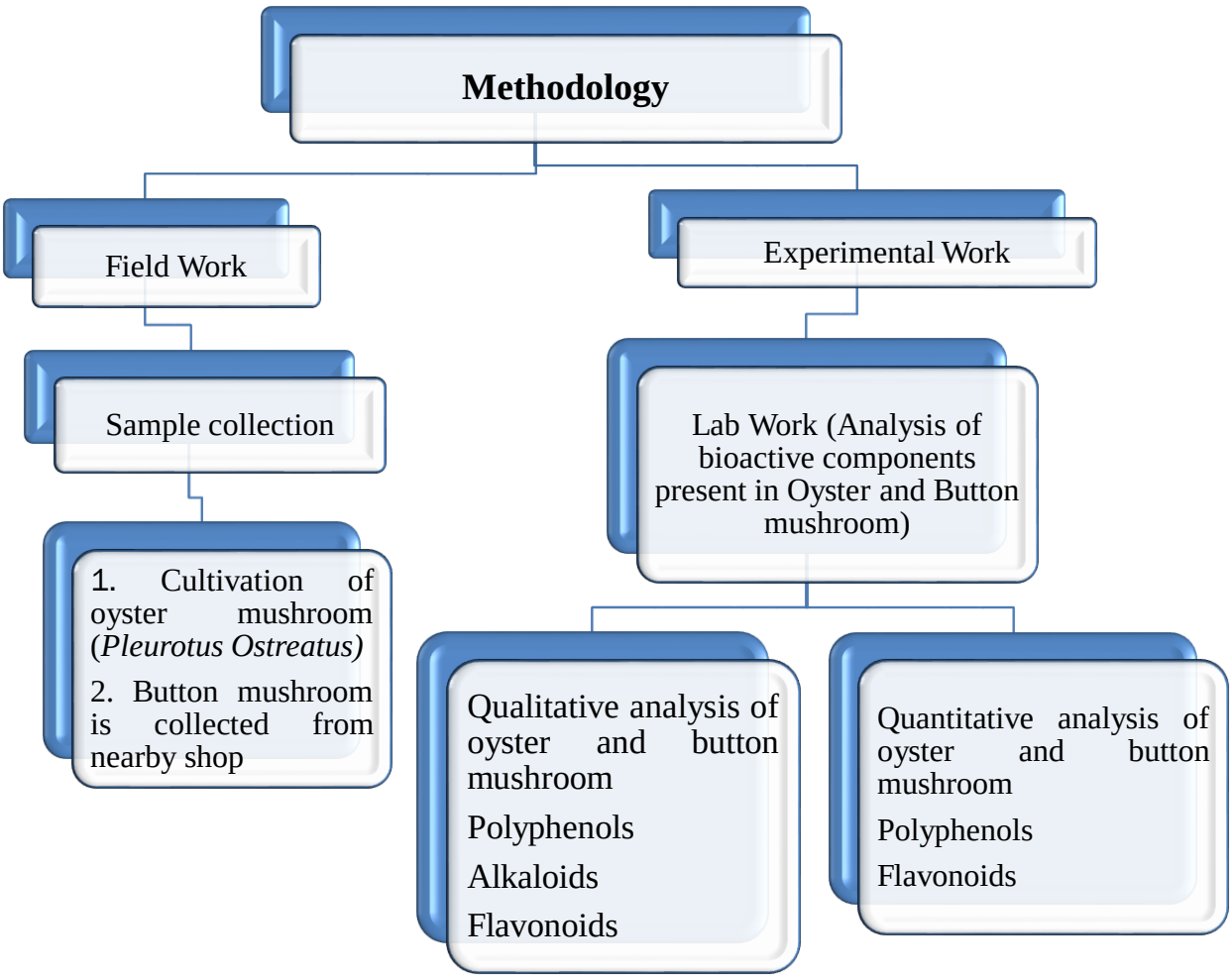



Figure-1 Methdology

## Field Work

Samples of Oyster and Button mushrooms were collected and then cultivation procedure of Oyster Mushroom was started including following steps:

### Step : 1 Preparation of Culture Media

Make a solution of 7.8gm PDA in 200 ml distil water. Mix it well and put it into the autoclave at 121 °C at 5 psi for 25 min. After autoclave place the jar in laminar airflow under UV light. Now fill the slant and petri plates with the PDA solution and let it solidify. Place one piece of cut mycelia form mother petri plates on each petri plates with PDA solution and place them into the incubators.

**PDA (Potato dextrose agar):** It is the most basic and widely used medium for growing mycelia of most cultivated mushrooms. It is

available commercially as a ready-to-use powder that can be used to make the medium in the laboratory with a concentration of 20gm/l of distilled water.

### Step : 2 Preparation of Spawn or Seed

Wheat seed with fungal mycelium called spawn or fungal seeds**.** Wash the wheat seeds in water. Dry the seeds under shade. Add Rose Bengal in the wheat seeds and boil the seeds in water for 50 minutes. Dry the seeds in air by spreading on polythene sheet. Coat the seeds with 2% calcium carbonate to adjust the pH around 7 and also keeping the seeds in individuals. Fill polythene bags with the grain and close it using cotton plug. Autoclave it for 2 hours at 126°C. After autoclave, add the cut pieces of mycelium into the wheat bags and mix it well so the pieces spread evenly. It should be done in the laminar airflow and after mixing put these bags inside the incubator. After 15-20 days mycelium grows on the grain and afterwards it can be used as spawn.

### Step: 3 Preparation of Substratum

Soak the Paddy Straw in Water for 24 Hours. Boil in water for one hour. Dry under the shade the pasteurized substrates. But maintains moisture content up to 65-70% Spray 10% calcium carbonate solution for maintain the pH around 7. Add Gypsum For High Yield. Now, the substrate mixture is ready for mushroom cultivation. Use polypropylene bags for the cultivation of mushroom.

### Step: 4 Method of Cultivation

The cylindrical block System is better for Oyster mushroom cultivation. Take the substrate mixture in polypropylene bag about 5 cm height as a first layer. Spread 50 gm of spawn on the substratum. This process called spawning. Make the second layer of substratum about 10 cm height. Spread 50 gm of spawn on the substratum, mostly on the periphery. Repeat this process for several times in the same manner. Finally, the spawn covered with 5 cm height of substratum. Make many holes on the surface of the bag for watering, good aeration and reduce temperature. The inoculated bags then transferred to an incubation chamber and the temperature is kept between 21 and 22 degrees Celsius. The mycelium can move through the substrate and start consuming it at that temperature. In about two weeks, the bags will begin to turn white as the mycelium colonizes them. Afterward, the loads must be transferred from the inoculation room to the fruiting chamber. A small hole must be pierced in the face of each bag. This exposes the mycelium and substrate to fresh air and humidity. The mushroom will then start growing. After one week, each of these

will produce a lovely bouquet of fresh mushrooms. After that, it's harvest time!

### Step: 5 Harvesting and Yield

Harvesting is done when the cap has the diameter of 8-10 cm. Picking is done by twisting gently so that it is pulled out without leaving any stalk and also the nearby fruiting bodies are not disturbed. When the base of the stipe is deeply immersed within the straw, cutting the base of stipe with sharp knife can be done. It is possible to get 500-800 g to a kilogram fresh mushrooms per kilogram of the dry substrate. The bags are kept in the growing chamber after the first harvest to allow other mycelium to grow and produce more fruiting bodies, which can then be harvested again.

## Experimental Work

### A. Qualitative and Quantitative analysis of Bioactive components of Button Mushroom and Oyster Mushroom

**Sample Preparation:** Drying of both the mushrooms under shading. After drying make powder of dry mushroom using mortar and pestle. Powdered mushrooms is then mixed with 50 mL distilled water and after mixing put the jars in the rotatory flask shaker for 24 hours. After 24 hours sample is ready for analysis.

**Reagents Required:** Sodium hydroxide, Ferric chloride, Wagner's reagent.

**Table-1 Qualitative analysis of oyster mushroom and button mushroom**

| Experiment | Observation | Inference |
|---|---|---|
| **Test for Flavonoids**<br>Alkaline reagent test:<br>Add few drops of sodium hydroxide solution to test solution | Development of intense yellow colour | Presence of Flavonoids |
| **Test for Alkaloids**<br>Wagner's test:<br>Two drops of Wagner reagent was added to extract and mixed well | Appearance of a reddish color indicates the presence of alkaloids. | Alkaloids present |
| **Test for Polyphenols**<br>This test depends on the variety of changes that happens when polyphenols respond with ferric chloride. A couple of drops of ferric chloride is added to the sample, and the development of variety change, typically a blue, green, or earthy colored tone, demonstrate the presence of polyphenols | Presence of earthy colored tone | Presence of Polyphenols |

**Structure of Alkaloids** **Phenolic Acids** **Structure of Flavonoids**

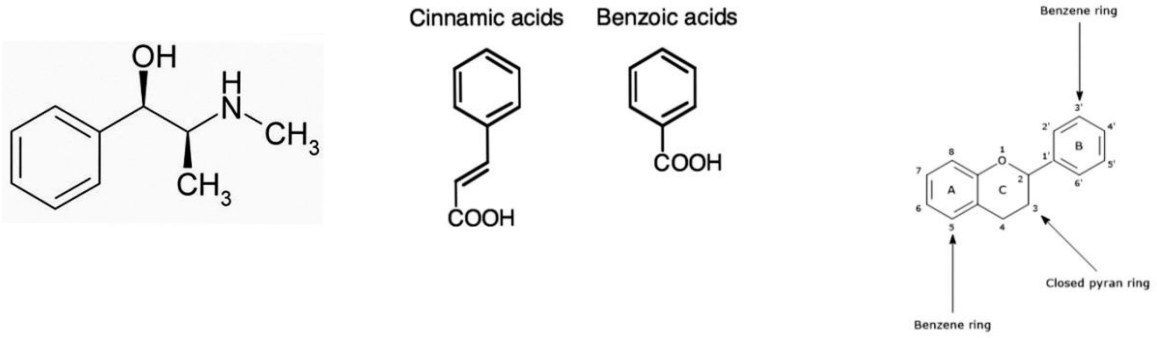



**Figure- 2**

## B. Quantitative Analysis of Oyster and Button Mushroom

**Preparation of Standards:** Prepared a series of standard solutions of a reference polyphenol compound. Commonly used standards include Gallic acid, catechin, or tannic acid. Concentrations typically range from 40,50,60 µg/mL

**Preparation of Sample Extracts:** Extracted polyphenols from mushroom samples using an appropriate solvent mainly distilled water.

**Folin-Ciocalteu Assay:** Took aliquots of appropriately diluted sample extracts and placed them in test cuvettes. Added an appropriate volume of Folin-Ciocalteu reagent to each tube. The Folin-Ciocalteu reagent is usually added in excess to ensure all polyphenols are oxidized. Mixed thoroughly and allow the reaction to proceed in the dark for a specified time (typically 30 minutes to 2 hours) at room temperature. During this time, the Folin-Ciocalteu reagent reacts with the polyphenols to form a blue-colored complex.

**Measurement of Absorbance:** After the reaction period, measure the absorbance of each sample at a specific wavelength using a spectrophotometer. The wavelength was measured at 765 nm. Recorded the absorbance values for both mushroom sample extracts and the standard solutions.

**Calculation of Total Polyphenol Content (TPC):** Constructed a calibration curve using the absorbance values of the standard solutions plotted against their known concentrations (µg/mL). Used the equation of the calibration curve to determine the concentration of polyphenols in sample extracts. Express the TPC in µg of Gallic acid equivalents (GAE) per gram or milliliter of sample extract (µg GAE/g or µg GAE/mL).

**Table-2 Optical Density of Polyphenols content in button mushroom and oyster mushroom**

| OD (Optical density) of Button Mushroom | Concentration (µg/mL) of Button Mushroom Absorbance=0.045× Concentration +0.002 | OD (Optical density) of Oyster Mushroom | Concentration (µg/mL) of Oyster Mushroom Absorbance=0.045× Concentration+0.002 |
|---|---|---|---|
| 0.000 | 0.000 | 0.000 | 0.00 |
| 1.352 | 30 | 1.834 | 40.71 |
| 1.357 | 30.53 | 1.836 | 40.53 |
| 1.376 | 30.31 | 1.817 | 40.33 |

**Table-3 Optical Density of Flavonoids Content in button mushroom and oyster mushroom**

| OD ( Optical density) of Button Mushroom | Concentration(µg/mL) of Button mushroom Absorbance =0.037× Concentration+ 0.015 | OD (Optical density) of Oyster Mushroom | Concentration (µg/mL) of Oyster Mushroom Absorbance= 0.037+ Concentration+0.015 |
|---|---|---|---|
| 0.000 | 0.000 | 0.00 | 0.00 |
| 0.0722 | 19.10 | 1.028 | 0.35 |
| 0.730 | 19.32 | 1.048 | 0.81 |
| 0.720 | 19.05 | 1.055 | 1.08 |

## RESULT AND DISCUSSION

In qualitative study we found out the presence of flavonoids, alkaloids and polyphenols in both Oyster mushroom *(Pleurotus ostreatus*) and Button mushroom (*Agarcius Bisporus).*

**Table-4 Qualitative Results of analysis of Oyster and Button mushrooms**

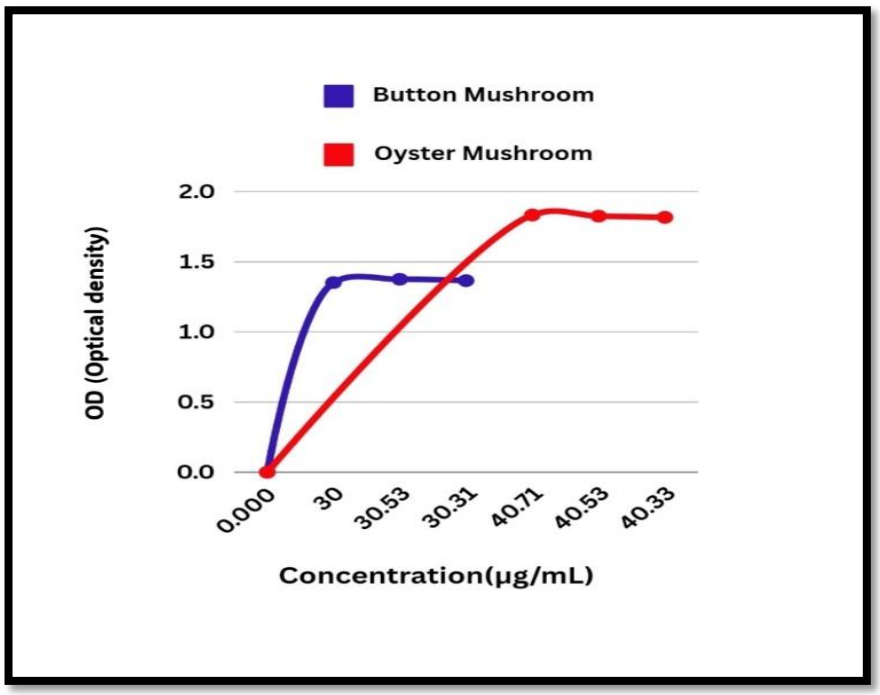
| Sr. No. | Qualitative test | Observation | Results |
|---|---|---|---|
| I. | Flavonoids test: Alkaline reagent test | Positive | Presence of flavonoids |
| II. | Alkaloids test: Wagner's test | Positive | Presence of alkaloids |
| III. | Test for Polyphenols | Positive | Presence of polyphenols |

## Quantitative results of Oyster and Button Mushrooms

### TPC (Total phenolic content)

In quantitative study the total phenolic content (TPC) of Button Mushroom sample, expressed in µg/mL of gallic acid equivalents (GAE), is approximately 30.28 µg/mL and Oyster Mushroom sample is approximately 40.52µg/mL.

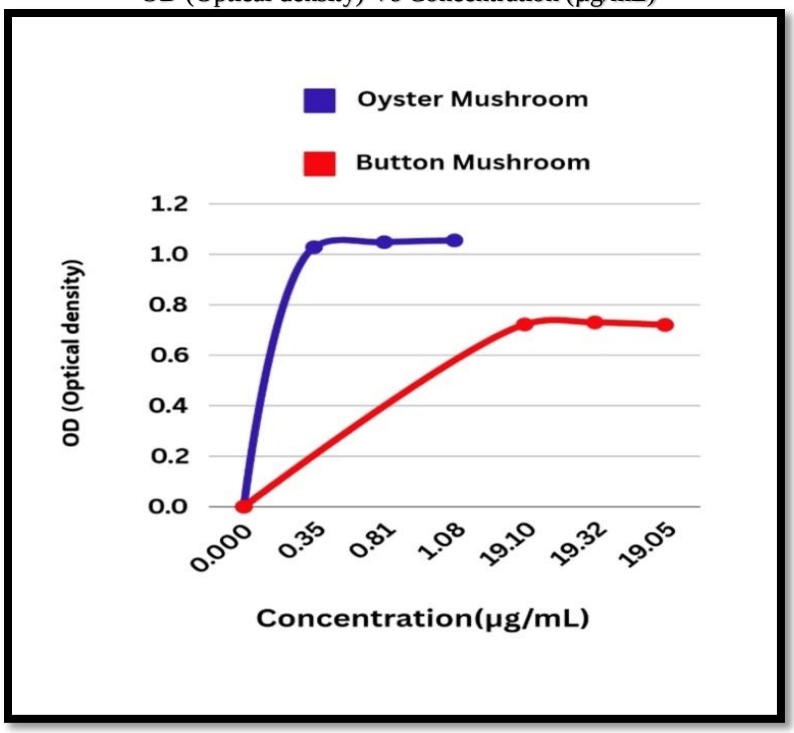
Chart-1 Polyphenols Content
OD (Optical density) Vs Concentration (µg/mL)




## TFC (Total Flavonoid Content)

The total flavonoid content (TFC) of the Button Mushroom sample is approximately 19.05 µg/mL and Oyster Mushroom sample is 0.74µg/mL.

Chart-2 Total Flavonoid Content
OD (Optical density) Vs Concentration (µg/mL)



This research aimed to cultivate the Oyster mushroom *(Pleurotus ostreatus) a*nd analyze the bioactive components of Oyster Mushroom *(Pleurotus ostreatus)* and compare them with Button Mushroom *(Agarcius Bisporus)*. Mushrooms are one of the most popular and versatile gifts of nature. It can be mixed into any food preparations or can be processed to give a new product. A lot of mushroom products are currently available in market such as mushroom pickle, seasonings, beverages, extracts, dried and canned mushrooms, mushroom supplements, cosmetics etc. Apart from the mushroom food products many innovative products are emerging in other industries as well such as mushroom based building materials, medicines, myceliabased platforms, biodegradable packaging, mycelium based leather etc.Mushrooms are easy to cultivate, have quick growth and nil carbon emission and waste generation.Oyster mushrooms can grow on a ton of different materials, including hardwood sawdust, supplemented sawdust, straw, the masters mix, coffee grounds, paper, and pretty much any other liginous material. Here we used paddy straw as substrate for oyster mushrooms. For spawn preparation of oyster mushroom we can use different grains like wheat, barley, sorghum and millet grains could be equally used in the production of good quality spawn for the cultivation of oyster mushrooms.

## Conclusion

Oyster mushroom can grow at moderate temperature ranging from 20° to 300° C and humidity 55-70% for a period of 6 to 8 months in a year.The fungi is a good source of income generation for the growers and also provides additional benefits through its processing.Mushrooms holds a bright future in every aspect owing to its diverse properties. Mushroom cultivation is highly compatible with a variety of other traditional agricultural and domestic activities, and can make a particularly important contribution to the livelihoods of the disabled, of women and the landless poor who, with appropriate training and access to inputs, can increase their independence and self-esteem through additional income generation. In qualitative analysis we found out the presence of flavonoids, alkaloids and polyphenols in button mushrooms and oyster mushrooms and in quantitative analysis it was observed that Polyphenol contents are found in concentrations are 30.28 µg/mL and 40.52 µg/mL for Button and Oyster mushrooms and the OD are between 1 to 2 as shown in table-2. Study of Optical Density of Flavonoids in different concentrations of oyster Mushroom and button mushroom have shown values from .072 to 1.08 as

given in table-3. So, we can conclude that both the mushrooms are good source of bioactive compounds and considered as super food.

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## Disclaimer Statement

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

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# About flowers on the cover

## Mangifera indica

**Scientific name:** *Mangifera indica*

**Family:** Anacardiaceae

**Order:** Sapindales

**Genus:** Mangifera

**Kingdom:** Plantae

Mangifera indica, commonly known as mango, is an evergreen species of flowering plant in the family Anacardiaceae. It is a large fruit tree, capable of growing to a height and width of 30 metres. There are two distinct genetic populations in modern mangoes – the "Indian type" and the "Southeast Asian type". Mango seed powder has an effective nutritional impact as it is rich in dietary fiber, protein, and micronutrients. Antioxidants included in mango seed powder are effective in the treatment of diabetes, diarrhoea, irregular bowel movements, allergies, cancer, urinary tract infections, and haemorrhoids.

# Momordica charantia

**Scientific name:** *Momordica*

**Family:** Cucurbitaceae

**Order:** Cucurbitales

**Genus:** Momordica; L.

**Kingdom:** Plantae

Momordica is a genus of about 60 species of annual or perennial climbers herbaceous or rarely small shrubs belonging to the family Cucurbitaceae, natives of tropical and subtropical Africa and Asia and Australia. Bitter Gourd Seeds with botanical name Momordica charantia, is considered to have medicinal value in lowering blood sugar levels in diabetic patients. Bitter gourd plant is a fast growing warm seasonal climbing plant that can be grown in any season in tropical climates. It is a source of most Vitamins, i.e., A, B, C, E, and Zinc, Potassium, and other essential nutrients. It controls blood sugar, boosts the immune system, and guards against parasites, in addition to being a wonderful source of anti-inflammatory, anti-fungal, and anti-parasitic properties.

# Datura stramonium

**Scientific name:** *Datura stramonium*

**Family:** Solanaceae

**Genus:** Datura

**Order:** Solanales

**Kingdom:** Plantae

Datura stramonium, known by the common names thornapple, jimsonweed, or devil's trumpet, is a poisonous flowering plant in the Daturae tribe of the nightshade family Solanaceae. Its likely origin was in Central America, and it has been introduced in many world regions. The seeds of Datura are analgesic, anthelmintic and anti-inflammatory and as such, they are used in the treatment of stomach and intestinal pain that results from worm infestation, toothache, and fever from inflammation. The juice of its fruit is applied to the scalp, to treat dandruff and falling hair.

# Prunus cerasoides

**Scientific name:** *Prunus cerasoides*

**Family:** Rosaceae

**Subgenus:** Prunus subg. Cerasus

Prunus cerasoides, commonly known as the wild Himalayan cherry, sour cherry or pahhiya is a species of deciduous cherry tree in the family Rosaceae. Its range extends in the Himalayas from Himachal Pradesh in north-central India, to south-western China, Burma and Thailand. The leaves, twigs and bark contain a cyanogenetic substance. The bark is used for plastering fractured bones. The smaller branches are crushed and soaked in water and taken internally to stop abortion. Its stem is antipyretic, refrigerant and useful in treating vomiting, leprosy and leucoderma.

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   December 12-15, 2024
2. **12th International Conference and Exhibition on Natural Products and Medicinal Plants Research, Rome, Italy**
   December 12-13, 2024
3. **29th Annual Meeting on Pharmaceutical Biotechnology, London, UK**
   January 06-07, 2025
4. **2nd International Conference on Pharmacognosy, Madrid, Spain**
   February 24-25, 2025
5. **8th International on Pharmacy and Pharmaceutical Conference, Prague, Czech Republic**
   March 13-14, 2025
6. **4th International Conference and Expo on Pharmaceutics & Novel Drug Delivery Systems, Vancouver, Canada**
   April 07-08, 2025
7. **18th World Drug Delivery Summit, London, UK**
   April 24-25, 2025
8. **7th International Conference and Exhibition on Pain Management, Zurich, Switzerland**
   May 19-20, 2025
9. **24th International Conference on Medicinal and Pharmaceutical Chemistry Vancouver, Canada**
   June 10-11, 2025
10. **18th International Conference and Exhibition on Pharmaco-vigilance & Drug Safety, Frankfurt, Germany**
    June 16-17, 2025
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    June 23-24, 2025

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**Prof. Durgesh Pant**
Director General