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EXPLORING THE ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF ETHANOLIC LEAF EXTRACTS OF *Ocimum sanctum* L.

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ABSTRACT:

The antimicrobial and antifungal activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. The effect of various chemical constitutions are on zonation of inhibition by extracts plant. Also higher concentration and medium effect given by ethanolic leaf extracts of *Ocimum sanctum* L. plant because the chemical constituents only present in the extract. In 200 µl concentration of extract against *Escherichia coli* ranged from 14 mm to 15 mm and *Bacillus subtilis* ranged from 20 mm to 21 mm. Those inhibition of the growth of microbe's colony and also inhibit the physiological reaction of microbes such as *E. Coli*, *Bacillus substilis* and Yeast.

KEYWORDS: *Ocimum sanctum* L., Ethanolic, E.Coli - *Escherichia coli*., *Bacillus subtilis,* Yeast - *Saccharomyces cerevisiae*, Microliter, Inhibitory concentration.

INTRODUCTION

In India thousands of plant species are known to have medicinal value (Mahalingam, *et al*., 2011). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Particularly in rural India, use of raw plant products as well as some concoction of plant products in Ayurveda medicines is sought after to a great proportion, because of cheap availability, and in urban areas too those are increasingly popular for cultural nuances that exist. They are obtained from different parts of the plant: flowers, leaves, seeds, bark and tubers and many have medicinal properties. In ethno-botanical literature of India, one of those popular ones is tulsi traditionally used for the treatment of diseases. Leaves possess antimicrobial activity. The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties,

Ocimum sanctum L. (Lamiaceae) is widely distributed in tropical and warm temperate regions. The species *Escherichia coli* is responsible for a variety of infections; affecting the skin, urinary tract, the eyes, and the ears. *Bacillus subtilis* is often found colonizing the natural micro biota, especially the skin. It causes a variety of skin and subcutaneous infections, post-surgical infections, and pneumonia.

MATERIALS AND METHODS :-

The fresh, green Tulsi plants (*Ocimum sanctum* L.) were collected and identification was done using the key in Flora of Gujrat by G.L.Shah. After proper identification the plant material were collected for the present study. Fresh leaves of plant species free from diseases were collected. Leaves were washed, dried and milled into coarse powder by mechanical grinder. Then, 30 g of dried *Ocimum sanctum* L. leaf powder was mixed and macerated with 300ml absolute Ethanol for 7 days. The extract solutions were then filtered with filter paper (Whatman No.1), concentrated under a rotary evaporator and finally pulverized until a powdery crude *Ocimum sanctum* L. leaf extract was obtained. The extracts were kept at 4°C until use. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth. Culture of test organisms were used as inoculum, The sterile discs were carefully placed on surface of agar medium and definite volume of sample was placed with help of sterile micropipette. For study of antimicrobial activity by well-diffusion method. These plates were then observed for zones of inhibition after 24 hours, 48 hours, 72 hours. The zone of inhibition formed around each well was then examined and measured in millimetres using digital vernier caliper and also Scalestipe.

OBSERVATION

Ocimum sanctum L (Lamiaceae) is widely distributed in tropical and warm temperate regions. It is an erect, much branched subshrub, 30–60 cm tall with hairy stems and simple opposite green or purple leaves that are strongly scented. Leaves have petioles and are ovate, up to 5 cm long, usually slightly toothed.

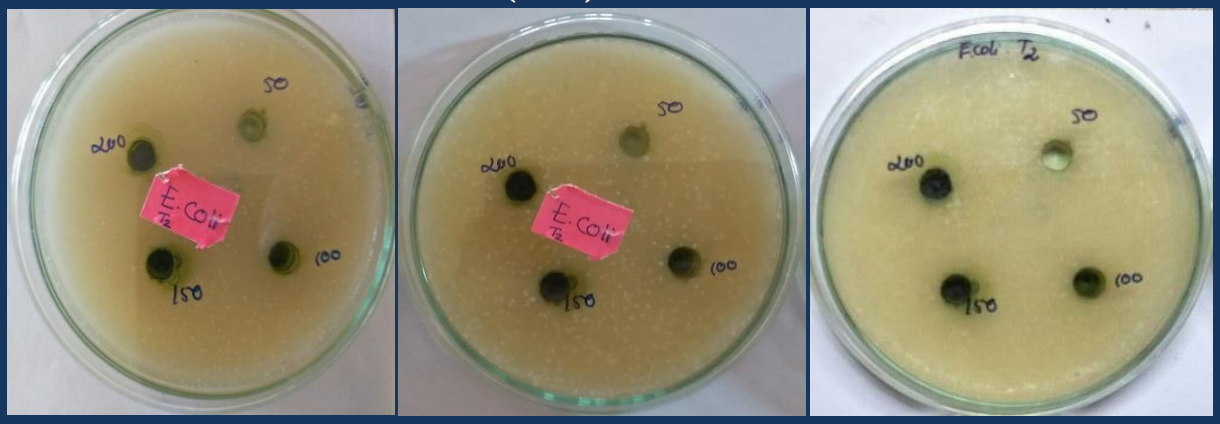
Antimicrobial susceptibility testing was done using the well-diffusion method according to the standard of the National Committee for Clinical Laboratory Standards. 5 mm diameter wells were punched into the medium using a sterile cork borer. With a total of three plates used for each extract for selecting bacterium. For each bacterial and fungal strain, controls were maintained where pure solvents were used instead of the extract. The plates are labeled and placed in an incubator set to 37∘C. After 24 hours of incubation, each plate was examined for inhibition zones.

The antimicrobial activity of Ethanolic extracts of leaves of *Ocimum sanctum* (Lam) with 50µl , 100µl , 150µl and 200µl concentration against selected Gram positive bacteria (*Bacillus subtilis*), Gram negative bacteria (*Escherichia coli*) and *Saccharomyces cerevisiae* (Yeast) the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. The results were expressed as 24 hour, 48 hour and 72 hour wise condition.

Table -1. Antibacterial Activity of *Ocimum sanctum* L. (Tulsi) Ethanolic extract leaf on *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae.*

Zones of inhibition (mm)												
Test organism	***Concentrations of extract* (µl)**											
	50(µl)			100(µl)			150(µl)			200(µl)		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
E. coli	**0**	**0**	**0**	**9**	**9**	**10**	**13**	**13**	**10**	**14**	**15**	**11**
Baccilus	**0**	**01**	**12**	**10**	**12**	**16**	**19**	**19**	**19**	**20**	**21**	**21**
Yeast	**0**	**01**	**7**	**9**	**15**	**19**	**13**	**17**	**15**	**18**	**15**	**0**

Photo plate:-1 The antibacterial activity of Ethanolic Extract of leaves *Ocimum sanctum* L.(Tulsi) on E. coli.

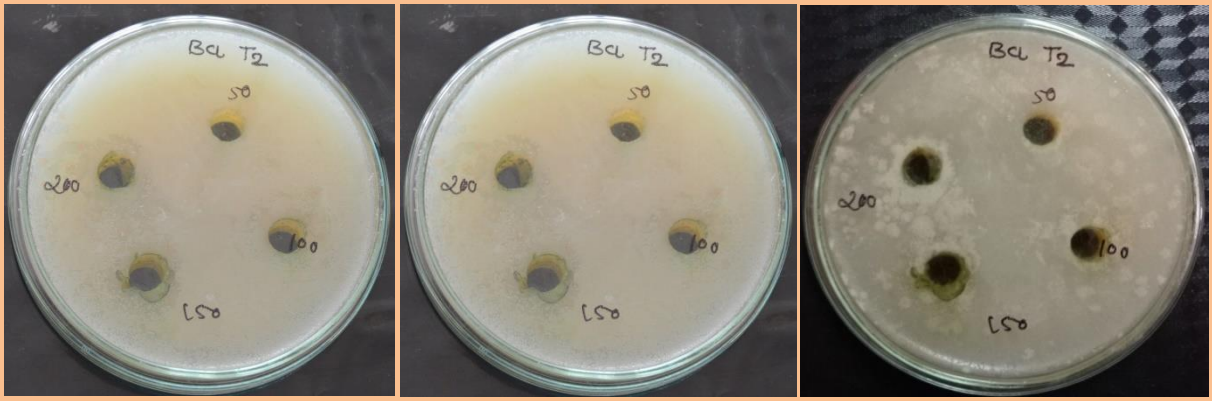


24hours

48hours 72hours

(T_2 = Tusiethanolic leaf extract, E.Coli - *Escherichia coli*., 50 = 50 µl, 100 = 100 µl, 150 = 150 µl, 200= 200 µl.)

Photo plate :- 2 The antibacterial activity Ethanolic of Extract of leaves *Ocimum sanctum* L. (Tulsi) on Bacillus subtilis



24hours

48hours 72hours

(T_2 = Tusiethanolic leaf extract, Ba - *Bacillus subtilis*, 50 = 50 µl, 100 = 100 µl, 150 = 150 µl, 200= 200 µl.)

Photo plate:-3 **The antifungal activity of Ethanolic Extract of leaf *Ocimum sanctum* L.(Tulsi) on yeast.**

24hours **48hours** **72hours**

(T_2 = Tulsi ethanolic leaf extract, Yeast - *Saccharomyces cerevisiae*, 50 = 50 µl, 100 = 100 µl, 150 = 150 µl, 200= 200 µl.)

The Ethanolic extract of leaves of *Ocimum sanctum* (Lam) gave no effect on 50 µl concentration against *Escherichia coli* and *Bacillus subtilis* ranges from 1 mm to 12 mm, for fungus *Saccharomyces cerevisiae* ranges from 1 mm to 7 mm in 0 to 72 hours, whereas these extract effect against *Bacillus subtilis* shows 0 mm to 5 mm to *Escherichia coli* and *Saccharomyces cerevisiae* (table- 1)

The Extract of leaves 100 µl concentration against *Escherichia coli* range from 9 mm to 10 mm and *Bacillus subtilis* ranges from 10 mm to 16 mm, for *yeast* range from 09 mmto 19 mm whereas these extract effect against *Saccharomyces cerevisiae* shows 1 mm to 3 mm to *Escherichia coli* and *Bacillus subtilis* found in 0 to 72 hours. (table-1) And 150 µl concentration of Ethanolic extract of *Ocimum sanctum* L. against *Escherichia coli* range from 10 mm to 13 mm and *Bacillus subtilis* ranged from 19 mm and for *yeast* it ranged from 13 mm to 17 mm, whereas extract effect against *Bacillus subtilis* shows 0 mm to 2 mm to *Escherichia coli* and *Saccharomyces cerevisiae*. (table-1) 200µl concentrations of extract against *Escherichia coli* range from 11 mm to 15 mm found in 0 to 72 hours, and *Bacillus subtilis* ranges from 20 mm to 21 mm and for *yeast* it ranged from 0 mm to 18 mm, whereas extract effect against *Bacillus subtilis* show 2 mm to 3 mm to *Escherichia coli* and *Saccharomyces cerevisiae*.

There was no inhibition of growth with vehicle control in 50µl on *Escherichia coli Bacillus subtilis* and *Saccharomyces cerevisiae* in 24 hour, but was effective after 24 hour. Antimicrobial activity of 200µl Ethanolic extract of leaves of *Ocimum sanctum* (Lam) was significantly effective against the gram positive *Bacillus subtilis* and gram negative *Escherichia coli* bacteria. After 72 hour the effect of Ethanolic extract can stop in *Escherichia coli* and No zone of inhibition was found (photo plate-1,2 and 3).

Results and Discussion

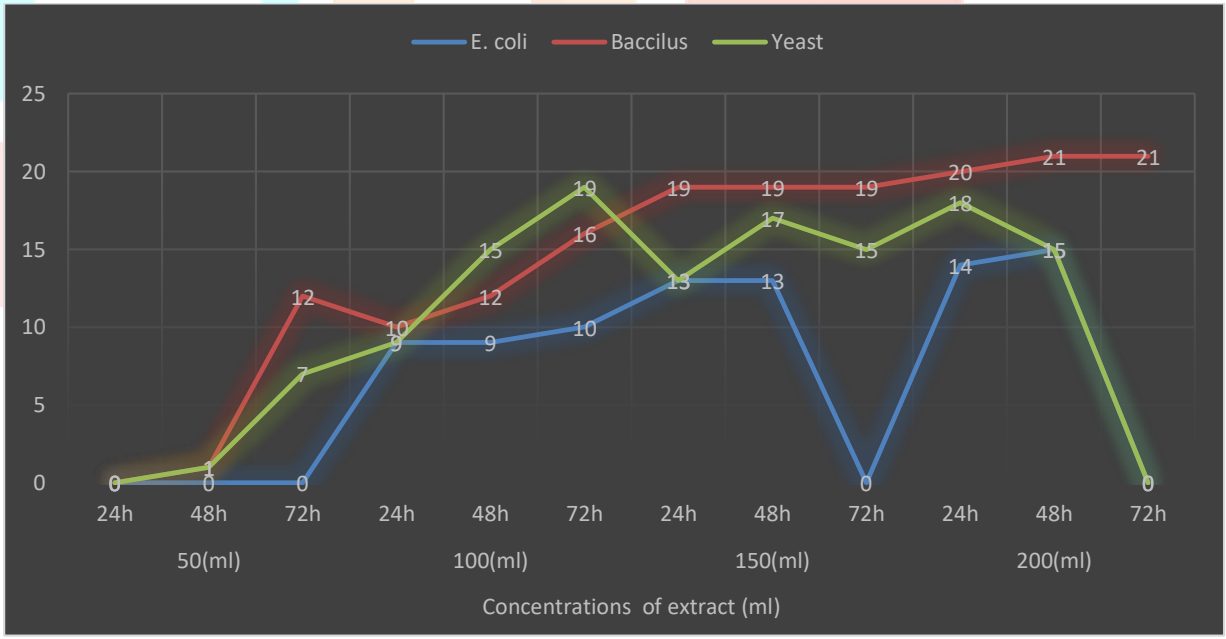
The antimicrobial activity of Ethanolic extracts of two plants (Tulsi) *Ocimum sanctum* L.. were investigated using agar well diffusion method against selected microbes such as *Escherichia coli, Bacillus subtilis,* and *Saccharomyces cerevisiae.*

The Ethanolic extract of leaves of *Ocimum sanctum* (Lam) 50µL,100µL, 150µL, 200 µL concentration against *Escherichia coli* range 0 mm to 14 mm found. And *Bacillus* ranges from 0 mm to 19 mm, for fungus *yeast* ranges from 0 mm to 18 mm. whereas these 50µL concentrate extract given no effect against *Yeast, Escherichia coli* and *Bacillus.*

Antimicrobial activity of 100µL Ethanolic extract of leaves of *Ocimum sanctum* (Lam) was significantly effective against the gram positive *Bacillus* and gram negative *Escherichia coli* bacteria. The Ethanolic extract of leaves of *Ocimum sanctum* (Lam) 50µL,100µL, 150µL, 200 µL concentration against *Escherichia coli* range 0 mm to 15 mm found. And *Bacillus* ranges from 1 mm to 21 mm, for fungus *yeast* ranges from 1 mm to 17 mm. whereas these 200µL concentrate extract given better effect against *Escherichia coli* and *Bacillus.*

The Ethanolic extract of leaves of *Ocimum sanctum* (Lam) 50µL,100µL, 150µL, 200 µL concentration against *Escherichia coli* range 0 mm to 9 mm found. But the zonation of inhibition show higher against to *Bacillus,* for fungus *yeast* also control by 50µL,100µL, 150µL concentration of Extract in 72 hours.

Graph 1.- The Comparison of zonation of inhibition of Ethanolic extracts Tulsi (*Ocimum sanctum* L.) effect on *Escherichia coli, Bacillus subtilis* and Yeast (*Saccharomyces cerevisiae*).



Comparative study of Ethanolic extract of *Ocimum sanctum* (Lam) effect:-

The Ethanolic extract of leaves of *Ocimum sanctum* (Lam) effect 50 µl concentration against *Bacillus subtilis* shows more zonation of inhibition to *Escherichia coli* and *Yeast.* also given reverse effect in time 72 hours, The Extract of leaves 100 µl concentration effect against *Yeast* shows 1 mm to 3 mm to *Escherichia coli* and *Bacillus subtilis* found in 0 to 72 hours. me. And 150 µl concentration of Ethanolic extract of *Ocimum sanctum* L. against *Bacillus subtilis* more to *Escherichia coli* and *Yeast.* and 200 µl concentrations of extract against *Bacillus subtilis* shows 2 mm to 3 mm to *Escherichia coli* and *Yeast.* Extract concentration 200 µl of

leaf effect against *Escherichia coli* shows 2 mm to 6 mm to *Bacillus subtilis*and *Yeast* (*Saccharomyces cerevisiae*)..

So The Ethanolic extract of leaves of *Ocimum sanctum* (Lam) 100µl, 150µl, 200µl concentration extract given no zonation of inhibition effect against *Escherichia coli*. all concentration of extract given better effect against *all* microbe. So Ethanolic extract is better to other. (Graph - 01)

CONCLUSION:

It is a multi-purpose medicinal herb commonly used in folk medicines to treat different diseases like upper respiratory tract infections, diarrhea, headaches, eye problems, skin disease, pneumonia, coughs, fevers, and conjunctivitis.

From this study, it is concluded that the Ethenol extracts contain some complex compounds that are responsible for antimicrobial activity. The various extract having various chemical constituents. The effect of various chemical constitutions are on zonation of inhibition are different in different extracts of plants. Those inhibit the growth of microbes colony and also inhibit the physiological reaction of microbes such as *E. Coli*, *Bacillus substilis* and *Yeast*. So the growth is microbes are inhibite and shows the zonation.

The Ethanolic extracts of 150µl to 200µl concentrations of extract plants to 48hr to 72hr are higher zonation of inhibition because its having more chemical constituents.

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