MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES



SCHOOL OF PHARMACY

DEPERTMENT OF PHARMACEUTICAL MICROBIOLOGY

A PROJECT REPORT

SCREENING FOR *IN VITRO* ANTIMICROBIAL ACTIVITY OF *OCIMUM GRAVEOLENS* CRUDE EXTRACT.

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Lastly I file my regards to my family and friends who were always close to me and giving me their support in every move I made.

ABSTRACT

Background

Despite the availability of effective chemotherapeutic agents the fight against diseases is very far from due to emergences of drug resistance against several conventional chemotherapeutic agents .Resistance of microbes to antimicrobial agents usually oblige the health care providers to shift to more efficacious but expensive drugs which are not affordable by majority in developing countries. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases.

Objective

The study was conducted in order to screen for *in vitro* antimicrobial activity of crude plant extract of *ocimum graveolens* specifically the antifungal and antibacterial activities.

Methods

The in vitro antimicrobial activity of crude plants extract *Ocimum graveolens* which were collected from lake zone region was tested on strains of standard reference microorganism namely *Candida albicans, Pseudomonas aurignosa, Escherichia coli , and staphylococcus aureus*. The antimicrobial activity of the crude plant extracts of varying concentrations 5,10 20 and 75mg/ml by dissolving in (Dimethylsulphoxide) and evaluated by microdilution method with modified MTT methods Microtitre plate with 96 wells was used. Sterile Mueller-Hinton broth, and Sabouraud broth was used as a culture media for bacteria and fungi respectively. About 120µl of culture media, Various concentration of extract ranging from 1 to 128 µl were serially added into given wells, finally a 5-µl of microbial sunspension with previously adjusted turbidity to match that of McFarland 0.5were also added in each wells .The plate was aerobically overnight incubated at 37^0c then 5µl of MTT was added to each well and incubated for another 4 hours before an addition of 20µl of detergent (sodium dodecyl sulfate) was added to the wells and incubated for 1 hour. After that absorbance reading of each well was taken by using spectrophotometer machine.

RESULTS

A concentration –dependent phenomenon was observed for the non flowering *O.graveolens* crude extract that is an increase in concentration was direct proportional to the increase in absorbance. This was demonstrated by a positive correlation between absorbance and concentration (R= 0.875, P=0.001).*Candida albicans, was* apparently the most resistant

microorganism at higher concentration and Pseudomonas demonstrated to be at least the most susceptible.

A negative correlation between absorbance and concentration was observed (Pearson R= -0.356, P= 0.001), for the flowering *O.graveolens* crude extract , the flowering *O.graveolens* crude extract , that is an increase of concentrations led to decrease in absorbance. Of the four tested microorganisms *Stphylococus aureus* was the most resistant to the crude extract while *Esherichia coli* was the most susceptible. However, T-test revealed no the statistical significance difference between the two test microorganisms with respect to the absorbance.

Conclusion

The flowering *Ocimum graveolens* crude plant extract exhibited relatively stronger antimicrobial activity on the test microorganisms compared to the non-flowering extract.

Pseudomonas isolates were the most resistant of the four test microorganism against the flowering crude extract while *E. coli* was the most susceptible. The extract has also shown activity on candida albicans

O. graveolens non flowering crude extract, seemed to support microbial growth, which leads to increase of absorbance as the extract's concentrations increase, contrary to the flowering crude extract.

INTRODUCTION & LITERATURE REVIEW.

Microorganism is a collective term given to bacteria (*Staphylococcus aureus*), viruses (influenza, which causes the "flu"), fungi (e.g., *Candida albicans*, which causes some yeast infections), and parasites (e.g., *Plasmodium falciparum*, which causes malaria

An antimicrobial agent Is a broad term used to describe any agent that interferes with the normal function of microscopic organisms, including bacteria, fungi, yeast, viruses and protozoa. Antimicrobial research is geared toward the discovery and development of novel chemical structures such as therapeutic antimicrobial agents. The continuing problem of development of resistance to existing antibacterial agents and the dearth of good and multipurpose antimicrobial agents motivates this effort toward innovation

Drug resistance That is the ability of an organism to resist the action of an inhibitory molecule or compound. Drug resistance has become a serious public health problem, since many disease-causing bacteria are no longer susceptible to previously effective drug therapy

Mechanisms involved in drug resistance:

1. Drug inactivation or modification: e.g. enzymatic deactivation of *Penicillin* G in some penicillin-resistant bacteria through the production of β-lactamases.
2. Alteration of target site: e.g. alteration of PBP—the binding target site of penicillins-in MRSA and other penicillin-resistant bacteria.
3. Alteration of metabolic pathway: e.g. some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid.

4. Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux) of the drugs across the cell surfaces

In the hospital environment, many factors combine to promote the development of drug resistance among bacteria. Increasing use of powerful new antibiotics gives selective advantage to the most resistant bacteria. In addition, advances in medical technology allow for the survival of sicker patient who undergo frequent invasive procedures. Poor infection control practices in hospitals allow for the unchecked spread of already resistant strains of bacteria.

A multifaceted worldwide effort will therefore be required to control drug resistance among disease-causing microorganisms.

Ongoing programs to decrease the unnecessary use of antibiotics, both in the clinics and in agriculture, will be necessary.

The increased use of vaccines to prevent infection can help limit the need for antibiotics.

Finally, the development of novel classes of antibiotics to fight emerging resistant bacteria will be required.

Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are combining the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers however have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes.

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. This review attempts to summarize the current status of botanical screening efforts, as well as in vivo studies of their effectiveness and toxicity. The structure and antimicrobial properties of phytochemicals are also addressed. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public

METHODS /TECHNIQUES FOR EVALUATION OF ANTIMICROBIAL ACTIVITY

Several methods/techniques have been employed for screening of antimicrobial agents. (EUCAST, 2003). Successful discovery of novel natural antimicrobials has necessitated the development of new bioassay techniques which are sensitive enough to detect small amounts of biologically active chemicals (Lampinen 2005). Standardized *in vitro* susceptibility tests are essential for screening plant extracts or compounds. Evaluation of the performance of a susceptibility test should include criteria such as ease of use, reproducibility test, sensitivity, and specificity (Struelens et al., 1995).

Some of the commonly employed techniques are:

1. Agar disk diffusion assay

Agar diffusion techniques have been widely used to assay plant extracts for antimicrobial activity (Freixa et al., 1996; Salie et al., 1996),
although there are limitations with the technique Disk diffusion is suitable for identification of leads but not effective for quantification of bioactivity (Hammer et al., 1999; Nostro et al., 2000; Langfield et al., 2004). These diffusion techniques generally do not distinguish

bactericidal and bacteriostatic effects. The MIC can not be determined and these are usually used for preliminary screening (Parekh et al., 2006; Tepe et al., 2004) that is, as qualitative tests, since the amount of extract that adheres to the disk is not quantitatively determined. The agar disk diffusion technique can only be used for antimicrobial susceptibility test of pure substances because when it is applied to mixtures containing constituents, which exhibit different diffusion rates, results may be unreliable (Silva et al., 2005)

2. Agar well diffusion:

The principle of the agar well diffusion is the same as that of the agar disk diffusion method. However here an extract is exposed into a well and left to diffuse in essence this technique is similar to ditch plate technique.

3. Agar plate proportional (APP):

The method determines the proportion of bacteria /microbial population that is resistant to the plant extract tested. When 1% or more of the microbes tested are resistant to the extract (drug), the population is considered resistant.

4. Microdilution method.

Microdilution method is carried out on microtitre plates using various media like Mueller-Hinton broth. A given amount of extract of various concentrations is put in wells, microorganism, (Bacteria/fungi) culture is added to the wells and incubated at 37oC for 24 hour (Lourens et al., 2004). the EUCAST recommended temperature is 35–37oC in air for 16–20 hour for nonfastidious organisms (EUCAST, 2003). The inoculum size for the microtitre plate was 1×10^6 cfu/ml (Lourens et al., 2004; Basri and Fan, 2005), which adjusted to a 0.5 McFarland turbidity standard (Baris et al., 2006) and inoculated within 30 min of standardization of inoculum, to avoid changes in inoculum density. Following overnight incubation. Plates were then examined for changes in turbidity as an indicator of growth. The first well that appears to be clear was taken to be the MIC of the extract. Some researchers use indicators (Umeh et al., 2005) or spectrophotometry to determine presence of growth in microtitre plates (Devienne and Raddi, 2002, Matsumoto et al., 2001) Indicators (usually tetrazolium salts or resazurin dye) are added after the incubation period and left for about 6 hour and changes in color or absence of color, depending on the indicator, is used to detect the MIC breakpoint. The use of calorimetric indicators eliminates the need for a spectrophotometric plate reader and avoids the ambiguity associated with visual comparison or measurement of growth inhibition rings on agar plates. When the spectrophotometric method is used the absorbance usually at 620 nm with the negative control as a blank, is used to detect the breakpoint (Salie et al., 1996). The concentration at which there is a sharp decline in the absorbance value (Devienne and Raddi, 2002), or the lowest concentration which gives a zero absorbance reading (Salie et al., 1996) is deemed to be the MIC

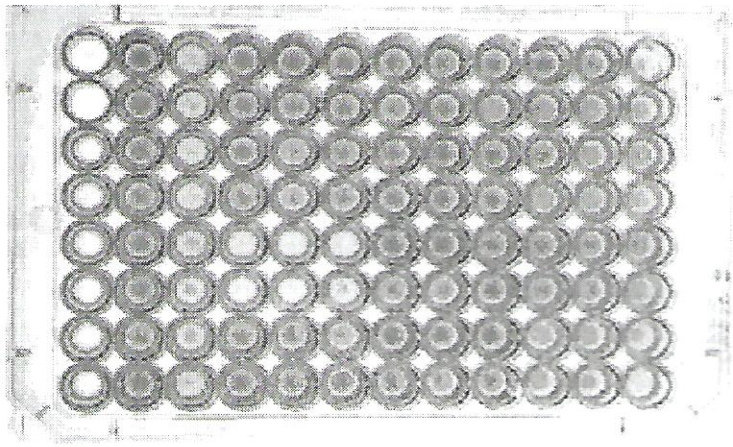
Advantage

It allows testing of multiple drugs with multiple concentrations. It is easy to perform and does not require special equipment or expensive supplies.

Its advantages over diffusion techniques include increased sensitivity for small quantities of extract which is important if the antimicrobial is scarce as is the case for many natural antimicrobial products, ability to distinguish between bacteriostatic and bactericidal effects; and quantitative determination of the bactericidal effects; and quantitative determination of the MIC (Langfield et al., 2004).

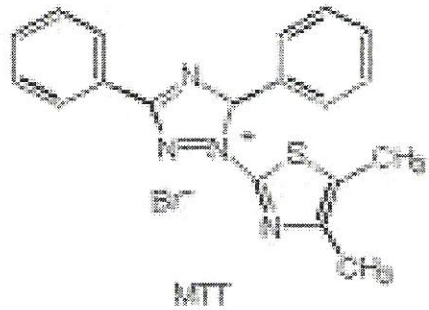
Disadvantage

- Observation of the MIC by observing turbidity may be biased as they depend on the observer, and it may need an extra person to countercheck.



Microdilution method with the modified MTT method

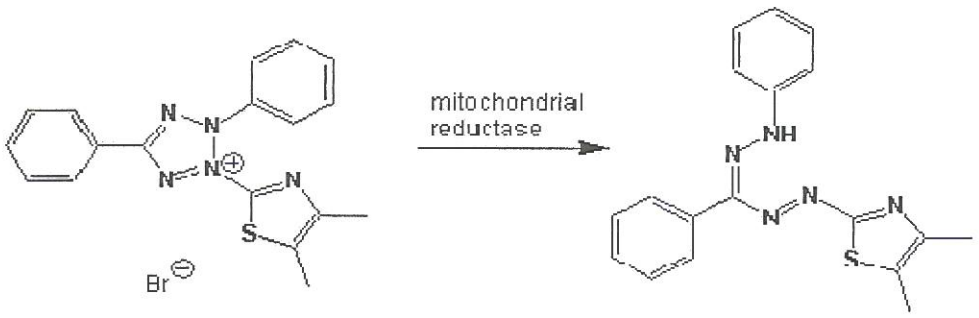
MTT, [3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyltetrazolium bromide] has been used to measure the activity of the oxidative pathways in many different systems and situations, and there is an extensive literature available on its many applications, including toxicity testing.



MTT

The **MTT assay** is the laboratory test and standard colorimetric assays (an assay which measures changes in color) for measuring the activity of mitochondrial dehydrogenase enzymes that reduce MTT to **formazan**, giving a purple color or blue. It can also be used to determine cytotoxicity of potential medicinal agents and other toxic materials (Mosmann et al.1983).

Yellow **MTT** (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple or blue formazan in living cells.



MTT Formazan (insoluble)

Yellow **MTT** (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple or blue formazan in living cells.

A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution.

The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum is dependent on the solvent employed.

DESCRIPTION OF THE PLANT

Plant name: *Ocimum graveolens.*

Ocimum graveolens is from family labiatae cultivated in tropical Africa, tropical America, Himalaya, India, Srilanka, Thailand, Indochina, Malaysia at an altitude from sea level up to 600m. Flowering and fruiting occur from January to December.

The genus Ocimum consists of more than 64 species, some of them are, *Ocimum americanum*, *Ocimum basilicum*_Linn , *Ocimum sanctum* Linn , *Ocimum gratissimum*, *Ocimum tenuiflorum*an, *Ocimum viride*_Willd., *Ocimum graveolens*_A. Br., *Ocimum micranthum*_Willd., and *Ocimum kilimandscharicum*_Gurke. (Tchoumbougnang, F Et al 2006)

The most important species in tropical Africa include *O. americanum* L., *O. basilicum* L. and *O. gratissimum* L., *O.graveolens* .All species are commonly found around village huts and

gardens where they are cultivated for their medicinal uses as febrifuge, insect repellent and as a condiment.

The plants belonging to the genus Ocimum contain numerous phytochemicals including methyl chavicol, linalool, methyl eugenol, and methyl cinnamate, thymol, and others. The essential oil, leaves, seeds, flowers, and roots of *Ocimum* spp. are used as medicines (Tchoumbougnang Et al 2006)

In traditional medicines, the oils and plant parts of several Ocimum species (have been suggested to possess anti-oxidant, cardioprotective, anti-fertility, anti-diabetic, hepatoprotective, anti-inflammatory, anti-fungal, anti-microbial, anti-emetic, anti-spasmodic, analgesic, anti-cancer activities.

The essential oil has been shown in vitro to have antibacterial activity against *Staphylococcusaureus, Salmonella enteritidis* and *Escherichia coli*, antiseptic activity against *Proteus vulgaris, Bacillus subtilis*, and *Salmonella paratyph*, and antifungal activity against *Candida albicans, Penicillium notatum*, and *Microsporeum gyseum.* (Beckstrom-Sternberg, Et al1994, van Duong, Nguyen. 1993, de Mena Guarero, M.G. 1994, McCorkle, C.M 1996, Takano, T. 1993,)

Oils from some *Ocimum* spp. have been shown to repel insects and have larvicidal activity against houseflies, blue bottle flies, and mosquitoes. Camphor, d-limonene, myrcene, and thymol are some of the compounds in the oil that may provide the repellent properties. Eugenol and methylchavicol may be responsible for the larvicidal activity. (Beckstrom-Sternberg, Et al1994, van Duong, Nguyen. 1993, de Mena Guarero, M.G. 1994, McCorkle, C.M 1996, Takano, T. 1993,)

The fruit of the plant of the genus Ocimum is known to improve the sugar tolerance of diabetics. Therefore has been used in the preparation of an obesity control agent. The *Ocimum canum* was used specially for treating diabetes.

However, *O. basilicum* has been used as an expectorant in animals. Also, *O. micranthum* has been combined with *Chenopodium ambrosioides* to treat stomach pain and colic in animals. It also has been used as a paste to massage the udder of livestock.

O. gratissimum is grown for the essential oil in its leaves and stems. Eugenol and to a lesser extent thymol extracted from the oil are substitutes for clove oil and thyme oil. (Sulistiarini, D 1999) Medicine: The whole plant and the essential oil have many applications in traditional medicine, especially in Africa and India. Preparations from the whole plant are used as stomachic and in treating sunstroke, headache and influenza. The seeds have laxative properties and are prescribed against gonorrhea. The essential oil is applied against fever, inflammations of the throat, ears or eyes, stomach pain, diarrhea and skin diseases. It is being tested as an antibiotic. The essential oil is also an important insect repellent. Other products: In Indonesia (Sumatra) a tea is made from the leaves, while in Thailand the leaves are applied as flavouring. In Indonesia the eugenol-type of O. gratissimum is used in the

ceremonial washing of corpses and is planted in graveyards. In India O. gratissimum, named 'ram tulsi', is widely used in religious ceremonies and rituals.

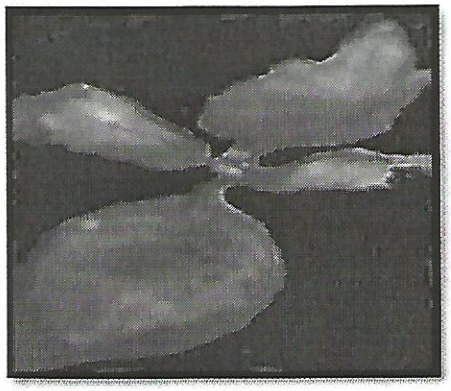
Little published information can be found about the use of oil from Ocimum and fresh plant materials in the treatment of animals. Therefore, efficacious doses are not known. (Beckstrom-Sternberg, Et al1994, van Duong, Nguyen. 1993, de Mena Guarero, M.G. 1994, McCorkle, C.M 1996, Takano, T. 1993,)

There is little available literature on the toxicity of *Ocimum* spp. However, *O. basilicum*, the species that appears to be used the most medicinally and the one for which the most analysis has been done, contains several potentially dangerous compounds. Some of these compounds are: safrole, rutin, caffeic acid, tryptophan, and quercetin. (Beckstrom-Sternberg, Et al1994, van Duong, Nguyen. 1993, de Mena Guarero, M.G. 1994, McCorkle, C.M 1996, Takano, T. 1993,)

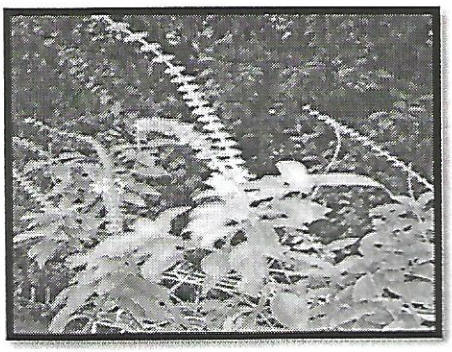
SOME OF THE PICTURES OF OCIMUM SPECIES



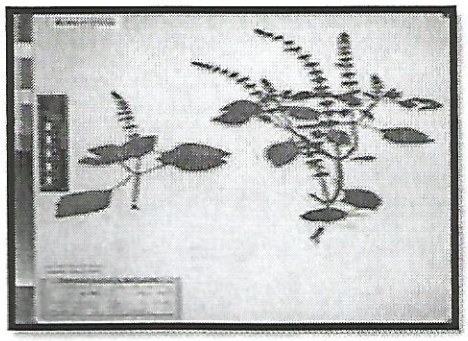
Ocimum canum



Ocimum basilicum



Ocimum gratissimum



Ocimum graveolens

PROBLEM STATEMENT AND RATIONALE

Despite the fact that the pharmaceutical industries have produced a number of new effective chemotherapeutic agents especially antibiotics, resistance to these drugs by microorganisms has increased. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.

Some plants have shown the ability to overcome resistance in some organisms and this has led to researchers' investigating their mechanisms of action and isolating active compounds. Particular focus is on establishing the effect of the plant(s) extracts in terms of their microstatic and microcidal action and the spectrum of organisms affected. This has enabled exploitation of plants for the treatment of microbial infections and in the development of new antimicrobial agents. This requires rigorous research and it is therefore imperative to follow standard methods to authenticate claims of antimicrobial action.

The use of higher plants and their extracts to treat infections is an age-old practice. Traditional medicinal practice has been known for centuries in many parts of the world, the science of life, prevention and longevity is the oldest and most holistic medical system available on the planet today. Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attributes.

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases.

OBJECTIVES

BROAD OBJECTIVES

- Screening for antimicrobial activity of *ocimum graveolens* crude extract.

SPECIFIC OBJECTIVES

- Assessment of antibacterial activity of *ocimum graveolens* methanolic crude extract.
- Assessment of antifungal activity of *ocimum graveolens* methanolic crude plant extract.
- Comparison of antimicrobial efficacies between the *O.graveolens* flowering and non flowering plant extracts.

METHODOLOGY

MATERIALS

Apparatus

- Spectrophotometer, manufactured by Barloworld scientific ltd in EU
- Analytical balance.
- Universal bottles
- Wire loop
- Bunsen burner
- Micro pipette
- Microtitre plates
- Measuring cylinder
- Autoclave
- Oven/incubator, Made by Memmert in Western Germany
- Refrigerator
- Sterile(Hood) chamber.
- Beaker
- Syringes

Solvent used

Media used.

- Mueller-Hinton broth. Manufactured by CarlRoth in Karlsrule German
- Sabouraud Dextrose broth ,Manufactured by OXOID LTDinEngland
- Methanol
- Distilled water
- DMSO

Chemical used

- MTT,(3-[4,5-Ddimethylthiazol-2-diphenyltrtrazolium bromide)
- SDS(sodium dodecyl sulfate in diluted hydrochloric acid)
- Fluconazole
- Ceftriaxone

Plants tested.

- *Ocimum graveolens* extracted with methanol (non flowering)
- *Ocimum graveolens* extracted with methanol (flowering.)

Test microorganisms

FUNGUS: *Candida albicans.*

BACTERIA: *Escherichia coli, staphylococcus aureus, Pseudomonus aureginosa.*

PROCEDURES

1. Preparation of culture media.

About 21 grams of Mueller- Hinton broth /Sabouraud broth were weighed and dissolved in one liter of distilled water, and sterilized in an autoclave.

Standard innocula of *Candida albicans, Staphylococcus auerus, pseudomonas aureginosa, Escherichia coli* was prepared in freshly culture media (Mueller-Hinton broth for bacteria, and sabouraud Dextrose broth for fungi).

Loopful of microbial suspension of each standard microbe was taken, immersed and uniformly homogenized by shaking for at least 3min into freshly prepared 20ml culture media (Mueller- Hinton broth or sabouroud broth) and incubated overnight at 37 ^{0}c

2. Preparation of crude plant extract.

The extract was prepared to various concentrations namely 5, 10, 20, and 75mg/ml, which were aspired from 750mg, 200mg, 100mg, 50mg of extract that were measured and dissolved in 10ml of DMSO solvent.

3. Preparation of MTT.

MTT reagent is a yellow powder.

25mg of MTT powder was weighed by using an analytical balance and diluted to1:10ml equivalent to 2.5mg/ml. The overall concentration was equivalent to 0.03mg/ml (minimum assayed concentration) to 147mg/ml as the highest concentration respectively.

2. McFarland turbidity standard

A McFarland 0.5 standard was prepared prior to beginning susceptibility testing. The McFarland standard is used to adjust the turbidity of the inoculums for the Susceptibility test.

ANTIMICROBIAL/SUSCEPTIBILITY ASSAY

Microdilution with modified MTTmethod.

The antimicrobial activity of the crude plant extracts of varying concentrations 5,10 20 and 75mg/ml by dissolving in (Dimethylsulphoxide) and evaluated by microdilution method with modified MTT methods Microtitre plate with 96 wells was used. Sterile Mueller-Hinton broth, and Sabouraud broth was used as a culture media for bacteria and fungi respectively. About 100µl of culture media, Various concentration of extract ranging from 1 to 128 µl were serially added into given wells, finally a 5-µl of microbial sunspension with previously adjusted turbidity to match that of McFarland 0.5were also added in each wells .The plate was aerobically overnight incubated at 37^0c then 5µl of MTT was added to each well and incubated for another 4 hours before an addition of 20µl of detergent (sodium dodecyl sulfate) was added to the wells and incubated for 1 hour. After that absorbance reading of each well was taken by using spectrophotometer machine.

Spectrophotometric analysis

Each well content, 35µl was taken and put into the cuvete and adjusted with distilled water 1000µl (1ml)

(A) Control negative.

DMSO solvent that was used to dissolve each crude extract is known not to possess any antimicrobial activities.

(B) Controlpositive

Pure antimicrobial agents with broad spectrum activity namely fluconazole (2mg/ml) as an antifungal agent and ceftriaxone (0.25g/ml) as antibacterial agents were employed .The control assay was prepared as previously described in microdilution method.

RESULTS AND DISCUSION

Strains of reference bacterial and fungal isolates were tested. Results from the antimicrobial activity evaluation on non-flowering *O. graviolens* crude extract revealed a concentration-dependent effect (**Figure 1).** That is an increase in concentration was directly proportional to increase in absorbance, buttressed by a positive correlation between concentration and absorbance (Pearson R= 0.875; p = 0.001). Furthermore, T-test revealed significant differences in respect to ABS among different assayed concentrations (F= 19.931; df = 30; p= 0.001).

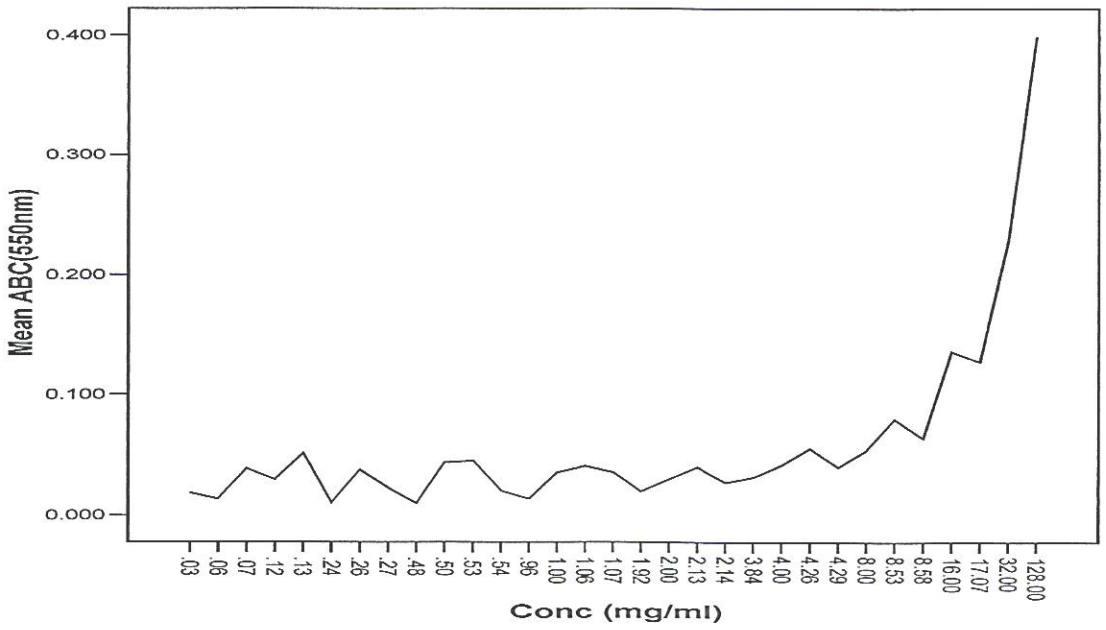


Figure1: Concentration-dependent effect of non-flowering *O. graveolens* crude plant extract

Figure1: Concentration-dependent effect of *O. graveolnes* crude extract on test microorganisms

Of all assayed/tested the strains of reference microorganisms, Mean absorbance showed that *Staphylococcus aureus* was the most resistant to the crude extract followed by *C. albicans* (**Figure 2)** below.

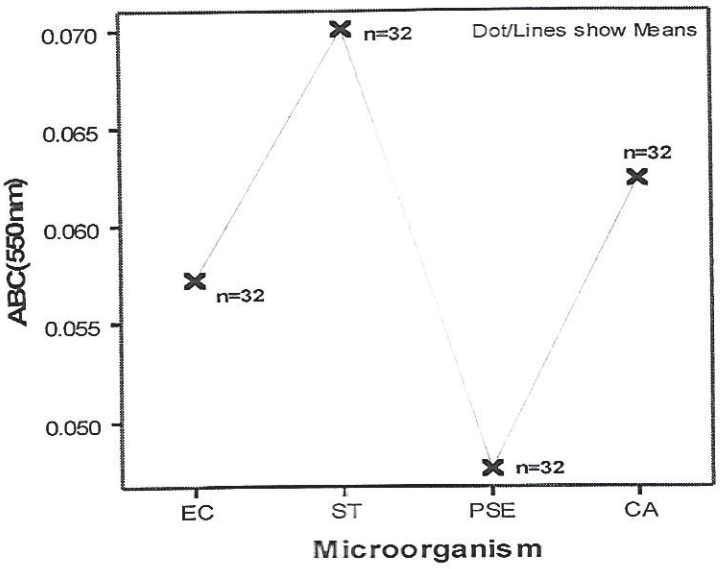


Figure 2: Different susceptibility profiles of test microorganisms to the crude extract

The overall observations show that *C. albicans* was the most resistant to the non-flowering *O. graviolens* methanolic crude extract (at higher concentrations) whereas *Pseudomonas* demonstrated to be the most susceptible, indicated on **Figure 3**. These observations were unexpected, since an increase in concentration would have correlated with a decrease of absorbance as the conversion of MTT to formazan by the surviving fraction of microorganisms would be declining as consequence and effect of the extract concentration.

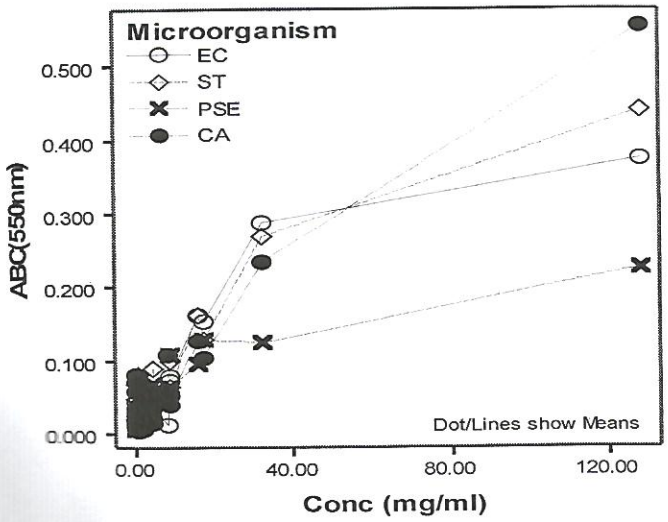


Figure 3: Effect of *O.graveolens* methanolic crude extract on test microorganisms

Results from the antimicrobial evaluation of *O.graveolens* flowering plant's crude extract exhibited dissimilar results to that of non-flowering plant. Spectrometric determination (ABS) of the extract's efficacy negatively correlated with the assayed concentrations (R= -0.356; p = 0.001) as depicted on (Figure4). below

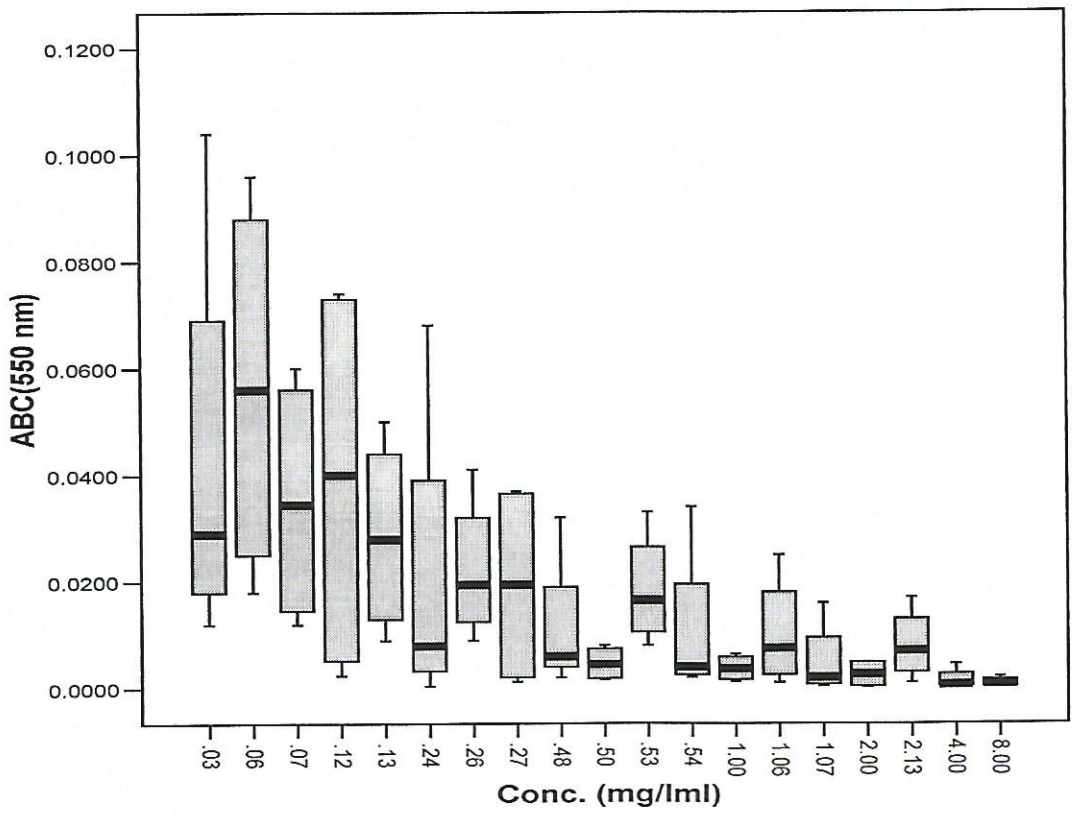


Figure 4: Negative correlation between extract's concentration and ABS (antimicrobial efficacy).

Of the four test microbes (reference strains of microorganisms), mean absorbance revealed that *Pseudomonas auruginosa was* the most resistant to the extract while *Escherichia coli* was the most susceptible (Figure 5). Moreover, analysis of variance (ANOVA) revealed statistically significant differences with respect to susceptibility among the test microbes in terms of ABS (F = 7.653; df = 3; p = 0.001).

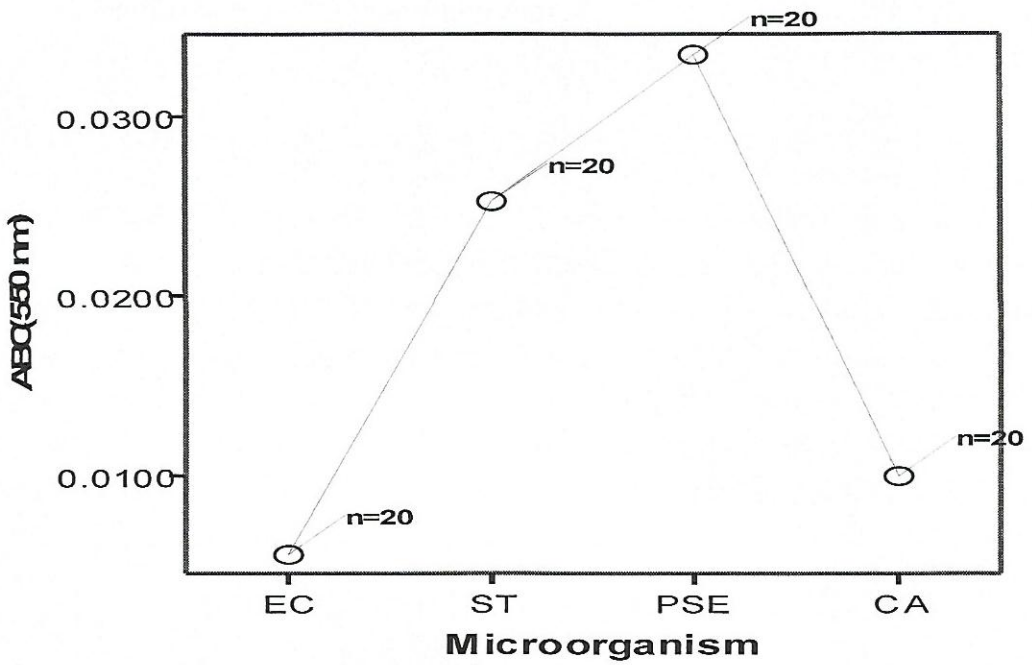


Figure 5: Different susceptibility profiles of test microorganisms to the O. graveolens flowering plant crude extract

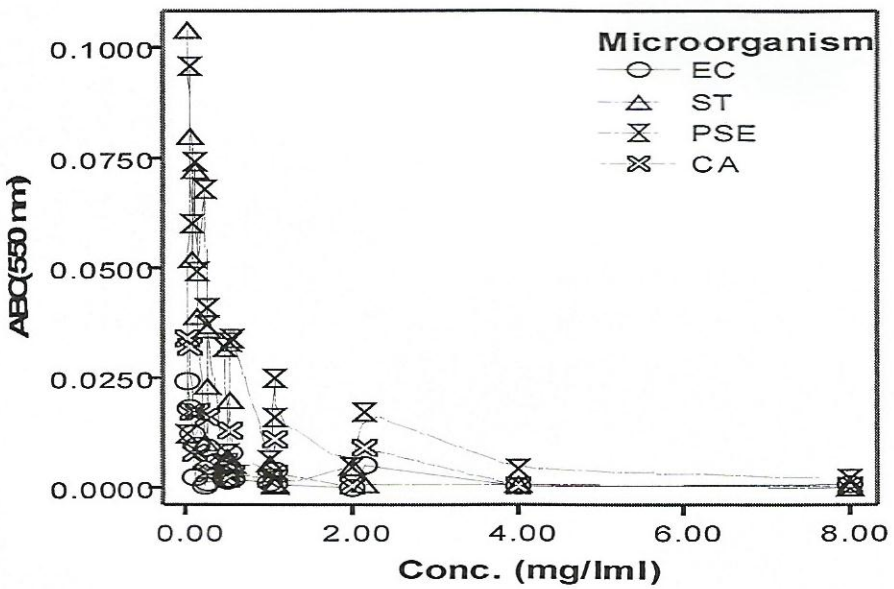


Figure 6: Effect of *O.graveolens* methanoilc flowering plant crude extract on test microorganisms

Figure 6 above shows contrary effect of the flowering plant extracts on the test microorganisms in comparison to that of non-flowering plant extract. The optic density /ABS decrease as extract's concentrations increase. The conversion of MTT to formazan

increases as the extracts concentrations decrease, thus increasing the number of surviving microorganisms that degrade the former more efficiently.

Discussion

Results of *Occimum graveolens* non flowering, may be due to reductive potential of some phytoestrogens, kaempferol and resverasterol, Antioxdants such as ascorbic acid ,vitamin E, and N-acetylcystein, these compounds if are present in the extract usually interfered with the MTT assay as they have potential to cause conflicting results by increasing Formazan formation despite the decrease in viable cells. These results indicate that the MTT tetrazolium assay may lead to false positive results when testing natural compounds with intrinsic reductive potential. However in some studies botanical extracts have shown to reduce MTT in the absence of living cells. Also probably time and period of collection and storage of extract and preparation of extract may cause the extract to lose some of the active constituents.

Results of *O.graveolens* flowering extract may be due to greater concentration of active constituents in the flowering part. Absence of compounds with reductive potential in the flowering parts, and proper collection as well as storage of the extract.

CONTROL TESTS RESULTS

CONTROLS NEGATIVE.

Solvents used were DMSO, (Dimethyl sulfoxide) and distilled water which have been documented in many Literatures to have no antimicrobial activity.

Control positive results

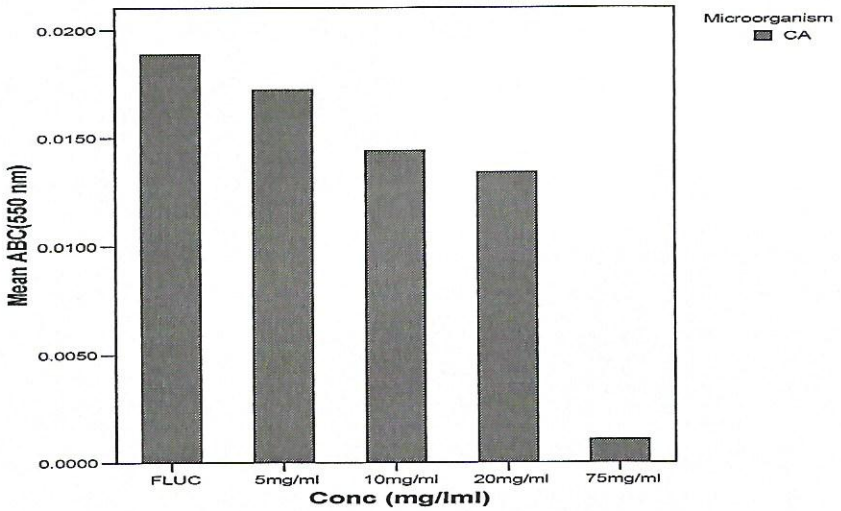


Figure 8: Concentration-dependent effect stock extract solutions and positive control

Figure 8 shows that of the 4 assayed concentrations of extract 5, 10, 20, and 75mg/ml. The strongest effect was displayed by *O. graveolens* extract at concentration of 75mg/ml against fungal isolates of *Candida albicans* than the standard fluconazole.

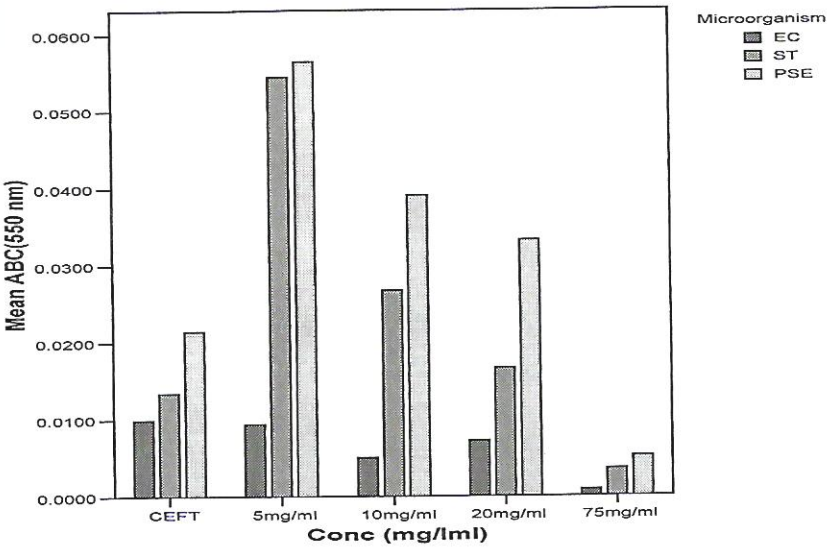


Figure 9: Control antibiotic and stock extract solutions against test microbes

Higher extract concentrations displayed higher antimicrobial effect (Figure 9). This was depicted by effect of the extract stock solution at, 75 mg/ml of the crude extract of flowering *O. graveolens*, which exhibited even stronger effect compared to the control positive/antibiotic (Ceftriaxone).

CONCLUSION AND RECOMMENDATIONS

The flowering *Ocimum graveolens* crude plant extract exhibited relatively stronger antimicrobial activity on the test microorganisms compared to the non-flowering extract.

Pseudomonas isolates were the most resistant of the four test microorganism against the flowering crude extract while *E. coli* was the most susceptible. The extract has also shown activity on *Candida albicans*.

O. graveolens non flowering crude extract, seemed to support microbial growth, which leads to increase of absorbance as the extract's concentrations increase, contrary to the flowering crude extract. Further studies should be conducted on non-flowering crude extract of *O. graveolens* in order to investigate on the possible causes that favor growth of microorganisms, which is an increase of absorbance (formation of formazan) with an increase of extract concentrations.

Lastly, we recommend that the experienced shortage of equipment and apparatus, which to a great extent attributed to the slow progression of the project, should be addressed. Moreover, because of the electrical power irregularities, of course anything to do with electricity was postponed.

The research has filled the knowledge gap in this field of study and it has shown to be very crucial for a pharmacist, like me. Therefore, we recommend that funds and resources should be allocated for this purpose. Because by discovering new drugs our country will save a lot from importing abroad and therefore the money will be allocated to other national necessities.

Finally we recommend that further studies should be carried out on these plants in order to get to know how the activity can be improved so that the drug can be declared in modern pharmaceutics.

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