ANTIMICROBIAL ACTIVITY OF *Ocimum tenuiflorum* (tulsi) BY USING LEAF EXTRACT AGAINST DIFFERENT BACTERIAL STRAINS

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ABSTRACT: -

From ancient times, plants are used as an important source of medicines. The large number of drugs that we use today are derived from plant materials like leaves, steams, roots etc. Ocimum sanctum is an aromatic plant and it belongs to the family Lamiaceae. The main advantage of using plant material for medicinal purpose is that they have very less side effects as compared to chemical drugs. Due to widespread emergence of resistance among microbial pathogens against currently available antibiotics, there is a need in medical world to discover novel antibiotics. In search of novel antimicrobial compounds, plants have been proven a good source. Tulsi plant is also known as "Queen of herbs", and it is also one of the most cherished plants. For the prevention and treatment of many diseases,tulsi is a common herbal and cheap remedy. Active principles from plant origin have been used as medicinal compounds, from ancient times.In hindu culture, Tulsi plant is worshipped and also used for its medicinal values. The objective of this research was to analyse the antimicrobial activity of Ocimum tenuiflorum by using leaf extract against different bacterial strains. The antimicrobial activity of Ocimum tenuiflorum leaves was measured by Kirby-Bauer method. Minimum Inhibitory Concentration (MIC) of various extracts and fractions of Ocimum tenuiflorum was also measured.The results of this study showed that Ocimum tenuiflorum leaves possessed antibacterial activities against isolated bacterial strains at the concentrations of 200 mg/ml, 400 mg/ml, and 800 mg/ml. From this study, it could be concluded that Ocimum tenuiflorum contains bioactive compounds that provides strong antibacterial activities against some microbes.

Keywords:- *Ocimum tenuiflorum, Antibacterial, E. coli, Bacillus subtilis, Medicinal plant.*

INTRODUCTION: -

Due to a wide range of medicinal properties, tulsi has made contribution to the field of science from ancient as also to modern research. *Ocimum tenuiflorum* belongs to the plant family *lamiaceae* and it is known as Holy Basil in English and Tulsi in Urdu (**Vidhani *et. al.*, 2016, Rajamurgan et. al., 2013**). Generally, tulsi is found in two forms- Vanya and Gramya. Vanya is found in wild form but the Gramya is grown in homes, both of them have similar use but the former one is little darker (**Hussain *et. al.*, 2013**). Many infectious disease can be treated with the help of tulsi leaves. The essential oils, mainly the eugenols are responsible for the antibacterial activities of tulsi (**C. Ramteke *et. al.*, 2012**). It was reported that tulsi leaves show strong antibacterial and antifungal activities(**Pandian *et. al.*, 2016**). Traditionally, *Ocimum tenuiflorum* is used as medicinal plant. It is found from clinical practices and pharmacological studies that tulsi possesses antioxidative and antimicrobial functions. In the treatment of diabetes, skin disease, bronchitis, hypertension, diarrhea dysentery, tulsi is also recommended (**Saharkhiz *et. al.*, 2013**).There is an urgency of finding new infection-fighting strategies because antibiotic resistance has become a global concern (**A.R. Singh *et. al.*, 2013**). There is an increase in occurrence of antibiotic resistance to many common bacterial pathogens like *Staphylococcus aureus, E.coli, Streptococcus pneumoniae, Pseudomonas aeruginosa, Streptococcus pyogenes, Klebsiella pneumoniae* etc., in recent

times.The phenomena of resistance shown by different microbes against some currently available antibiotics leads researchers to discover novel sources of antimicrobial drugs (**P. Goyal *et. al.,* 2011**).

For its religious sanctity, tulsi has been grown in Indian sub-continent. The diverse medicinal properties of tulsi such as hypoglycemic, anti-hyperlipedimic, hypotensive, anti-inflammatory and antimicrobial are mentioned in an Indian traditional medicine scripture"Charak Samhita". The various compounds like beta-caryophyllene, derivatives of eugenol, rosmarinic acid, vanillin, gallic acid, ursolic acid and vanillic acid are present in tulsi leaves(**A. Choudhary *et. al.,* 2020**). Traditionally, tulsi is widely used as traditional herbal medicine to treat carminative, malaria, wounds, microbial infections and stomachic. It is also utilized in food industries as well as perfume industries (**Hussain *et. al.,* 2013**).

Fig. 1:- *Ocimum tenuiflorum*

MATERIALS AND METHODS: -

The study was conducted in the Department of Life Sciences, Institute of Applied Medicines and Research, Duhai, Ghaziabad in 2021.

Objectives: -

Present research is focused on investigating the susceptibility of several clinically significant Bacterial strains against some crude extracts prepared from *Ocimum tenuiflorum* (tulsi) leaves. The Minimum Inhibitory Concentrations (MICs) were also determined from the methanolic extracts showing significant activity against the bacterial strains selected for the susceptibility assay.

Collection of Plant Material and Identification: -

The leaves of Ocimum sanctum was collected from Duhai, Ghaziabad, India. The authentication of plant material was done by Dr. Jyoti Tyagi, faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad. The sampling was carried out during the month of March. Fresh leaves were collected, detached from plant, washed under running tap water and then shed-dried for 14-15 days. The dried leaves were ground to powder and then the powder was stored in an air tight container until required for analysis .

Test Microorganisms: -

Common human pathogenic bacterial strains of *E.coli, Bacillus subtilis* were used for assessment of antibacterial activity. These strains were obtained from the stock cultures of Institute of Applied Medicines and Research, Duhai, Ghaziabad.

Chemicals used: -

Methanol, Dimethyl sulfoxide (DMSO), Nutrient agar Media, whatmann filter paper.

Methanol Extraction of Plant Material:-

80 gm., of shed-dried powder of *Ocimum tenuiflorum* leaves was taken in 200 ml of methanol, in a beaker and then mixed thoroughly with the help of a glass rod. The prepared methanolic extract was covered with polythene bag for 8 to 10 days. Then, the methanolic extract was filtered with the help of a muslin cloth. The solvent was evaporated for 7 days at room temperature. At last, 150.5g of crude extract was obtained and stored in air-tight bottles. This crude extract was stored in refrigerator until further use.

Loading of Extracts in Vials: -

In the experimentation, the four vials were used. In each vial 1ml Dimethyl sulfoxide (DMSO) was added with the help of micropipette. The one vial contains 0.1g, second vial contains 0.2g, third vial contains 0.4g and fourth vial contains 0.8g of methanolic extract of *Ocimum tenuiflorum* leaves. Whatmann filter paper No. 4 was used to make discs with the help of punching machine. Then, these discs were added to each vials for 24 hours at room temperature.

Preparation of Petri Plates: -

In a 500 ml of conical flask, 7g of nutrient agar powder was added in 250 ml distilled water and mixed thoroughly. Then this nutrient agar media was autoclaved at 121^0C temperature for 15 minutes. Once the nutrient media is autoclaved, it is allowed to cool but not to solidify. The nutrient agar media was poured in sterilized petri plates inside the laminar air flow chamber. Then the plates were allowed to solidify. After the solidification of nutrient agar media in petri plates, the plates were labelled according to the bacterial strain used and the concentration of extract used. Kirby-Bauer method was used to perform Antibiotic Susceptibility test. A cotton swab was dipped in the pure culture broth of *E.coli*, and then this culture was spreader over the solidified nutrient agar plates with the help of this swab. Then, the discs of appropriate concentrations were placed over these plates. Same procedure were followed for *Bacillus subtilis*. Paraffin was applied on the petri plates and then these plates were incubated in an incubator at 37^0C, in an inverted positon for 24 hours. After 24 hours of incubation, the results were recorded.

RESULTS AND DISCUSSION: -

For the evaluation of antimicrobial nature of plant extracts and minimum quantity of antibacterial compound required to kill or stop multiplication of all microorganisms present in the medium, MIC value is used (1). Based on the growth inhibition zone diameter obtained by 100 mg/ml, 200 mg/ml, 400 mg/ml and 800 mg/ml *Ocimum* methanol extract concentration, bacterial strains were tested. Better diffusion of the extracts into the medium was obtained by this method and thus enhancing contact with the organisms. The antimicrobial activity of extracts of O. sanctum was used against two pathogenic organisms, *Escherichia coli* and *Bacillus subtilis*. The data is presented in Table 1.

The results shown that the *Ocimum* extracts at final concentrations of 200 mg/ml, 400 mg/ml and 800 mg/ml was active against the two types of micro-organisms, while, at the concentration 100 mg/ml for the methanol extracts was not efficient to kill some types of treated micro-organisms. On the other hand, it was found that the final concentration of 200 mg/ml, 400 mg/ml, 800 mg/ml, methanol extract of tulsi was effective to kill the treated micro-organisms. It was also indicated by results that the best solvent for extracting antibacterial substances from the leaves of tulsi plant is methanol (12). *Bacillus subtilis* was inhibited by the methanol extract with inhibition zone of 35 mm while *E.coli* was inhibited by the methanol extract with inhibition zone of 37 mm. These results shows that the antimicrobial property of *Ocimum tenuiflorum* leaves support the traditional use of the plant in therapeutic use against microbial infections.

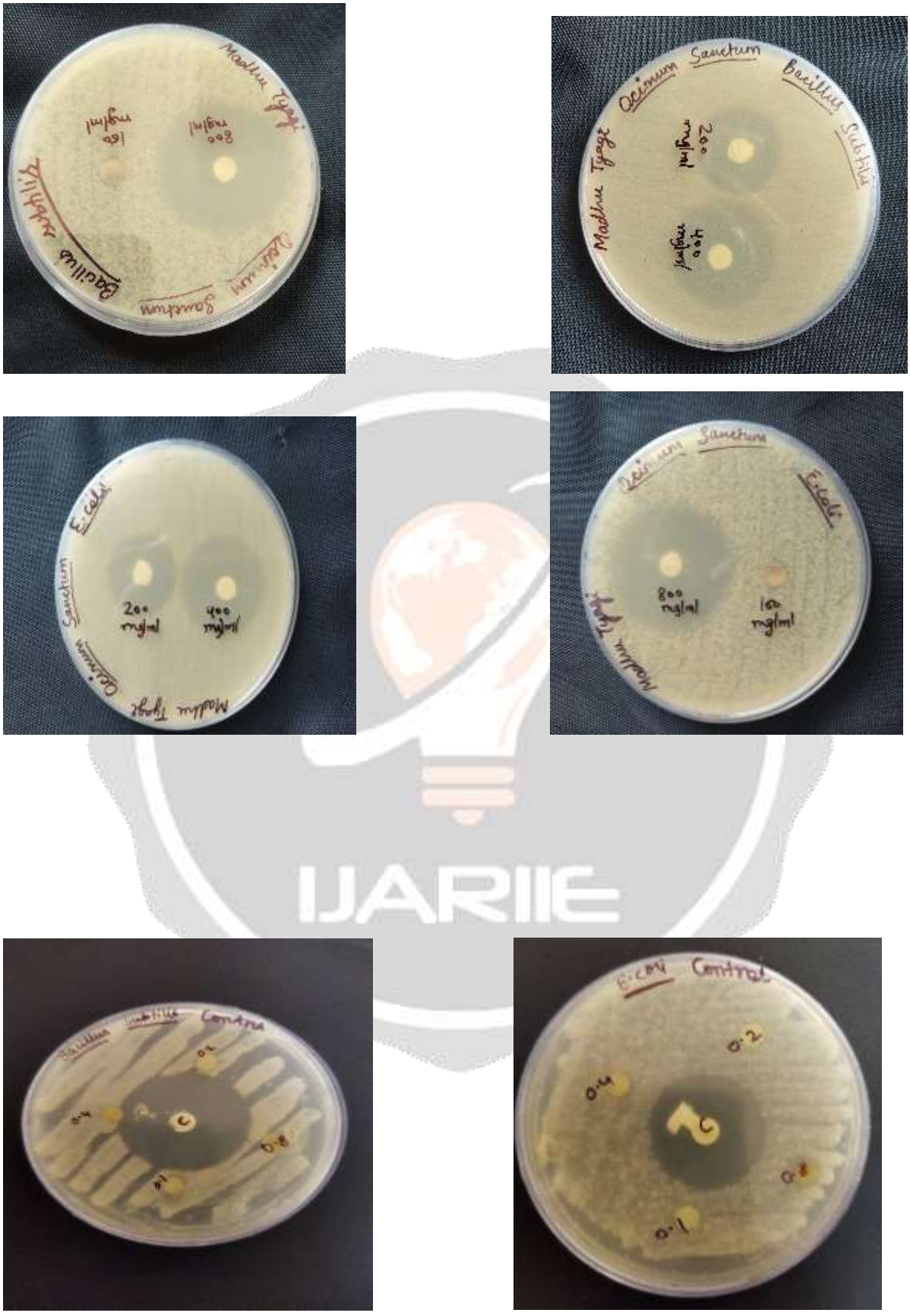

fig.2 - Nutrient Agar Plates showing Zone of Inhibition

Table 1: - Anticimcrobial activity of *O. tenuiflorum*: -

Sample	Solvent	Zone of Inhibition (mm)									
		Bacillus subtilis					*Escherichia coli*				
Ocimum tenuiflorum (Tulsi)	Methanol	100 mg/ml	200 mg/ml	400 mg/ml	800 mg/ml	Positive Control gentamicin	100 mg/ml	200 mg/ml	400 mg/ml	800 mg/ml	Positive control ceftioxime
		0 Mm	24 mm	27 mm	34 mm	36 mm	0 mm	26 mm	29 Mm	33 mm	34 mm

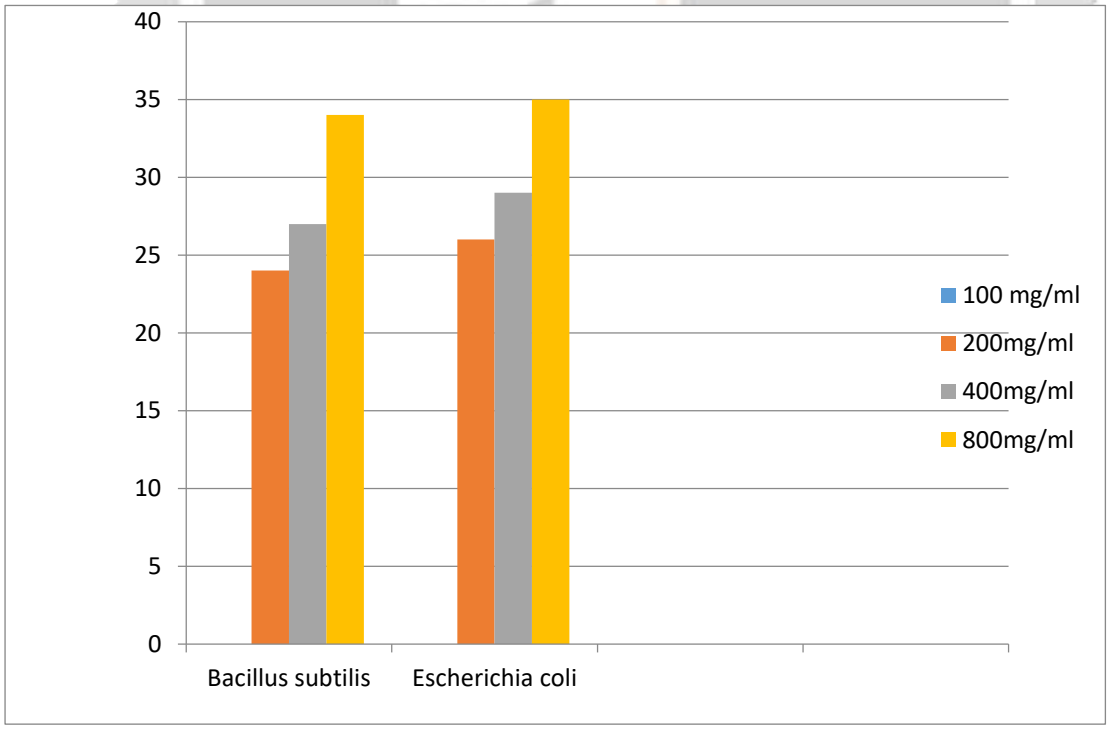

Fig. 3- Graph showing zone of inhibition introduced by tulsi leaf extract against *E.coli, Bacillus subtilis*

CONCLUSION: -

From the results of this study, it can be concluded that Ocimum tenuiflorum contain bioactive compound and found to have a strong antibacterial activity specifically in the methanolic extract of leaves. Further, it can also be confirmed that these herbs can be used as traditional medicines. *Ocimum tenuiflorum* can be used as a source of novel antibacterial drugs.

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