

**ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *OCIMUM GRATISSIMUM L*****Thirunethiran Karpagam^{1*},**S.R.Subashini¹, G.Gayathri¹, S.Jannathul Firdous¹, A.Shanmuga Priya¹¹Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli - 620 002, (INDIA)**ABSTRACT**

In recent years interest in medicinal plants has increased considerably. Antibacterial activity Ethanol, Chloroform and Ethyl acetate extract of leaves, root and stems of *Ocimum gratissimum.L* were tested against *E. coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Then the zone of inhibition after 24 hours was calculated. Chloroform solvent of root extract was found to be more effective than leaf and stem extracts against *E. coli*. The phytochemical analysis using the proton NMR spectral data of petroleum sample suggested the presence of benzoid group, methoxy group and an amide group, a compound named N-Salicyloyl-2-amino-1-metboxy propane which may be the cause of antibacterial activity in the root of *Ocimum gratissimum L*.

Keywords: *Ocimum Gratissimum L*, *E.coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*, NMR, GCMS.

Correspondence to Author**Thirunethiran Karpagam**Department of Biochemistry, Shrimati
Indira Gandhi College, Tiruchirappalli
- 620 002, (INDIA)**Email:**karpagam_murugan@yahoo.com**INTRODUCTION**

Allopathic system of medicine has been practiced for the last 500 years. "Modern Western Medicine" might have been the transfer of ayurvedic knowledge, its modification, processing led to the synthesis of new product as "Modern Medicine". Though the Allopathic Medicine has positive aspects, on the other hand it also has negative aspects. But natural medicine has no such side effects and has the ability to counteract those effects. It is note worthy that 80% of world's population still relies on herbal medicine. The medicinal value of herbal plants is due to the presence of some chemical substance or

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substances that produce a definite physiological action on the human body. The most important of these substances are essential oils, fatty oils, glucosides, resins, gums, mucilages, tannins, steroids, flavonoids and alkaloids^[1].

Medicinal plants stimulate the immune system and are used in the treatment of upper respiratory tract infections, headache, neuralgia, diarrhea, skin disease & pneumonia. Onajabi., 1986^[2] reported that the *Ocimum* oil is active against several species of bacteria and also Decoction of leaves is used in seminal weakness & is remedy for gonorrhoea.

MATERIALS & METHODS

Collection of Plants

Ocimum gratissimum L was collected from in and around Kolli Malai, Tamil Nadu, India.

Procurement of Test microorganism

The Test microorganisms Gram +ve Bacteria:

Staphylococcus aureus and Gram-ve Bacteria: *E. coli* and *Klebsiella pneumoniae* were procured from

Department of Biotechnology, KCAS, Tiruchirappalli.

Preparation of plant extract

Dry plant material (Leaf, stem, root (5g)) was used for taking extract. Solvents like Ethanol, Chloroform, Ethyl acetate was used to take extract from the dry plant material which was used for screening the antibacterial activity^[3].

Innoculum preparation

The test organism was inoculated nutrient broth (HiMedia) and incubated at 37°C over night. Nutrient agar was prepared under sterile condition.

Antibacterial assay

The ethanolic, chloroform, ethyl acetate solvent of leaf, stem, and root extract were tested for antibacterial activity. Sterile cotton swabs or L-rods were used to lawn culture of the organism. 50 µl of each extract was poured on a sterile disc of 5 mm diameter and allowed to dry for 30 minutes under aseptic condition. The paper disc, thus prepared, was placed equidistantly on an inoculated agar petriplates. The plates were incubated at 37°C for 24 hours & Zone of inhibition, was recorded^[4].

Preparation of extract for TLC Analysis

100 gm of dry plant material was ground well with 600 ml of Ethanol and the extract was kept for one week, with slight shaking for alternative days^[5]. After a week, filtered extract material was kept in the boiling water bath at 40°C for 2 hours. Finally 3ml water was added to get a clear yellow solution,

which was separated by separating funnel by using Petroleum Ether, Chloroform & Ethyl Acetate solvents and stored. The stored samples were subjected to thin layer chromatography, Gas Chromatography, NMR, Mass Spectroscopy^[6].

Thin layer chromatography

The extract were subjected to TLC. Silica gel coated TLC plates were kept in a chamber containing mobile phase solvent namely n-hexane, chloroform and petroleum ether. (8:2:2)

The sample extract was loaded in a TLC plate at 0.1mm distance from the margin line. After complete running of the solvent the plate were dried and kept in another chamber containing sodium nitroprusside crystals and allowed to develop.

NMR (Nuclear magnetic resonance)

This is sensitive and useful in structural analysis of active components ¹H resonance frequency of 400 MHz was used for standard NMR. It gives information of structural elucidation of natural products.

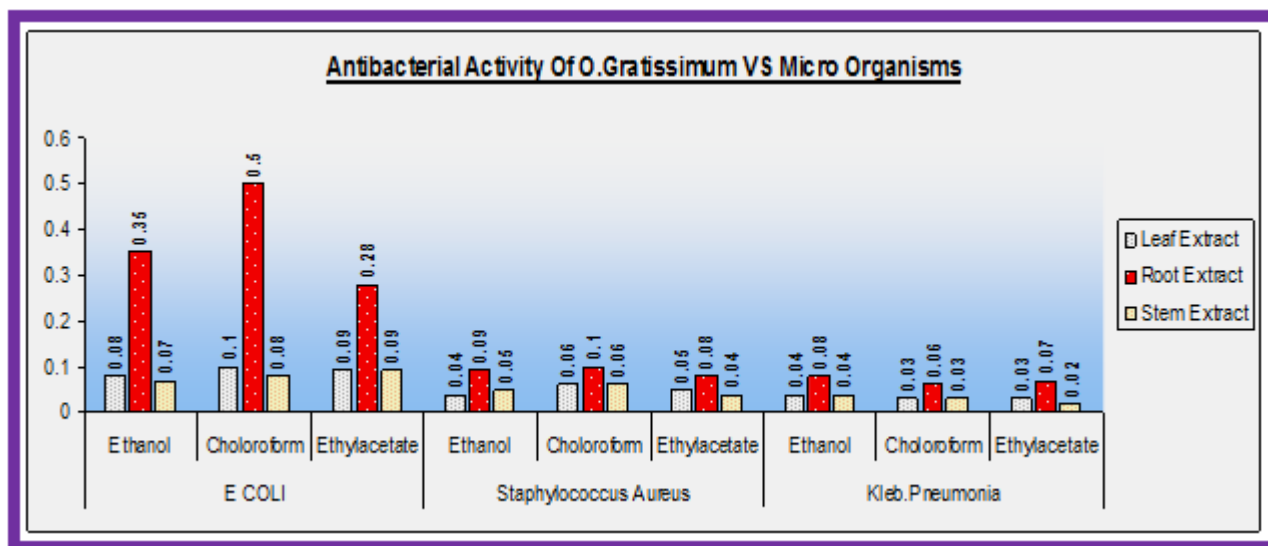
GCMS

Compounds that are adequately volatile, small and stable in high temperature in GC conditions can be easily analyzed by GCMS. The sample was injected into GC column. A carrier gas (argon, helium, nitrogen, hydrogen) propels sample down the column. The sample gets vaporized and separated in column. The separated components were analyzed and detected by the MS detector and provides information that identifies structure of each compound^[7].

RESULTS AND DISCUSSION

The medicinal plants possess varying degrees of phytoconstituents with antimicrobial action and have direct impact on physiological activity.

Figure I

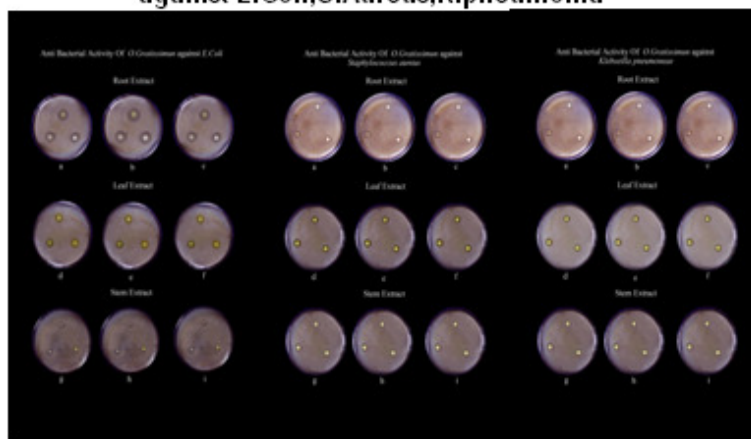


In our present study, the antibacterial activity was found to be higher in chloroform solvent of root extract of *Ocimum gratissimum L* against *E.coli* than the leaf extract of ethanol, chloroform, ethyl acetate, solvent, but the root extract of other solvent showed moderate activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Ndounga & Quamba,, 1997^[8]. in their study

reported that the antibacterial activity of leaf extract (ethanolic, chloroform and ethyl acetate) of *Ocimum gratissimum L* exhibited some degree of activity against isolates of *E. coli*. The stem and leaf extract showed mild antimicrobial activity for *Staphylococcus aureus* and *Klebsiella pneumoniae* than *E.coli*. Our results are inconsistent with above report.

Figure II

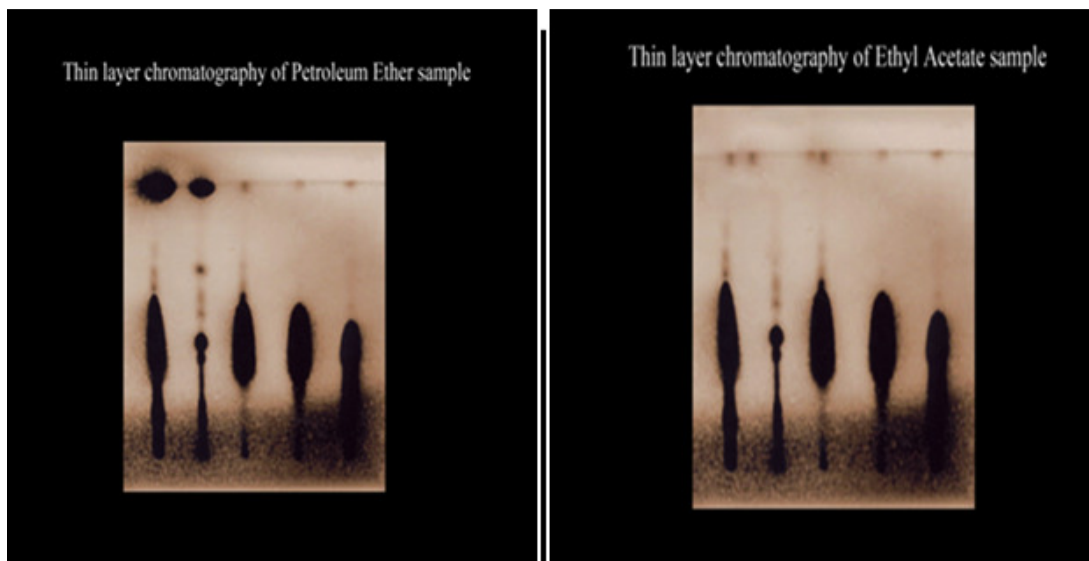
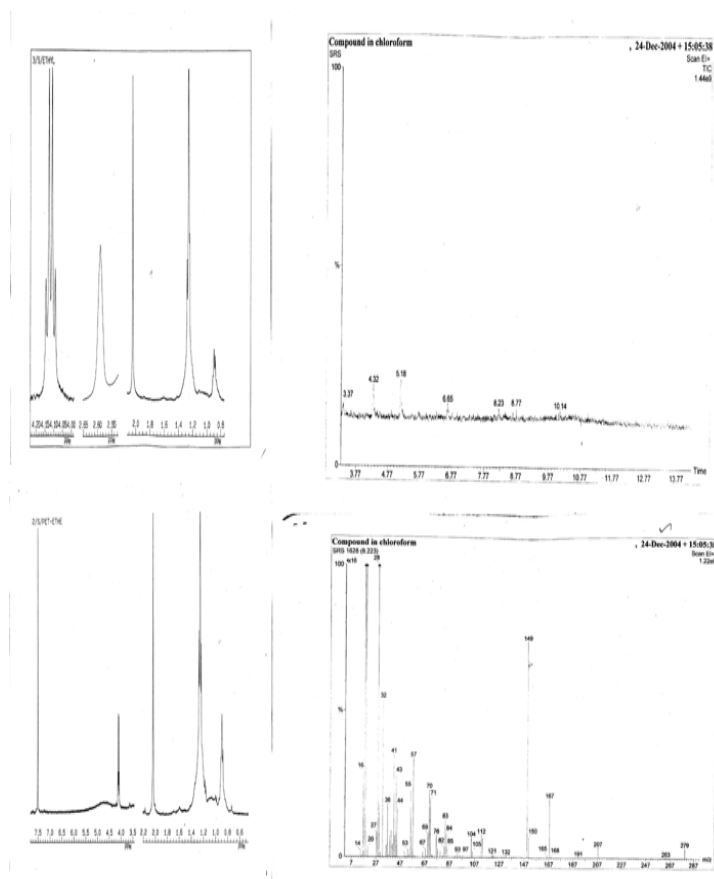
Antibacterial activity of Root,Leaf & Stem extracts gratissimum against E.Coli,S.Aureus,K.pneumonia



For phytochemical analysis root extract of petroleum ether and ethyl acetate were used as sample in Thin layer chromatography. The samples separated were scraped and analyzed for NMR and GCMS^[9]. The biologically active natural products

could be investigated from medicinal plants by using NMR and GCMS techniques^[6]. Petroleum ether showed effective results than ethyl acetate. So the petroleum ether sample was preceded for analysis.

Figure III

Figure IV NMR Study of *Ocimum gratissimum L*

methoxy group. The H¹NMR showed signals at δ 7.5(S, 4H), δ 4.1(d,1H), δ 2.5 (S, 11-1), δ 1.25(M,1H), δ 2.0(d,4H), δ 0.9(S,3H) were assigned an amide group. The mass spectra had given prominent fragments at m/2-191 [M-28⁺]. These two data's suggested a compound namely N-salicyloyl-2-amino-methoxy propane^[10]. Grayer and Kite 2000^[11] reported in his study that amino propane and amide is having antibacterial activity. Our results are also in consistent with the reports given by him.

CONCLUSION

From the results it was concluded that *Ocimum gratissimum.L* has antibacterial effect. The chloroform solvent of root extract was found to be more effective against *E. coli*. The proton NMR spectral data of ethyl acetate sample suggested the presence of non-aromatic group and alkaloids and the same analysis of petroleum ether sample suggested the presence of benzenoid group, methoxy group and an amide group of compound named N-Salicyloyl-2-amino-1- methoxy propane present in the root extract. Presence of N-Salicyloyl-2-amino-1- methoxy propane must be the cause of antimicrobial activity.

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