

## **Antibacterial Activity of Essential oil from *Ocimum gratissimum* on *Listeria monocytogenes*.**

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### **Abstract**

The antibacterial effect of *Ocimum gratissimum* extracted from aromatic plant was investigated against *Listeria monocytogenes* serotype 4a. Agar well diffusion and tube dilution methods were used and the data recorded demonstrated antibacterial activity of the essential oil Eos against the test bacteria. The bacteria was grown at 37°C in a chemically defined or a complex medium, containing essential oil obtained from *Ocimum gratissimum*. At concentration from 20 to 250µg/ml, the essential oil, progressively inhibited the bacterial growth. The bacteria cultivated on chemically defined medium were more sensitive to essential oil at concentration of 50, 62.5 and 100µg/ml in relation to those cultivated in complex medium at 37°C. The agar well diffusion was also evaluated. The results yield a zone of inhibition of 25mm. 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: Antibacterial *Ocimum gratissimum*, Essential oil Eos, *Listeria monocytogenes*

### **Introduction**

Medicinal plants has 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 coil*, 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 *O. 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).

*Listeria monocytogenes* is a gram positive bacteria, resists the deleterious effects of freezing, drying and heat. It causes Listeriosis in human and other animal and birds. The disease is primarily transmitted through various foods, milk, milk products, meat and meat products, fish, eggs and egg products, fruits and vegetable. It is particularly difficult to control, since it is ubiquitous and widespread in the environment, and since it possesses physiological characteristics that allow growth under conditions that are usually adverse for most other pathogenic bacteria. It can be isolated from soil, silage and other environmental sources.

The onset of Listeriosis 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 include 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; Dimitrijevic and Teodorov, 1998; Curtis, 2000). Sometimes in susceptible persons, fewer than  $10^3$  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 is therefore aimed at determining the rate of susceptibility of *L. monocytogenes* to the essential oils of a native plant of Nigeria for using as food additives in foods or therapeutic agents.

## Materials and Methods

### Plant Materials

The fresh leaves of *Ocimum gratissimum* was collected from Owerri in South eastern Nigeria. The leaves were cut into pieces and subjected to steam distillation. The distillate was then extracted with petroleum ether, which was removed carefully and the essential oil obtained. The oil was then stored at  $-20^{\circ}\text{C}$  until needed.

### Preparation of stock solutions

The stock solution (10mg/ml) of essential oil from *O. gratissimum* was made in a chemically defined medium (Roitmon *et al.*, 1972). From the stock solution dilutions were made to obtain 250, 150, 125, 100, 62.5, 50, and 20  $\mu\text{g/ml}$ .

### Microorganism

The strain used in this work was *Listeria monocytogenes* type 4a (food origin) obtained from Hebrews University Israel. The bacteria was maintained by weekly transfers in a chemically defined medium and tryptic soy broth (TSB) and distributed in 5ml volumes in screw capped tubes. Cells were grown at  $37^{\circ}\text{C}$  for 48h and cultures were kept at  $4^{\circ}\text{C}$ .

### Antibacterial activity

For experiment *Listeria monocytogenes* type 4a was incubated in a defined or complex medium containing different concentrations of essential oil, which were added only once to the culture. Cells were grown in 13x100mm tubes containing 1ml of defined medium and the start inoculum consisted of bacteria in logarithmic growth phase ( $1 \times 10^6$ cfu/ml). After 24-48h at  $37^{\circ}\text{C}$ , the cell growth was estimated by counting in a haemocytometer (Improved Double Neubauer). As negative control, defined medium alone was used. All experiments were performed in triplicate and the results were expressed as average values.

### Agar-gel diffusion

The antibacterial test of essential oil of *O. gratissimum* was tested on *Listeria monocytogenes* types 4a using the agar gel diffusion. 0.2 ml of a 24h broth culture of *Listeria monocytogenes* was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of 6.0mm in diameter were aseptically punched on each agar plate using a sterile cork bore. Fixed volume (0.1ml) of the essential oil of the plant extracts was carefully placed in each well. The plates were incubated at  $37^{\circ}\text{C}$  for 24h. The zone of inhibition of each well was obtained by measuring the underside of the plate in two planes with a ruler calibrated in millimeter. The control was placed with 0.1ml of the extracting solvent and incubated.

## Results and Discussion

Table I showed the effect of essential oil from *O. gratissimum* on growth *Listeria monocytogenes* cultivated in defined and complex media at 37<sup>0</sup>C during 48h. It is possible to observe in this table that the composition of the culture did not interfere with the growth inhibition of the bacteria when incubated at 37<sup>0</sup>C. On the other hand, *Listeria monocytogenes* cultivated at 37<sup>0</sup>C in defined medium containing 50, 62.5 and 100µg/ml of essential oil was inhibited more efficiently than when cultivated in complex medium.

The Agar gel diffusion test results showed the presence of a wide zone of inhibition surrounding the agar wells indicating antilisteric activity.

**Table 1: Effect of essential oil of *O. gratissimum* on growth of *Listeria monocytogenes* type 4a cultivated in defined and complex media at 37<sup>0</sup>c during 48h.**

Bacterial growth inhibition (%)		
Concentration of essential oil (µg/ml)	Chemically defined medium	Complex medium
	37 <sup>0</sup> C	37 <sup>0</sup> C
250	97.3	98.0
150	96.7	98.1
125	96.9	98.1
100	96.3	74.3
62.5	72.1	49.0
50	74.0	30.0
20	21.0	20

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 components and active ingredients found in these plants such as; eugenol, linalol, methyl cinnamate, camphor and thymol. It has been demonstrated that the eugenol isolated from *O. 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), nematocidal (Chatterje *et al.*, 1982) or which have fungistatic properties (Reuveni *et al.*, 1984)

In the present study, the inhibitory concentration of the essential oil from *O. gratissimum* on *Listeria monocytogenes* was studied. In table 1, the bacteria was progressively inhibited as the concentration of the essential oil increases to 250µg/ml. This study showed that cells treated with high concentration of the essential oil caused more than 50% of cell death since eugenol is the principal constituent of the essential oil. This result suggest that it could be responsible for this effect. The medium constitution and incubation temperature may be the adjuvant factors, responsible for the action of the essential oil as antibacterial agent.

The result of the agar gel diffusion showed wide zone of inhibition when the essential oil was treated with the test bacteria. This result agrees with previous studies on inhibition of bacteria species by essential oils (Eos) extracted from plants. (Aureli *et al.*, 1992)

In conclusion, the study has showed that *O. gratissimum* have properties that can inhibit the growth of psychrophils and heat resistant organisms and there should be need for the use of this plant and its derivatives for the primary purpose of flavouring foods and antimicrobial activity.

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