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

T Mbata, A Saikia

Citation

T Mbata, A Saikia. *Antibacterial Activity And Phytochemical Screening Of Crude Ethanolic Extract Of Leaves Of Ocimum Gratissimum L On Listeria Monocytogenes.*. The Internet Journal of Microbiology. 2007 Volume 4 Number 2.

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 medcine 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, 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 (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 repellant.

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 (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.

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 gycosides, and steroidal terpenes at different concentrations.

Figure 1

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

Concentration of extract (mg/ml)	Zone of inhibition (mm)
	Listeria monocytogenes
250	25.0
200	20.0
150	18.0
100	15.2
50	6.2

Figure 2

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

Plant	*Listeria monocytogenes*	
	MIC (mg/ml)	MBC (mg/ml)
O. gratissimum	9.25	2.15

Figure 3

Table 3: Phytochemical analysis of leaf extract of

Extracts compounds	Ethanolic fresh leaf
Alkaloids	+
Tannins	+
Glycosides	+
Anthraquinones	-
Saponin	+
Resins	+
ardiac glycoside	+
Steroidal ring	-
Steroidal terpens	+
Flavonoids	+

+/- = Presence or absence of the component tested

DISCUSSION AND CONCLUSION

DISCUSSION

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 (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), 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 (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 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.

CONCLUSION

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.

ACKNOWLEDGEMENT

The authors are grateful to Staff of IITA, Ibadan, Nigeria for their technical assistance.

References

1. Abayomi, S. (1982). The state of medical plants reach in Nigeria. University of Ife Press, pp 200.
2. Akinyemi K.O., Mendie U.E., Smith S.T., Oyefolu A.O. and Coker A.O. (2004). Screening of some medical plants for anti-salmonella activity. J Herb Pharmocother. 5(1), pp 45-60.
3. Akujobi C., Anyanwu B.N., Onyeze C. and Ibekwe V.I. (2004). Antibacterial Activities and Preliminary Phytochemical Screening of Four Medical Plants. J Appl Sci. 7(3), pp 4328-4338.
4. Aureli P., Constantini A. and Zolea S. (1992). Antimicrobial activity of some plant essential oils against Listeria monocytogenes. J Food Prot. 55, pp 344-348.
5. Baron J.E. and Fingold S.M. (1990). Method for testing antimicrobial effectiveness. In: Bailey Scotts Diagnostic Microbiology. Mosby, CV. (8th ed), Missouri. pp 171-194.
6. Begum J., Yusuf M., Chowdhury U. and wahab M.A. (1993). Studies on essential oils for their antibacterial and antifungal properties. Part 1. preliminary screening of 35 essential oils. J Sci Ind Res. 28, pp 25-3.
7. Chatterje A., Sukul N.C., Laskel S. and Ghoshmajumadar S. (1982). Nematicides principal from two species of Lamiaceae. J Nematol. 14, pp 118-120.
8. Chavan S.R. and Nikam S.T. (1982). Mosquito larvicidal activity of Ocimum basilicum Linn. India J Med Res. 75, pp 220-222.
9. Chogo J.B., and Crank G. (1981). Chemical composition and biological activity of the Tanzania plant Ocimum suava. J Nat Prot. 44(3), pp 308-311.
10. Correa M.P. (1932). Dicionario das plantas uteis do Brasil. IBDF, Rio de Janeiro.
11. Craveiro A.A., Fernandes A.G., Andrade C.H.S., Matos F.J.A, and Alencar W.J. (1981). Oleos essenciais de plantas do Nordeste, Imprensa Universitaria. Universidade Federal do Ceard, Fortaleza.
12. Curtis G.D. (2000). Detection by Classical Cultural Techniques.. Encyclopedia of Food Microbiology, Bacteriology Department, John Ratclife Hospital, Oxford, UK. pp 119-1207
13. Desphande R.S. and Tipnis H.P. (1977). Insecticidal activity of Ocimum basilicum L. Pesticides., 11, pp 11-12.
14. Dimitrijevic M. and Teodorovic V. (1998). Importance of food animal origin in genesis of alimentary Listeriosis. Veterinarski Glasniki., 52(1-2), pp 79-85.
15. El-said F., Sofowora E.A., Malcolm S.A. and Hofer A. (1969). An investigation into the efficacy of Ocimum gratissimum as used in Nigeria native medicine. Planta Medica., 97, pp 195-200.
16. Elujoba A.A. (2000). Studies on the antidiarrhoea

activity of Ocimum gratissimum. University of Ile Ife Press.
17. Esimone C.O., Adikwu M.U. and Okonta J.M. (1998). Preliminary antimicrobial screening of the ethanolic extract from the lichen Usnea subfloridans (L). J. Pharm. Res. Dev. 3(2), pp 99-101.
18. Harbone N.V. (1994). Phytochemical methods. A guide to modern techniques of plant analysis, 2nd Edition, Chapman and Hall London. pp 425
19. Ilori M., Sheteolu A.O., Omonibgehin E.A., and Adeneye A.A. (1996). Antibacterial activity of Ocimum gratissimum (Lamiaceae). J. Diarhoeal Dis. Res.14, pp 283-285.
20. Iwalokun R.A., Gbenle G.O., Adewole T.A., Smith S.I., Akinsinde K.A., and Omonigbehin E.O. (2003). Effects of Ocimum gratissimum L. essential oil at sub-inhibitory concentration on virulent and multidrug resistance Shigella strains from Lagos, Nigeria, APMIS., 3(4), pp 477-482.
21. Janine de Aquino L., Xisto S.P., Orionaida de Fatima L.F., Realino de Paula J., Pedro H.F., Hasimoto de Suza L.K., Aline de Aquino L. and Maria de Rosario R.S. (2005). Antifungal activity from Ocimum gratissimum L. towards Cryptococus neoformans. Mem. Inst. Oswaldo Cruz., 100(1), pp 55-58.
22. Lopez P., Sanchez K., Batlle R. and Nerin C. (2005). Solid and vapour phase anti-microbial activities of six essential oils susceptibility of selected food borne bacterial and fungal strains. J. Agric Food Chem., 53(17), pp 6939-6946.
23. Maijala R., Lyykikainen O., Johansson T., Autio T., Aaito T., Haavisto L. and Honkane-Buziski T. (2001). Exposure of Listeria monocytogenes with an epidemic caused by butter in Finland. International J. of Food Microbiol., 70(1-2), pp 97-109.
24. Marsden J.L. (1994). Industry perspective on Listeria monocytogenes in food: raw meat and poultry. Dairy, Food and Environmental Sanitation., 14(2), pp 83-86.
25. Martin S.E. and Fisher C.W. (2000). Listeria monocytogenes. In R.K. Robinson, C.A. Batt, and P.D. Petal (Eds). Encyclopedia of Food Microbiol., Academic Press, San Diego, CA. pp 1228-1238.
26. Mshana N.R., Abbiw D.K., Addae-Mensah I., Adjanohoun E., Ahji M.R.A., Enow-Orock E.G., Gbile Z.O., Odei M.A., Adenlami H., Oteng-Yeboah A.A., Sarppony K., Sofowora A. and Tackie A.N. (2000). Traditional medicine and pharmacopoeia contribution to the revision of Ethnobotanical and floristic studies in Ghana, Scientific, Technical and Research Commission of the Organization of African Unity
27. Nakamura C.V., Nakamura T.V., Bando E., Melo A.F.N., Cortez D.A.G. and Dias Filho B.P. (1999). Antibacterial activity of Ocimum gratissimum L. essential oil. Mem. Inst. Oswaldo Cruz., 94, pp 675-678.
28. Ntezurubanze L.I., Scheffer J.J.C., Looman A. and Baerhiem Svends. (1984). Composition of essential oil of Ocimum kilimandscharicum grown in Rwanda. Plant. Medica., pp 385-388.
29. Nweze E.I., Okafor J.I. and Njoku O. (2004). Antimicrobial activities of Methanolic Extracts of Trema guineensis (Schumm and Thorn) and Morinda lucida Benth used in Nigerian Herbal Medicinal Practice. J. Biol. Res. Biotech., 2(1), pp 39-46.
30. Nwosu M.O. and Okafor J.I. (1995). Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. Mycoses., 38, pp 191-195.
31. Obi V.I. and Onuoha C. (2000). Extraction and Characterization Method of Plants and Plant Products. In: Biological and Agricultural Techniques. Ogbulie, J.N. and O.J Ojiako (eds). Websmedia Publications, Owerri. pp 271-286.
32. Onajobi F.D. (1986). Smooth muscle contracting lipid soluble principles in chromatographic fractions of Ocimum gratissimum. J.Ethnopharmacol.,18, pp 3-11.
33. Opara A.A. and Ansa M.A. (1993). The antibacterial activity of Tea and Coffee on selected organisms. J. Med. Lab. Sci., 3, pp 45-48.
34. Patrick R.M., Ellen J.B., Michael P.E., Fred L.T. and Robert H.Y. (1995). Listeria. In: Manual of Clinical Microbiology. 6th ASM Press, Washington DC, pp 341-347.
35. Pessoa L.M., Morais S.M., Bevilaque C.M.L. and Luciano J.H.S. (2002). Antihelmintic activity of essential oil of Ocimum gratissimum Linn, and eugenol against Heamonchus contortus. Vet Parasitol., 109, pp 59-63.
36. Reuveni R.A., Fleisher and Putievsky E. (1984). Fungistic activity of essential oils from Ocimum basilicum chemotypes. Phytopath.,110, pp 20-22.
37. Roitman C.M., Roitman I., and Azevedo H.P. (1972). Growth of an insect Trypanosomatid at 37oC in a defined medium. J. Protozool., 19, pp 346-349.
38. Singleton P. (1999). Bacteria in Biology, Biotechnology and Medicine, 4th edition. John Wiley and Sons Ltd, New York. pp 324-337
39. Snedecor G.W. and Cochran W.G. (1967). A statistical method. Iowa State University Press, Ames, Iowa, USA.
40. Tilton R.C., and Howard B.J. (1987). Antimicrobial susceptibility testing. In: Carson D, Birchor S, Clinical and Pathogenic Microbiology. Mosby Co., St Louis, pp 121-115.
41. Trease G.E. and Evans W.C. (1989). Pharmacognosy 13th Edition. Bailliere Tindall Ltd London.

Author Information

T.I. Mbata
Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University

A. Saikia
Department of Horticulture, Assam Agriculture University