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Research Article

Antibacterial Activity of *Ocimum Sanctum* Extracts against Four Food-Borne Microbial Pathogens

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Abstract: Aim of this study was to evaluate the antibacterial activity of aqueous extract, oil extract, chloroform extract and alcohol extract obtained form leaves of *Ocimum sanctum* against four food borne microbial pathogens, *Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli* and *Listeria monocytogenes.* In general, extracts obtained by all extraction methods showed antimicrobial activity against all tested microorganisms. Lowest and highest antibacterial activity was shown by aqueous extraction and chloroform extraction of residue obtained after aqueous extraction. Highest antibacterial activity was shown by chloroform extraction of residue obtained after aqueous extraction against *Salmonella enteritica. Listeria monocytogenes* was found to be the most resistant organism to all types of extracts. Higher antibacterial activity was shown against gram negative bacteria compared to gram positive bacteria tested. Acording to the results of the study, chloroform extraction was found to be the best extraction method to extract phytochemicals from *Ocimum* leaves. In conclusion, *Ocimum* extracts found to be containing chemical compounds useful in food preservation and development of drugs against food borne microbial pathogens.
Keywords: *Ocimum sanctum*, antibacterial, food borne pathogens

INTRODUCTION

Plant extracts has been used to treat for microbial disease from ancient time in traditional medical systems. Ability of using most of the medicinal plants for the treatments for various diseases may lie in the antioxidant and antimicrobial effect of phytochemicals [1]. Antimicrobial activities of some phytochemicals have been investigated and the possibility of using them in the development of new antimicrobial drugs also been documented [2]. Due to the development of resistance in pathogenic microorganisms to antibiotics used in modern medical science, there is a growing attention towards plant extracts as a source of new antimicrobial drug discoveries. As such investigations on the composition, activity, as well as validation of the use of extracts obtained from medicinal plant is important [3].

Ocimum sanctum is a grassy annual plant originated from Iran, Afghanistan and India [4, 5, 6]. Some of the phytochemicals of medicinal importance present in *Ocimum sanctum* have already been identified [7]. Some of these phytochemicals have been shown to possess useful biological activities belonging mainly to phenolic, flavonoid, and carotenoid compounds [8]. The ability of this plant to be used in traditional medicine in the treatment of headches, cough, diarrhea, constipation, warts, kidney malfunctions, nasal polyps and ulcers has also been reported [4,5,9]. Further, its action as insecticide, nematicide, fungicide and antimicrobial compound also has been reported [7, 10, 11, 12, 13, 14]. As such, extract of these chemicals from *Ocimum sanctum* plant possess useful pharmacological applications. However, reports on the antibacterial activity of *Ocimum sanctum* extracts against food borne pathogens are limited.

Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli and Listeria monocytogenes are food borne pathogenic bacteria causing gastroenteritis of human. *Salmonella* is one of the major food and water borne pathogenic bacteria which cause the disease called salmonellosis [15, 16]. *Vibrio parahaemolyticus* is a gram negative bacterium considered as a major cause of gastroenteritis specially associated with the consumption of raw seafood [17]. *Escherichia coli* is a bacterium present in the intestinal tract of warm blooded animals as its normal micro flora. Most of the *E. coli* strains are nonpathogenic but some serogroups, such as enterohemorrhagic O157:H7, are pathogenic and cause severe diarrhea and fever [18]. *Listeria monocytogenes* is an opportunistic intracellular pathogen causes the disease called listeriosis [19].

The aim of present study was to investigate the antibacterial activity of different extracts of *Ocimum sanctum* leaves against above four food borne microbial pathogens.

MATERIALS AND METHODS

Aqueous extraction

Fresh leaves of *Ocimum sanctum* were harvested and macerated. Then 200 g of leaves were mixed with 500 mL of water and kept 8 h at ambient temperature. Then

whole mixture was filtered using a cheese cloth and obtained extracts was centrifuged. After centrifugation, supernatant was labeled as "Extract A" and used for antibacterial activity assay. Residue obtained from filtration and centrifugation were mixed and used for $CHCl_3$ extraction.

Isolation of essential oil from *Ocimum sanctum*

Essential oil was isolated from *Ocimum sanctum* leaves by 3 h hydrodistillation of 200 g of leaves which were placed in 500 mL of water. The essential oil was separated, dried over anhydrous sodium sulphate, labeled as "Extract B" and stored at -20 ^{0}C until used for other tests [14, 20]. Residue of oil extraction was used at the chloroform extraction.

Chloroform extraction

The residue obtained after aqueous extraction and oil extraction were separately used for the chloroform extraction. Those were mixed with equal volume of chloroform and kept 24 h at ambient conditions. Then, mixture was filtered using cheese cloth and extract was obtained. Extract obtained from residue of aqueous extraction was labeled as "Extract C" and extract obtained from residues of oil extraction was labeled as "Extract D". The obtained extracts were used for antibacterial activity assay and both residues were separately used for alcoholic extraction.

Alcoholic extraction

Both types of residues obtained after chloroform extraction were separately treated with equal volume of methanol and kept for 24 h at ambient conditions. Then the mixtures were filtered using cheese cloth and extracts were obtained. Extract obtained from residues left after extraction of "Extract C" was labeled as "Extract E" and extract obtained from residues left after extraction of "Extract D" was labeled as "Extract F". The obtained extracts were then used for antibacterial activity assay.

Preparation of microbial cultures

Four bacteria used in this study *Vibrio parahaemolyticus, Salmonella enterica, Escherichia coli,* and *Listeria monocytogenes* were separately enriched by culturing for 24 hours at 37 ^{0}C in an universal culture medium, Tryptic Soy Broth Yeast Extract medium (TSBYE). Each culture was then divided to two potions and one potion was stored at refrigerated conditions. The other potion was serially diluted (10^{-1}–10^{-10}) in sterile distilled water and enumerated in Tryptic Soy Agar (TSA) plates. Bacterial concentration was estimated by calculating the average number of colonies on plates containing 30 to 300 colonies. Then using the stored cultures dilutions of each culture containing 10^3 CFU / mL were prepared and used in the antimicrobial activity test.

Antibacterial activity assay

Prepared cultures of *Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli* and *Listeria monocytogenes* were used for this assay. Form each cultures, one mililiter were cultured on nutrient agar plates. Then with the help of a sterile cork borer wells of 4 cm diameter were prepared on each of those plates. Fifty micro liter of each *Ocimum sanctum* extracts were separately added to wells in plates which were inoculated with each bacteria. Then those were incubated at 37 ^{0}C for 24 h. after incubation clear zone of inhibition were observed around the wells. Diameters of those inhibition zones were measured. Antibacterial activity of each *Ocimum sanctum* extracts against four tested pathogenic microorganisms was evaluated using those measurements.

Results and discussion

Antibacterial activity of different *Ocimum sanctum*a extracts against four food borne microbial pathogens, *Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli* and *Listeria monocytogenes* were studied. Results of the study are shown in the table 1.

Table 1: Antibacterial activity of different *Ocimum sanctum* extracts against *Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli* and *Listeria monocytogenes*

Extract	Microorganism			
	Salmonella enteritica	***Vibrio parahaemolyticus***	***Escherichia coli***	***Listeria monocytogenes***
Extract A	+	+	+	+
Extract B	+ +	+	++	+
Extract C	+++++	++++	++++	+++
Extract D	++++	++++	+++	+++
Extract E	+++	+++	++++	+++
Extract F	++	+++	++++	+

+,++,+++,++++, and +++++ are zone diameter less than 5 mm, 5-10 mm, 10-15 mm, 15-20 mm and higher than 20 mm respectively

According to the results, all different types of extracts obtained from *Ocimum sanctum* leaves shown to be with antibacterial activity against all tested food borne microbial pathogens. In a similar study [14] *Ocimum* extract has found to be with antimicrobial properties against *Staphylococcus aureus, Escherichia*

coli, Psudomonas aeruginosa and *Salmonella typhimurium.* In that study, significantly higher antibacterial activity of *Ocimum sanctum* extract has shown against *Staphylococcus aureus.* In the present study highest antimicrobial activity was shown against *Salmonella enteritica*.

In the present study, lowest and highest antibacterial activity was shown by cold liquid extraction and chloroform extraction of residue obtained after cold liquid extraction (table 1). These results are in agreement with the similar studies carried out by other authors for other microbes [14]. Highest antibacterial activity was shown by chloroform extraction of residue obtained after cold liquid extraction against *Salmonella enteritica.* Out of the four bacteria tested, *Listeria monocytogenes* was found to be the most resistant organism to all types of extracts. Extract obtained by oil extraction also showed good antibacterial activity. This can be mainly due to the presence of eugenol, a phenolic compound, which has been reported to have antimicrobial properties [21, 22, 23].

As per the results of the present study, *Ocimum* extract has shown antimicrobial properties against both gram negative bacteria (*Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli)* and gram positive bacteria (*Listeria monocytogenes*). *Ocimum* extract shown higher antibacterial activity against gram negative bacteria compared to gram positive bacteria in the present study. These results are in agrement with the results of some similar studies [14]. However some reports on higher antibacterial activity against gram positive bacteria than gram negative bacteria by *Ocimum* extract also available [4].

In general, acording to the results of the study, leaves of *Ocimum sanctum* were found to be containing chemical compounds which can be used as antimicrobial compounds against food borne microbial pathogens.

CONCLUSION

Ocimum extract obtained by different methods showed antimicrobial activity against four food borne microbial pathogens tested. Those were found to be more effective in controlling gram negative bacteria than gram positive bacteria. Chloroform extraction was found to be the best extraction method to extract phytochemicals from *Ocimum* leaves. Extracted essential oils were also found to be with antimicrobial properties. So, *Ocimum* extract can be useful in food preservation and in the development of drugs against tested food borne microbial pathogens.

REFERENCES

1. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO; Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci Res Essay, 2007; 2: 163-166.
2. Nascimento GGF, Locatelli J, Freitas PC, Silva GL; Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol., 2000; 31(4): 247-256.
3. Nair R, Chanda S; Activity of some medicinal plants against certain pathogenic bacterial strains. Indian J Pharmacol., 2006; 38(2): 142-144.
4. Mann CM, Cox SD, Markham JL;The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of Melaleuca alternifolis (tea tree oil). Lett. Applied Microbiol., 2000; 30(4): 294-297.
5. Volak J, Jiri S; Plant medicinal interpreter. 3rd edition, Tehran University Publications, Tehran, 1997: 20.
6. Zargari A; Medicinal Plants. 4th edition, Teheran University Press, Tehran, 1990: 1-57.
7. Deshpande RS, Tipnis HP; Insecticidal activity of *Ocimum basilieum* L. Pesticides, 1997; 11: 11-12.
8. Cook NC, Samman S; Flavonoids-chemistry, metabolism, cardio protective effect and dietary sources. J Nutr Biochem., 1996; 7(2): 66-76.
9. Sikmon JE, Morales MR, Phippen WB, Vieira RF, Hao Z; Preservatives on new crops and new uses, in a source aroma compounds and popular curlinary and ornamental herbs. ASHS press, Alexandria, VA, 1990: 495-505.
10. Chaterjee A, Sukul NC, Laskal S, Ghoshmajumdar S; Nematicidal principles from two species of lamiaceae. J Nematol., 1982; 14(1): 118-120.
11. Reuveni R, Flelsher A, Putievsky E; Fungistatic activity of essential oils from *Ocimum basilieum* chemotypes. Phyropathol J., 1984; 110: 20-22.
12. Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T; Anti HIV-1 activity of herbs in Labiate. Biol Pharm Bull., 1998; 21(8): 829-833.
13. Wannissorn B, Jarikasem S, Siriwangehaiand T, Thubthimthed S; Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia, 2005; 76(2): 233-236.
14. Mishra P, Mishra S; Study of antibacterial activity of *Ocimum sanctum* extract against Gram positive and Gram negative bacteria. American Journal of Food Technology, 2011; 6(4), 336-341.
15. Wang H, Blais BW, Brooks BW, Yamazaki H; *Salmonella* detection by the polymyxin-cloth enzyme immunoassay using polyclonal and monoclonal detector antibodies. International

Journal of Food Microbiology, 1996; 29(1): 31-40.

16. Nowak B, Muffling TV, Chaunchom S, Hartung J; Salmonella contamination in pigs at slaughter and on the farm: A field study using an antibody ELISA test and a PCR technique. International Journal of Food Microbiology, 2007; 115(3): 259 – 267.
17. Pham HN, Ohkusu K, Miyasaka J, Sun XS, Ezaki T; Rapid and specific identification of 5 human pathogenic Vibrio species by multiplex polymerase chain reaction targeted to dna J gene. Diagnostic Microbiology and Infectious Disease, 2007; 59(3): 271-275.
18. Stender H, Oliveira K, Rigby S, Bargoot F, Coull J; Rapid detection, identification, and enumeration of *Escherichia coli* by fluorescence in situ hybridization using an array scanner. Journal of Microbiological Methods, 2001; 45(1): 31–39.
19. Liu D; Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. Journal of Medical Microbiology, 2006; 55(Pt 6): 645–659.
20. Ezekwesili CN, Obiora KA, Ugwu OP; Evaluation of anti-diarrhoeal property of crude aqueous extract of *Ocimum gratissium* L. (Laniatae) in rats. Biokemistri, 2004; 16(2): 122-132.
21. Sen P; Therapeutic potentials of Tulsi from experience to facts. Drugs News Views, 1993; 1: 15-21.
22. Iwalokun RA, Gbenle GO, Adewole TA, Smith SI, Akinsinde KA, Omonighehin EO; Effect of *Ocimum gratissium* L. essential oils at sub inhibitory concentration on virulent and multidrug resistant Shigella strains from Lagoe, Nigeria. APMIS, 2003; 111(4): 477-482.
23. Jamine DAL, Xisto SP, Orionaida de FLP, Jose RDP, Pedro HF; Antifungal activity from *Ocimum gratissium* L. towards Cryptococcus neoformans. Mem Inst Oswaldo Cruz, 2005; 100: 55-58.