PRELIMINARY PHYTOCHEMITRY AND ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* L.

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ABSTRACT

Ethanolic extract of the leaves of *Ocimum gratissimum* was screened for its phytochemical and antibacterial properties on *E. coli* and *Listeria monocytogenes* at varying concentrations. The Agar gel diffusion method was used to assay for the antibacterial properties on the test isolate. The results showed that the ethanolic extracts at different concentrations inhibited the growth of *E. coli* and *L monocytogenes*. The concentration of 250mg/ml inhibited the isolate with highest diameter zone of inhibition ranging from 22mm to 25mm. The extracts inhibited the growth of the bacterial isolate in a concentration dependent manner with MICs of 9.25mg/ml. Phytochemical analysis of the leaf extracts revealed the presence of antimicrobial active agents such as alkaloids, phenolics, glycosides, resins, steroids, and tannins. These established a good support to the use of this plant in herbal medicine and as base for the development of new drugs and phytomedicine.

Keywords: Ocimum, Phytochemistry, Antibacterial activity, E. Coli.

INTRODUCTION

Medicinal plants have contributed immensely to health care in Indian subcontinent. This is due in part to the recognition of the value of traditional medical systems, particularly in Asian origin, and the identification of medicinal plant from indigenous pharmacopoeias and traditional knowledge, which have significant healing power.

Among all plant families, members of the Lamiaceae have been used for centuries as folk medicine. *Ocimum gratissimum* L (Lamiaceae), is used in the treatment of different diseases, for example upper respiratory tract infections, diarrhoea, headache, fever, ophthalmic, skin disease and pneumonia (Correa, 1932; Onajobi, 1986; Ilori *et al.,* 1996). The *Ocimum* oil is also active against several species of bacteria (*Staphylococcus aureus, Listeria monocytogenes, Escherichia coli*, Shigella, Salmonella and Proteus) and fungi (*Trichophyton rubrum, T. mentagrophytes. Cryptococcus neoformans, Penicillum islandi cum*, and *Candida albicans* (Akinyemi *et al.,* 2004, Janine de Aquino Lemos *et al.,* 2005, Lopez *et al.,* 2005). Various species of *Ocimum gratissimum* for example *O. viride* Linn, *O. suave* Linn, *O. basilicum* Linn and *O. canum* Sims have been reported for their numerous medical uses (Mshana *et al.,* 2000).

It is said to have numerous properties, such as the tannins and sweet smelling volatile oil known to have antibacterial agent (Elujoba 2000). The volatile oil also stops spasm, the hyperactivity of the gastrointestinal tract, by combining with the antibacterial activity and thus lowers the amount of times the muscle of the stomach and gastrointestinal tracts contracts stopping the diarrhoea (Elujoba, 2000) that are usually adverse for most other pathogenic bacteria. It can be isolated from soil, silage and other environmental sources (Patrick *et al.,* 1995).

The onset of Listeriosis is usually preceded by influenza-like symptoms (Martin and Fisher 2000). The onset time to serious forms of Listeriosisis is known but may range from a few days to 3 weeks. The manifestation of Listeriosis is septicemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion or stillbirth. At present the infective dose of *L. monocytogenes* is unknown, although it is believed to vary with the strain and susceptibility of the victim (Curtis, 2000). Sometimes in susceptible persons, fewer than 103 cfu/g or ml may cause disease (Martin and Fisher, 2000).

This study was designed to evaluate the antibacterial efficacy of *Ocimum gratissimum* on *Listeria monocytogenes* associated with ready-to-eat dairy products and to determine the active principles in the plant extract. The other bacterial strain E. coli was used to compare the properties against L. monocytogenes.

MATERIALS AND METHODS

Fresh leaves of *Ocimum gratissimum* L was collected from Dr, P. D. K. V. Agricultural University

Campus, Akola in the moth of March 2009. The plant was identified by local taxonomist and a specimen copy was deposited in Department of Botany, Shri Shivaji College, Akola (MS) India.

The fresh leaves were harvested and properly washed in tap water and then rinsed in sterile distilled water. The leaves were blended fresh using electric blender. The soluble ingredients were then extracted by solubilization using ethanol as solvent.

The ethanol extract of the active ingredient of the leaves were carried out using the method as described by (Harbone, 1994). 25g of the grinded fresh leaves were soxhlet extracted using 250 ml of 95% ethanol. The extraction lasted for 6 hours. The volatile oil obtained was concentrated by evaporation using water bath at 100^{o}C for 1 hour.

The method of Akujobi *et al.,* (2004) was adopted. The crude extract was diluted with 30% dimethylsulphoxide (DMSO) to obtain concentration of 250, 200, 150, 100, and 50mg/ml.

Test Microorganism

The strain used in this work was *L. monocytogenes* type 4a (Food origin) and *E. coli* obtained from Laboratory of Mycology and Plant Pathology, Department of Botany, RTM, Nagpur University. The bacteria was maintained by weekly transfers in tryptic soy broth (TSB) and distributed in 5ml volume in screw-capped tubes. Cells were grown at 37^{o}C for 48 hours and cultures were kept at 4^{o}C.

Antibacterial Test

The antibacterial tests of the plant extracts were tested on the test isolate using the agar-gel diffusion inhibition test. In the agar-gel diffusion inhibition test as described by Opara and Anasa (1993), 0.2 ml of a 24 hours broth culture containing 1 X 10^{6} cells/ml of organism was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of about 6.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the petri dish. Fixed volumes (0.1 ml) of the extract were then introduced into the wells in the plates. A control well was in the center with 0.01 ml of the extracting solvent. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract to occur (Esimone *et al.,* 1998) and then incubated at 37oC for 24 hours. The resulting zones of inhibition were measured using a ruler Calibrated in millimeters. The average of the three readings was taken to be the zone of inhibition of the bacterial isolate in question at that particular concentration (Abayomi, 1982).

Maximum Inhibitory Concentration (MIC)

The MIC of the potent extracts was determined according to the macro broth dilution technique (Baron and Finegold 1990). Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of leaf extracts and incubated at 37^{o}C for 24 hours. The MICs were read as the least concentration that inhibited the growth of the test organisms.

Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was subcultured onto extract free agar plates, incubated for further 24 hours at 37^{o}C. The least concentration, at which no growth was observed, was noted as the MBC.

Phytochemical Screening

Phytochemical screening was carried out according to the methods described by Trease and Evans (1989).

Statistical Analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA), as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Table 1 shows the results of the antibacterial effect of the extracts on the test isolate. In general, the zone of inhibition decreased with decrease in concentration of the leaf extract. The highest zone of growth inhibition occur with a zone diameter of 25mm at a concentration of 250mg/ml, while the lowest zone of growth inhibition occur with a zone diameter of 6.5mm at a concentration of 50mg/ml.

Table 2 shows the MIC and MBC of the extract on the test isolate. The MIC results indicated that ethanolic extract of the fresh leaf on test organism had MIC of 9.25mg/ml, while MBC had 2.15mg/ml for *L. monocytogenes* and 2.50 mg/ml for E. coli.

Table 3 shows the preliminary phytochemical profile of the ethanolic leaf extract of the plant. The phytochemical screening showed that the leaf extract of *Ocimum gratissimum* contain alkaloids, resins, tannin, phenolics, glycosides, saponin, and steroidal terpenes at different concentrations.

Table1: Antibacterial activity of ethanolic extract of *O. gratissimum* against *E. coli* and *L. monocytogenes*.

Concentration of leaf extract (mg/ml)	Zone of inhibition (mm)	
	E. coli	L. Monocytogenes
250	22.00	26.00
200	18.00	20.00
150	16.00	18.50
100	11.00	15.00
50	06.50	08.00

Table 2: Maximum inhibitory concentration and minimum bactericidal concentrations of ethanolic leaf extracts of *Ocimum gratissimum* (mg/ml)

Plant	MIC (mg/ml)	MIC (mg/ml)	
O. gratissimum	09.25	E. coli	L. monocytogenes
		2.50	2.15

Table 3: Preliminary phytochemical analysis of Fresh ethanolic leaf extract of *O. gratissimum*.

Phytochemicals	Ethanolic fresh leaf extract
Alkaloids	+
Tannins	+
Phenolics	+
Saponins	+
Glycosides	+
Resins	+

Several species and varieties of plants of the genus *Ocimum* have been reported to yield oil of diverse nature, commonly known as basilic oils. Craveiro *et al.,* (1981) and Janine de Aquino Lemos *et al.,* (2005) reported some chemical compounds and active ingredients found in these plants such as; eugenol, linaol, methyl cinnamate, camphor and thymol. It has been demonstrated that the eugenol isolated from *Oximum gratissimum* presented antimicrobial (Iwalokun *et al.,* 2003; Janine de Aquino Lemos *et al.,* 2005), Insecticidal (Deshpande and Tipnis, 1977; Chavan and Nikam 1982), antihelminthic (Pessoa *et al.,* 2002), nematicidal activities (Chatterje *et al.,* 1982), or fungistatic properties (Reuveni *et al.,* 1984).

In the present study, the antibacterial profile and phytochemical screening fresh leaf of *Ocimum gratissimum* on *Listeria monocytogenes* was studied. The results obtained from this study showed that the ethanolic extract of the plant inhibited the growth of the test isolates at varying concentrations. This is similar to the findings of Obi and Onuoha (2000), who reported alcohol to be best solvent for the extraction of most plant active principles of medical importance.

The low minimum inhibitory concentrations observed for ethanolic extracts of the fresh leaf on *Listeria monocytogenes* is of great significance in the health delivery system, since it could be used as an alternative treatment to orthodox antibiotics in the treatment of diseases due to this isolate, especially as they frequently develop resistance to known antibiotics, and will reduce the cost of obtaining health care. The result obtained for MBC (2.15mg/ml) after plating on various dilutions of extracts is more reliable and promising compared to MIC results obtained usually turbidity as an index.

Preliminary phytochemical screening revealed the presence of alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpens and flavonoids. These are believed to be responsible for the observed antibacterial effects. Some workers have also attributed to their observed antimicrobial effect of plant extracts to the presence of these secondary plant metabolites (Nweze *et al.,* 2004). The presence of these phytochemical bases in *O. gratissimum* accounts for it usefulness as a medicinal plant.

The study has showed that the observed antibacterial effect of *Ocimum gratissimum* leaf on the bacterial isolate, though *in vitro* appear interesting and promising:

The use of this plant and its derivatives for the primary purpose of flavouring and preserving foods will be of interest for further study.

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