Antibacterial Activity And Phytochemical Screening Of Crude Ethanolic Extract Of Leaves Of Ocimum Gratissimum L On Listeria Monocytogenes.

T Mbata, A Saikia

Abstract
Ethanolic extract of the leaves of Ocimum gratissimum was screened for its phytochemical and antibacterial properties on 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 Listeria monocytogenes. The concentration of 250mg/ml inhibited the isolate with highest diameter zone of inhibition of 25mm. The extracts inhibited the growth of the bacterial isolate in a concentration dependent manner with MICs of 9.25mg/ml, while MBCs had 2.15mg/ml. Phytochemical analysis of the leaf extracts revealed the presence of antimicrobial active agents such as alkaloids, cardiac glycosides, flavonoids, glycosides, resins, steroidal terpens, 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.

INTRODUCTION
Medicinal plants have contributed immensely to health care in Nigeria. 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, which have significant healing power.

Among all families of the plant kingdom, members of the Lamiaceae have been used for centuries in folk medicine. Ocimum gratissimum L (Lamiaceae), commonly known as “alfavaca” is naturally used in the treatment of different diseases, for example upper respiratory tract infections, diarrhea, 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, Penicillium islandi cum, and Candida albicans (El-said et al., 1969; Begum et al., 1993; Nwosu and Okafor 1995; 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).

Recent studies on Ocimum gratissimum proved to be a useful medication for people living with Human Immuno deficiency Virus (HIV), and Acquired Immuno Deficiency Syndrome virus AIDs (Elujoba, 2000). Spice or sweet basil is also thought to be an antispasmodic, carminative stimulant and insect repellent.

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 Listeriosisis is usually preceded by influenza-like symptoms (Martin and Fisher, 2000). The onset time to serious forms of Listeriosis 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 (Marsden, 1994; Dimitrijeric and Teodorov, 1998; Curtis, 2000). Sometimes in susceptible persons, fewer than 103 cfu/g or ml may cause disease (Martin and Fisher, 2000).

Over the past few years there have been published many studies that associate the consumption of foods contaminated by L. monocytogenes (Maijala et al., 2001). As suggested by the World Health Organization report, transmission of food borne Listeriosis to human is as a result of environmental contamination involving both food supply and food processing plant.

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.

MATERIALS AND METHODS

COLLECTIONS AND IDENTIFICATION

Fresh leaves of Ocimum gratissimum L was collected from Owerri in South Eastern Nigeria during the month of May. A branch of the plant was identified by trained plant taxonomists at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

PROCESSING OF PLANT SAMPLES

The fresh leaves were harvested and properly washed in trap 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.

EXTRACTION OF PLANT MATERIAL

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 100oC for 1 hour.

PREPARATION OF CRUDE EXTRACT

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 Listeria monocytogenes type 4a (Food origin) obtained from Hebrew University Isreal. 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 37oC for 48 hours and cultures were kept at 4oC.

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 106 cells/ml of organism was asceptically 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 asceptically 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 were determined according to the macro broth dilution technique (Tilton and Howard, 1987; 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 37oC 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 37oC. The least concentration, at which no growth was observed, was noted as the MBC.
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PHYTOCHEMICAL SCREENING
This 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
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 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 6.2mm 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.

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

Table 1: Antibacterial activity of the ethanolic leaf extracts of Ocimum gratissimum on Listeria monocytogenes

<table>
<thead>
<tr>
<th>Concentration of extract (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>250</td>
<td>25.0</td>
</tr>
<tr>
<td>200</td>
<td>20.0</td>
</tr>
<tr>
<td>150</td>
<td>18.0</td>
</tr>
<tr>
<td>100</td>
<td>15.2</td>
</tr>
<tr>
<td>50</td>
<td>6.2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. gratissimum</td>
<td>9.25</td>
<td>2.15</td>
</tr>
</tbody>
</table>

Table 3: Phytochemical analysis of leaf extract of

<table>
<thead>
<tr>
<th>Extract compounds</th>
<th>Ethanolic fresh leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Steroidal ring</td>
<td>-</td>
</tr>
<tr>
<td>Steroidal terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
</tbody>
</table>

+/- = Presence or absence of the component tested

DISCUSSION AND CONCLUSION
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, linal, methyl cinnamate, camphor and thymol. It has been demonstrated that the eugenol isolated from Ocimum gratissimum presented antimicrobial (Ntezurubanza et al., 1984; Nakamura et al., 1999; Iwalokun et al., 2003; Janine de Aquino Lemos et al., 2005), Insecticidal (Deshpande and Tipnis 1977; Chogo and Crank 1981; Chavan and Nikam 1982), antihelmintic (Pessoa et al., 2002), nematidal activities (Chatterje et al., 1982), or fungistic 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 Oluoha (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 (Singleton, 1999), 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 terpenes 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 its 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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References


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