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Study of Antibacterial Activity of *Ocimum sanctum* Extract Against Gram Positive and Gram Negative Bacteria

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ABSTRACT

This study was carried out to observe the antibacterial activity of aqueous extract, chloroform extract, alcohol extract and oil obtained from leaves of *Ocimum sanctum* against the selected bacteria i.e., *E. coli, P. aeruginosa, S. typhimurium* and *S. aureus*. The antibacterial activity of *Ocimum* was evaluated by liquid inhibition test. *E. coli, P. aeruginosa, Staphylococcus aureus* and *Salmonella typhimurium* were not found resistant against the *Ocimum* extract since reduction in optical density were observed from 0.20 to 0.85. Chloroform extract were found most effective against *P. aeruginosa* where 0.85 reductions in O.D were observed. Extract obtained from *Ocimum sanctum* were observed equally effective against the gram negative and gram positive bacteria. Present investigation reveals that *Ocimum sanctum* may be a better alternative as a preservative in Food Industries since it is equally effective against pathogenic gram positive and gram negative bacteria.

Key words: *Ocimum sanctum*, antibacterial, *E. coli*, *S. aureus*

INTRODUCTION

Ocimum is a grassy and annual plant. The leaves of this plant are oval with sharp tip. It is a native of Iran, Afghanistan and India (Mann *et al*., 2000; Volak and Jiri, 1997; Zargari, 1990; Mirheidar, 1990). *Ocimum* respectively, named basil is an aromatic herb that has been used traditionally as medicinal herbs in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunctions (Sikmon *et al*., 1990). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plants materials typically result from the combination of secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins, fatty acids, gums, which are capable of producing definite physiological action on body (Joshi *et al*., 2009). These plants also have therapeutic effects for nasal polyps (Mann *et al*., 2000), an upper respiratory tract diseases and it has been used as a bathing solution for treatment of ulcers (Volak and Jiri, 1997). Recent interest on *Ocimum* has resulted from its inhibitory activity against HIV-1 reverse transcriptase and platelets aggregation induced by collagen and ADP (adenosine 5'-disphosphate) (Yamasaki *et al*., 1998; Okazaki *et al*., 1998). It is also a source of aroma compounds and essential oils containing biological active constituents that

posses insecticidal (Deshpande and Tipnis, 1997), nematicidal (Chaterjee *et al*., 1982), fungistatic (Reuveni *et al*., 1984) and antimicrobial properties (Yamasaki *et al*., 1998; Wannissorn *et al*., 2005). *Ocimum* species contain a wide range of essential oils rich in phenolic compounds and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Deshpande and Tipnis, 1997). These Phenolic compounds and flavonoids are potent antioxidants, free radicals scavengers and metal chelator (Cook and Samman, 1996). In previous studies the essential oil of *Ocimum* species reported to have antibacterial activities. Very few data have been reported regarding the antibacterial activity of leaf. Also no such review is available related with the preservative action of leaves. The purpose of this study is to identify the antibacterial activity of the different extract of leaf against the growth of Gram positive and Gram negative bacteria (Gram positive bacteria are encased in a plasma membrane covered with a thick wall of peptidoglycan while Gram negative bacteria are encased in a triple layer. The outermost layer contains lipopolysaccarides LPS) in detail.

MATERIALS AND METHODS

The study has been carried out in year 2008-2009. The fresh leaves of *Ocimum sanctum* were collected from local market. The collected leaves were macerated. Macerated leaves were divided in two parts; first part was subjected to cold aqueous extraction and second part was subjected to essential oil extraction.

Cold aqueous extract of *Ocimum*: Macerated leaves of 200 g were mixed with 500 mL of water and kept for 8 h. After 8 h the whole mixture was filtered by using cheese cloth and obtained extract was centrifuged. Supernatant obtained by centrifugation was used for antibacterial activity assay whereas residue obtained from both filtration and centrifugation was mixed thoroughly and were further used for $CHCl_3$ extraction.

Isolation of essential oils: The 200 g of leaves and 500 mL of water have been placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistilltaion for 3 h. The obtained essential was separated, dried over anhydrous sodium sulphate and stored at -20°C before storage (Ezekwesili *et al*., 2004). Residue obtained from oil extraction was further used for Chloroform extract.

Chloroform extraction of *Ocimum*: The residue obtained after oil extraction and cold aqueous extraction was separately treated with $CHCl_3$, in both cases equal volume of chloroform and residue (v/w) were mixed thoroughly and kept for 24 h for $CHCl_3$ extraction. After 24 h the mixture was filtered by using cheese cloth. Extract obtained from residue of cold aqueous and oil extraction were termed as $CHCl_3^{\#}$ and $CHCl_3^{\#\#}$, respectively whereas residue obtained from both cases were further used for alcoholic extraction.

Alcoholic extraction of *Ocimum*: The residue obtained after chloroform extract was separately treated with methanol. Method of alcoholic extraction of *Ocimum* was same as was described in section chloroform extraction of leaves. Extract obtained from residue of cold aqueous and oil extraction were termed as $CH_3OH^{\#\#}$ and $CH_3OH^{\#\#\#}$ respectively.

Microorganism used for assay

- *Staphylococcus aureus* : 2079 HAL 1956
- *Pseudomonas aeruginosa* : 2056
- *Salmonella typhimurium* : ATCC 23564
- *Escherichia coli* : Enterotoxigenic AT 2056

Liquid inhibition: For liquid inhibition test, Nutrient broths were prepared by boiling peptone (5 g), Beef extract (5 g) and 2.5 g of NaCl in 500 mL of distilled water. Boiled broth was autoclaved at 121 psi. Four test tubes having 9.8 mL of broth and 0.2 mL of bacteria i.e., *E. coli*, *P. aeruginosa, S. aureus* and *S. typhimurium* were prepared in duplicates. O.D of each tube was observed at 550 nm. Similarly the 24 test tubes having 9 mL of Nutrient broth and 1 mL of *Ocimum* extract (essential oil/cold aqueous extract/chloroform/methanol extract separately) in duplicates were prepared, O.D of each test tubes were estimated at the same wavelength. Another 24 test tubes in duplicates having 8.8 mL of broth, 1 mL of extract and 0.2 mL of bacteria were prepared. All test tubes were incubated at 37°C for 24 h and after that the O.D were taken. All readings were taken against the blank (9 mL of nutrient broth and 1 mL solvent i.e., water, chloroform or methanol correspondingly) (Shukla and Sunadaram, 2002).

Statistical analysis: The data obtained were analyzed statistically for Analysis of Variance (ANOVA) using completely randomized design with least significant difference (LSD) at $p \leq 0.05$ using Co.Stat 6.303, Co Hart Software USA.

RESULTS

Strains of *E. coli, P. aeruginosa, Stphylococcus aureus* and *Salmonella typhimurium were* selected to study the effect of antibacterial activity of *Ocimum sanctum* extract. The antibacterial activity of *Ocimum* extract was analyzed by using Liquid inhibition test. *Ocimum sanctum extract* was found significantly effective against the growth of *Stphylococcus aureus* since reduction in optical density for all extracts tested were observed from 0.40 to 0.78 (Table 1). $CHCl_3^{\#\#}$ extract was found comparatively more effective against *S. aureus* than any other extract tested (Table 1) while $CH_3OH^{\#\#\#}$ extract was found least effective against all selected strains. Similarly *Ocimum sanctum* extracts were found significantly effective against *E. coli* where reduction in OD were observed

Table 1: Study of effect of extracts of *O. sanctum* against the activity of *S. aureus* (n = 3)

Extracts of *O. sanctum*	N.B. 9 mL +1 mL of solvent	N.B. (8.8 mL) +Extract of *O. sanctum* (1 mL) + *S. aureus* (0.2 mL) (after 0 h)	N.B. (8.8 mL) + Extract of *O. sanctum* (1 mL)+ *S. aureus* (0.2 mL) (after 24 h)	Total reduction in O.D of *O. sanctum* (1 mL) +*S. aureus* (0.2 mL) (after 24 h)
Oil	00	0.66 ± 0.01^a	0.04 ± 0.002^a	0.62
C.E.	00	0.88 ± 0.015^b	0.38 ± 0.011^b	0.50
$CHCl_3^{\#}$	00	0.70 ± 0.02^c	0.16 ± 0.003^c	0.54
$CHCl_3^{\#\#}$	00	0.98 ± 0.021^d	0.20 ± 0.002^d	0.78
$CH_3OH^{\#\#\#}$	00	0.54 ± 0.023^e	0.14 ± 0.013^e	0.40
$CH_3OH^{\#\#\#\#}$	00	0.67 ± 0.031^f	0.19 ± 0.003^f	0.48

Values in same column with different superscripts differ significantly ($p<0.05$), $CHCl_3^{\#}$: Extracts obtained from residue of cold aqueous extract, $CHCl_3^{\#\#}$: Extracts obtained from residue of oil extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#}$ extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#\#}$ extracts

Table 2: Study of effect of extracts of *O. sanctum* against the activity of *E. coli* (n = 3)

Extracts of *O. sanctum*	N.B. 9 mL +1 mL of solvent	N.B. (8.8 mL)+ Extract of *O. sanctum* (1 mL) + *E. coli* (0.2 mL) (after 0 h)	N.B.(8.8 mL)+ Extract of *O. sanctum* (1 mL) + *E. coli* (0.2 mL) (after 24 h)	Total reduction in O.D of *O. sanctum*+ *E. coli* (after 24 h)
Oil	00	0.54±0.01[a]	0.14±0.002[a]	0.40
C.E.	00	0.80±0.015[b]	0.20±0.01[b]	0.60
$CHCl_3^{\#}$	00	0.62±0.02[c]	0.29±0.004[c]	0.33
$CHC1_3^{\#\#}$	00	0.90±0.012[d]	0.21±0.003[d]	0.69
$CH_3OH^{\#\#\#}$	00	0.46±0.031[e]	0.20±0.01[e]	0.26
$CH_3OH^{\#\#\#\#}$	00	0.59±0.02[f]	0.16±0.001[f]	0.43

Values in same column with different superscripts differ significantly ($p<0.05$), $CHCl_3^{\#}$: Extracts obtained from residue of cold aqueous extract, $CHCl_3^{\#\#}$: Extracts obtained from residue of oil extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#}$ extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#\#}$ extracts

Table 3: Study of effect of extracts of *O. sanctum* against the activity of *P. aeruginosa* (n = 3)

Extracts of *O. sanctum*	N.B. 9 mL+1 mL of solvent	N.B. (8.8 mL)+Extracts of *O. sanctum* (1 mL) + *P. aeruginosa* (0.2 mL) (after 0 h)	N.B. (8.8 mL)+Extracts of *O. sanctum* (1 mL)+ *P. aeruginosa* (0.2 mL) (after 24 h)	Total reduction in O.D of *O. sanctum* (1 mL) +*P. aeruginosa* (after 24 h)
Oil	00	0.78±0.02[a]	0.07±0.001[a]	0.71
C.E.	00	1.04±0.032[b]	0.67±0.006[b]	0.37
$CHCl_3^{\#}$	00	0.86±0.022[c]	0.52±0.004[c]	0.34
$CHCl_3^{\#\#}$	00	1.14±0.041[d]	0.29±0.007[d]	0.85
$CH_3OH^{\#\#\#}$	00	0.76±0.04[e]	0.20±0.004[e]	0.50
$CH_3OH^{\#\#\#\#}$	00	0.83±0.051[f]	0.13±0.01[f]	0.70

Values in same column with different superscripts differ significantly ($p<0.05$), $CHCl_3^{\#}$: : Extracts obtained from residue of cold aqueous extract, $CHCl_3^{\#\#}$: Extracts obtained from residue of oil extracts, CH_3OH $^{\#\#\#}$Extracts obtained from residue left after $CHCl_3$ extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#\#}$ extracts

Table 4: Study of effect of extracts of *O. sanctum* against the activity of *S. typhimurium* (n = 3)

Extracts of *O. sanctum*	N.B. 9 mL+1 mL of solvent	N.B.(8.8 mL) + Extracts of *O. sanctum*+*S. typhimurium* (after 0 h)	N.B.(8.8 mL) + Extracts of *O. sanctum*+*S. typhimurium* (after 24 h)	Total reduction in O.D of *O. sanctum* (1 mL)+ *S. typhimurium* (after 24 h)
Oil	00	0.60±0.04[a]	0.20±0.003[a]	0.40
C.E.	00	0.76±0.02[b]	0.49±0.008[b]	0.37
$CHCl_3^{\#}$	00	0.68±0.04[c]	0.40±0.03[c]	0.28
$CHCl_3^{\#\#}$	00	0.96±0.009[d]	0.29±0.001[d]	0.67
$CH_3OH^{\#\#\#}$	00	0.52±0.031[e]	0.20±0.01[e]	0.32
$CH_3OH^{\#\#\#\#}$	00	0.65±0.008[f]	0.19±0.007[f]	0.46

Values in same column with different superscripts differ significantly ($p<0.05$), $CHCl_3^{\#}$: Extracts obtained from residue of cold aqueous extract, $CHCl_3^{\#\#}$: Extracts obtained from residue of oil extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#}$ extracts, $CH_3OH^{\#\#\#}$: Extracts obtained from residue left after $CHCl_3^{\#\#}$ extracts

from 0.26 to 0.69 (Table 2). *Ocimum* extract showed maximum activity against the growth of *P. aeruginosa* where total 0.85 reduction in OD was observed when treated with $CHCl_3^{\#\#\#}$ extracts (Table 3) while $CHCl_3^{\#\#}$ was found least effective for *P. aeruginosa* where only 0.34 reduction in OD was observed. The same trend was observed when tested against *S. typhimurium* (Table 4). The therapeutic potential of the essential oils extracted from fresh leaves of *Ocimum sanctum* L. has been found to be largely due to eugenol (major constituents of the essential oils) which is a phenolic

compound (1-hydroxyl-2-methoxy-4-allylbenzene) (Sen, 1993; Rajeshwari, 1992; Mukherjii, 1987). Eugenol which was analyzed to be the major compounds present in the essential oil of this plant has been reported to present antibacterial activity (Jamine *et al.*, 2005; Loughrin and Kasperbaucer, 2001; Iwalokun *et al.*, 2003) insecticidal activity (Chavan and Nikam, 1982) and nematicidal (Chaterjee *et al.*, 1982). Mann *et al.* (2000) reported that the essential oil of *Ocimum* had highest antibacterial activity against gram positive bacteria as opposed to gram negative bacteria while present findings were in contradiction with Mann *et al.* (2000) and essential oil obtained from *Ocimum sanctum* was found equally effective against gram positive and gram negative bacteria (Table 1-4). Nakamura *et al.* (1999) reported that essential oil obtained from *Ocimum gratissium* had no any bacterial activity against the growth of *P. aeruginosa* while in present investigation *P. aeruginosa* was not found resistant against the *Ocimum sanctum* extracts. The present finding supports the Joshi *et al.* (2009) that the crude extracts of *Ocimum sanctum* is effective against the *S. aurues* and other selected gram positive microorganisms. Cold aqueous extract of *Ocimum* also showed potent antibacterial activities against the selected strains. Hence it may be interpreted from the present findings that *Ocimum* extract may be a better alternative as natural food preservatives in Food Industries.

CONCLUSION

Ocimum respectively, named basil is an aromatic herb that has been used traditionally as medicinal herbs in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunctions. Present investigation reveals that *Ocimum sanctum* may be a better alternative as a preservative in Food Industries since it is equally effective against pathogenic gram positive and gram negative bacteria. In present investigation the concentration *O. sanctum* was kept constant for all selected strains hence the same experiments may be carried out with varying concentration of *O. sanctum* in future.

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