Phytochemical Screening And *In Vitro* Antimicrobial Activity Of Methanolic Extract Of *Ocimum sanctum* Against Some Selected Microbes.

[1]Bilal Javed Bukhari and [2]Dr.A.P.Singh

[1.] Research Scholar, Centre for Biotechnology Studies, Awadhesh Pratap Singh University, Rewa, (M.P.). India.

[2.] Professor, Department of Botany, Govt. Model Science College Rewa, (M.P.). India.

Abstract: The present study was to evaluate the qualitative estimation of phytochemicals and antimicrobial activity of methanolic extracts of leaves of *Ocimum sanctum* against some selected microbial strains. The qualitative analysis was carried out to investigate the presence or absence of Alkaloids, Carbohydrates and Glycosides, Phenolic compounds and Tannins, Proteins and Amino acids, Flavonoids, Terpenoids, Saponins, Phlobotannins and Steroids. The antimicrobial potential of methanolic extract of *Ocimum sanctum* was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. Antimicrobial activity was studied using disc diffusion method against three Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) bacteria pathogens.

Keywords: *Ocimum sanctum*, Phytochemicals, Antimicrobial activity, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*

I. Introduction

Medicinal plants are a vital therapeutic aid for a variety of ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Yadav, 2012). Plant extracts have been used for different purposes for several thousands of years. Antibacterial screening of medicinal plants offers clue to develop newer drugs (Ali *et al.*, 2017). In India, from ancient times, different parts of medicinal plants have been used to cure specific aliments. Today, there's a widespread interest in medicines derived from plants. Natural antimicrobials can be derived from plants, animal tissues, or Microorganisms (Gordon and David, 2001). Plants are the friends of man in survival, giving him food and fuel and medicine from the days beyond drawn of civilization (Bose and Choudhary 1991). In recent years, the utilization of plants in ancient drugs has raised the interest in ethnobotanical studies throughout the planet. In fact, the World Health Organization (WHO) estimates that seventieth of populations from several countries area are utilizing ancient drugs to cure numerous ailments (Jiofack *et al*., 2010).

Ocimum sanctum also known as Tulsi is a sacred plant of Hindu religion worshiped all over the India belonging to the Family Lamiaceae is a erect, branched fragmented shrub with the height of about 30- 60cm when mature. Its leaves are simple, aromatic, branched, opposite, obtuse, elliptical and have dentate margins (Joseph 2013). The leaves of the plant are thought to be very sacred and often form a regular part of the Hindu religious rituals (*Tirtha* or *Prasada*). *Ocimum sanctum* having two varieties i.e. black (*Krishna Tulsi*) and green (*Rama Tulsi*), their chemical constituents are same. Both the varieties also possess similar medical properties (Das and Vasudevan 2006). They are up to 5cm long. Flowers are elongate raceme in close whorls and purple in colour. Seeds are radish yellow and fruits are small (Kumar 2012).

They are cultivated in different parts of the world and are widely known for their medicinal properties (Buddhadev 2014). Tulsi is also described as: Vanya (wild) and Gramya (Grown in homes) (Kumar 2012). It is planted after rainy season and harvested after few months. Plant is useful in the treatment of cold, cough, malaria, dengue, bronchitis, asthma, sore throat, influenza, heart disorders, eye diseases, mouth infections, insect bites, stress, and kidney stones etc. (Joseph 2013). A number of therapeutic properties have been accredited to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani system of medication (Jeba *et al*., 2011).

The present study was aimed to investigate the phytochemical constituents and antibacterial effects of *Ocimum sanctum* against three Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) bacteria pathogens.

II. Materials and Methods

2.1 Collection and identification of Plants

The plant used for study was collected from various localities of Rewa district in Madhya Pradesh, India. The plant was identified and authenticated by Dr. A.P. Singh, Professor, Department of Botany, Govt. Model Science College, Rewa.

2.2 Drying

Plant materials were washed, dried in shade, grinded to fine powder and stored in airtight container at room temperature in the dark until used (Tetyana *et al.*, 2002). After that the powdered samples were dried in hot air oven at 40°C for an hour just before starting the extraction process to remove any moisture content.

2.3 Extraction Process

Soxhlet extraction

The powdered samples were subjected to extraction using organic solvent methanol following the method of Nair *et al.*, (2005). Around 40 g of the powder was packed in a thimble of filter paper prepared manually. The thimble was then inserted into the Soxhlet apparatus and extraction was done by using 250 ml methanol as a solvent. The temperature was maintained at 45°C and extraction was continued for 48 hours. Then the methanol extract was collected and powder from the thimble was dried, weighed and finally discarded. The obtained methanolic extract was dried and stored at 4°C for further studies.

Rotary evaporator

The extracts were then concentrated by rotary evaporator. The crude extract obtained after evaporation were stored in air tight glass containers for phytochemical and antimicrobial screening .

2.4 Screening of phytochemical components

Phytochemical investigation of methanolic extract of leaves of *Ocimum sanctum* was carried out to investigate the presence or absence of Alkaloids, Carbohydrates and Glycosides, Phenolic compounds and Tannins, Proteins and Amino acids, Flavonoids, Terpenoids, Saponins, Phlobotannins and Steroids as per the standard methods (Brain and Turner, 1975; Evans 1996).

2.5 ANTIBACTERIAL ACTIVITY TEST

Microbial species used in the present study were collected as pure cultures from the microbial type culture collection (MTCC) of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Three Gram negative and three Gram positive bacterial strains used in this study *viz Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* respectively were grown on nutrient broth (Himedia) at 37°C for 18-24 hrs and were maintained on respective agar slant at 4°C.

Preparation of the culture media

Nutrient Broth and Nutrient Agar (Himedia)

Nutrient Broth media was prepared by suspending 13.0 grams of nutrient broth in 1000 ml distilled water and heated to dissolve the media completely and then dispensed into conical flasks. Media was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Nutrient Agar media was prepared by suspending 28 grams in 1000 ml distilled water and heated to dissolve the media completely and then dispensed into conical flasks. Media was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Media was allowed to cool around 45-50°C.

Antimicrobial assay

The antimicrobial assay of crude extracts was performed by disc diffusion method (Bauer *et al*., 1966). Petri plates were sterilized in Hot Air Oven at 160^0C for 1 hour. Cultured organism (1 ml) was poured in petri plates with the help of pipette and 25 ml of cooled media was added to the plate and left for some time to solidify. For testing the antimicrobial activity of crude extracts, uniform filter paper discs (6 mm diameter) were formed, sterilized and dipped in methanolic extract of *Ocimum sanctum*. The filter paper discs were placed in Petri dishes and the plates were labeled. The plates were incubated at 37^0 C for 24 h. After the incubation, the plates were examined for inhibition zone. The inhibition zones were then measured. The tests were repeated three times to ensure reliability.

III. Results and discussion

Phytochemical screening of the methanolic extracts of *Ocimum sanctum* revealed the presence of alkaloids, saponins, flavonoid, phenolic compounds and tannins (Table 1). Different phytochemicals have been found to possess a wide range of activities, which may help in protection against various diseases. For example, Alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and possess good antibiotic properties (Aiyer and Kolammal 1962). Flavonoids have been referred to as nature's biological reaction modifiers, in view of their characteristic capability to adjust the body's response to hypersensitivities and infection and they show their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer properties (Argal and Pathak 2006).

Table 1: The analysis of phytochemical constituents in the methanolic extracts of leaves of *Ocimum sanctum*

S.No.	Phytochemical constituents	Test/Reagent Used	Methanolic Extract
1.	Alkaloids	Mayer's Reagent	+
2.	Carbohydrates and Glycosides	Fehling solution	-
3.	Phenolic compounds and Tannins	Ferric chloride solution	+
4.	Proteins and Amino acids	Ninhydrin Test	-

5.	Flavonoids	Alkaline Reagent Test	+
6.	Terpenoids	Acidic Reagent Test	-
7.	Saponins	Foam/Froth Test	+
8.	Phlobotannins		-
9.	Steroids		-

"+" indicate presence and "–" indicate absence of phytochemical constituents.

The antimicrobial potential of methanolic extract of *Ocimum sanctum* was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale (Hi-media). The results showed that the methanolic extract of leaves of *Ocimum sanctum* possess good antimicrobial activity against *Pseudomonas aeruginosa* followed by *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* (Table 2).

Table 2: Antimicrobial activity of methanol extracts of leaves of *Ocimum sanctum* against three Gram positive and three Gram negative bacterial species tested by disc diffusion method.

S.No.	**Microorganism**	**Zone of inhibition (mm)**
1.	*Staphylococcus aureus*	14 ±1.24
2.	*Bacillus subtilis*	16 ±1.24
3.	*Bacillus cereus*	-
4.	*Escherichia coli*	17 ±0.94
5.	*Pseudomonas aeruginosa*	18 ±1.70
6.	*Proteus vulgaris*	17 ±0.94

Values were expressed as Mean ± Standard Error Mean

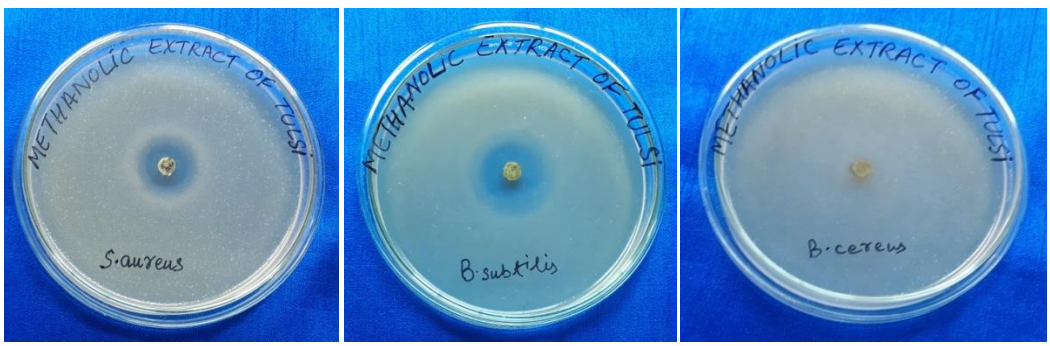

Fig 1: Antimicrobial activity of methanolic extracts of leaves of *Ocimum sanctum* against three Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) bacterial strains.

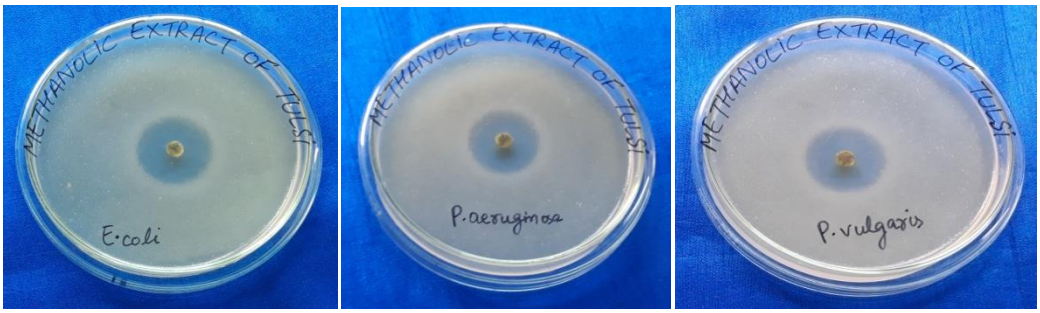

Fig 2: Antimicrobial activity of methanolic extracts of leaves of *Ocimum sanctum* against three Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) bacterial strains.

IV. Conclusion

It has been recommended that phytochemical extract from plants hold promising impacts to be utilized in allopathy as they are potential source of antiviral, antitumoral and antimicrobial agents (Nair *et al*., 2005). Methanolic extract of leaves of *Ocimum sanctum* posses antimicrobial potential against both gram positive and gram negative bacteria. It is thus used as a good antimicrobial agent. The slight differences in the inhibitory effect might be due to qualitative and quantitative differences in the antibacterial principles or compounds present in them. Based on the present investigation, it is revealed that the methanolic extract of leaves of *Ocimum sanctum* contains medicinally important compounds. Study will lead to scientific findings and production of novel drugs so as to improve the human public health importance.

V. References

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