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# Ability of *Azadirachta indica* and *Ocimum gratissimum* to inhibit the growth of some hospital isolates

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## Abstract

The study on the ability of *Azadirachta indica* and *Ocimum gratissimum* to inhibit the growth of some hospital isolates was carried out following several reports on the antimicrobial potentials of these plant leaves among locals. Some fresh leaves of *Azadirachta indica* and *Ocimum gratissimum* were processed into an aqueous extract as well as an ethanolic extract and preserved at 4°C. samples of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans* which served as test microorganisms were obtained from the Federal Medial Centre Umuahia Abia State Nigeria. Antimicrobial sensitivity test of test isolates was determined using the *Kirby-bauer* disc diffusion method Disc diffusion method of the extracts . A 0.5 Mc farland solution of the 4 organisms were prepared and inoculated into an already prepared Mueller hinton agar plates containing the paper disc of 400mg/ml aqueous and ethanolic extracts. A control experiment was set up with 2mg/ml gentamycin disc. After 48 hours incubation, Ethanolic extract of *Azadirachta indica* showed the highest zones of inhibition of 7.5mm on *Staphylococcus aureus*, followed by ethanolic extract of *Ocimum gratissimum* 7.0mm on *Escherichia coli*. While the aqueous extracts of both *Azadirachta indica* and *Ocimum gratissimum* showed least zones of inhibition on *Candida albicans*. The overall were more antibacterial effective than antifungal. Therefore, the Ethanolic extract of *Azadirachta indica* and *Ocimum gratissimum* possess more antibacterial effect and less antifungal effect.

**Keywords:** Antimicrobials; Antibiotics; Sensitivity; Isolates; Extracts; *Ocimum gratissimum*; *Azadirachta indica*; Mc-Farland Standard; Antibacterial; Antifungal.

## 1. Introduction

With the increasing incidence of diseases caused by bacteria and other microorganisms, as well as the development of drug resistance, there is an urgent need to search for alternatives from plant sources to combat these pathogens. The development of a new antimicrobial drug is difficult, taking into account of poor selective toxicity and fast development of resistant viral variants with the existing drugs. Frequencies of bacterial resistance to antibacterial drugs are increasing (Levy, 2002). The ease of national and international travel means that resistant organisms can be transported easily, making it a global problem. Despite all efforts by health bodies, the threat of bacterial and other infectious diseases persist, making the search for more effective and efficient drugs ever more pressing. For their basic medical needs, four out of five people turn to traditional medicine. Many bioactive chemicals found in medicinal plants can be

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used to treat bacteria that are resistant to drugs (MDR). This has not been examined in great detail up to this point (Kebede et al., 2021).

Of interest is *Azadirachta indica* leaves which has been used locally to cure several infections (Saradhajyothi, and Subbarao, 2011). The evergreen and fast growing height of 15- 20 meters and whose twigs is used as chewing stick (Almas and Ansal-Lafi, 1995). It is native to Indian subcontinent and grows in tropical and semi tropical regions. It is commonly known by different such as *Neem* (in Bengali), Tamar (in Burmese), *Grossblacttigerzedrach* (in German), *Muarubaini* (in Swahili; meaning the tree of the 40, as it is used to treat different diseases) and *Dongoyaro* (in Northern Nigeria; literarily, "tall boy") (Batool et al,. 2018).

Another plant of interest is the *Ocimum gratissimum* leaf or the whole herbs are popular treatment for diarrhea (Batool et al,. 2018).. It is an aromatic herbaceous plant also known as basil, basil-clove, or *alfavaca*. It belongs to the *Lamiaceae* family (genus: Ocimum and species: *gratissimum*) (Nweze and Eze,2009). In fact, the antimicrobial activity of the water-saturated oil had been shown to be proportional to the thymol content in preparations where *Ocimum gratissimum* is used as cold infusion (Batool et al,. 2018).. Therefore, the antimicrobial effect of the extracted thymol is probably sufficient explanation for the anti-diarrheal effect. However, in certain preparations, *Ocimum gratissimum* when boiled with water to form decoction will contain little of the steam-volatile thymol. Such aqueous decoctions were shown to be devoid of antimicrobial activity, but they do relax the guinea pig ileum and rat jejunum in-vitro (Refaz, et al.,2017).

### *Aims and Objectives*

The aim of this research is to test the compounds extracted *Azadirachta indica* and *Ocimum gratissimum* for antibacterial and antifungal activity.

The specific objectives are as follows:

- Selection of the best extractant for the plant material.
- Determination of antibacterial and antifungal activities of extracts.

## 2. Materials and Methods

### 2.1. Collection and identification of plants materials

The plant material that was used in this work is freshly harvested leaves of *Azadirachta indica* and *Ocimum gratissimum*. They were obtained from Umudike/Umuahia, Abia State. Mr. ibe Ndukwe of the Department of forestry and Environmental management of the Michael Okpara University of Agriculture, Umudike taxonomically identify the plants.

### 2.2. Preparation of the Plant Extracts

The freshly collected plants were rinsed in water and were dried using a moisture extracted oven at 50$^{o}$C and the drying period lasted for 48 hours. The dried leaves were pulverized into powder using Thomas Wiley Mill Model ED.5 (Oyagede et al., 1993) from Soil Science laboratory, National Root Crop Research Institute (NRCRI), Umudike.

### 2.3. Ethanol Extract Preparation

Exactly 50.0 grams of the pulverized *Azadirachta indica* and *Ocimum gratissimum* leaves were weighed using satanic AG Gottingen Electronic weighing balance. The weighed samples were soaked in 500ml of ethanol contained in a conical flask. The mixture was swirled. After 48 hours of interval stirring, the mixtures were filtered using whatman Number 1 filter paper, into a clean beaker (Azoro, 2002). The filtrates were dried by evaporating in steam bath at 60$^{o}$C and the dried solids were reconstituted to obtain the various concentrations used in the study. All the extracts were subjected to antimicrobial activity (Ogbuka 2022). Table 1 shows the percentage ethanol extracts.

### 2.4. Aqueous extract preparation

Exactly 50.0 grams of the pulverized *Azadirachta indica* and *Ocimum gratissimum* leaves were weighed and macerated in 500ml of distilled water. The mixtures were vigorously swirled. After 48 hours duration with interval stirring, the mixture was filtered using Whatman No1 filter paper (Azoro, 2002) into a clean beaker, and the mixture was concentrated to dryness by evaporation using bath at 60$^{o}$C. The filtrate had the following colour after filtration: Ethanol extract was dark brown, Aqueous extract was dark green. The extract was stored in refrigerator at 4$^{o}$C for use. The

yields were recovered as percentage of the quantity of the initial plant material (50.0g). Table 1 Shows the percentage of the aqueous extracts.

$$\text{yield} = \% \frac{\text{yield in (g)}}{50.0\text{g}} \times \frac{100}{1}$$

Yield in (g)/50.0g x100/1.

### 2.5. Yield of plant extracts

The yields of the plants extract (ethanol and aqueous) were recovered and calculated as percentage of the quantity of initial powdered sample of the plant materials as shown in Table 1. The ethanol extract of *Azadirachta indica* gave the highest yield of 21g representing 42%. While the ethanol extract of *Ocimum gratissimum* yielded 20g, representing 40%. The percentage yield of aqueous extract of *Azadirchta indica* and *Ocimum grassimum* were 28% and 30% respectively.

**Table 1** Yield of the crude extracts of *Azadirachta indica* and *Ocimum gratissimum*.

| Plant Species | Extract Type | Weight of powdered sample(g) | Weight of extracts (g) | Percentage yield of etract(%) |
|---|---|---|---|---|
| *Azadirachta indica* | Aqueous | 50.0 | 14 | 28 |
| | Ethanol | 50.0 | 21 | 42 |
| *Ocimum gratissimum* | Aqueous | 50.0 | 15 | 30 |
| | Ethanol | 50.0 | 20 | 40 |

### 2.6. Test Organisms

The bacterial strains used in this study were obtained from the Federal Medical Centre (FMC) Umuahia, Abia State. Among four microorganisms investigated, were one gram positive bacteria Staphylococcus *aureus,* two Gram negative bacteria viz *Escherichia coli, Pseudomonas aeruginosa*, and one fungus: *Candida albicans*. The microorganism were maintained at $4^0$C on a nutrient agar slants (Prakash et.,al. 2013).

### 2.7. Preparation of plant extracts concentration

The aqueous and ethanol crude extracts of both plants were reconstituted by weighing 0.2g of each and dissolving in 2ml of distilled water and 2ml of 90% ethanol respectively. Each dilution gave a concentration of 100mg/ml.

Plant extracts' sterility Test: It was determined whether microorganisms could grow in each ethanol and aqueous extract. Each of them was injected with 0.5 ml on sterile Mueller Hinton Agar, and the experiment was then incubated at 37˚C for duration of 18–24 hours. After four days of incubation at 25˚C, the fungal growth was evaluated on Sabouraud Dextrose Agar. A growth log was kept on the plates. The absence of growth in the extracts following incubation, as specified by CLSI criteria, demonstrates sterility and is assessed for antibacterial activity (Wikler et al., 2011).

### 2.8. Disc diffusion of assay

The disc diffusion method as reported by Wikler et al., (2011).was adopted by the determination of the antimicrobial activity of extracts. Whatman No. 1 filter paper was used. The filter paper was cut into circular disc using a perforator given a diameter of 6 mm; the disc was treated by boiling for 30 minutes so as to denature and destroy completely the chemical used in its preservation and also to prevent the inactivation of the extract when embedded into the discs. After it has boiled, the disc was transferred into a glass Petri dish and kept in the oven until it become dry. After drying, it was stored in a sterile vial bottle and autoclaved for 15 minutes at $121^0$C and 15 Psi. it was stored for use.

### 2.9. Media preparation and antimicrobial activity

Muller Hinton agar was prepared by weighing 38g of the powdered agar into 100ml of distilled water in a clean conical flask. It was stirred until it becomes a mixture. It was then covered with foil and was autoclaved at $121^0$C, 15 Psi for 15 minutes. The medium was cooled at $47^0$C and 20ml of the molten medium was poured into a sterile Petri dish and allow solidifying. A sterile wire loop was used to pick a colony of the test organism and placed into 2ml of normal saline and the standardized to 0.5 McFarland solution. A sterile swab stick was dipped into the test tube containing the organism

and it was used to spread the organism on the solidified Muller Hinton agar in an inoculating chamber that was preset. The prepared disc was carefully transferred unto the inoculated culture plates using sterile forceps. The placed disc included 400mg/ml of aqueous and ethanol extract, sterile water and gentamycin 2mg/ml. after 48 hours incubation at $37^0$C, the zone of inhibition was measured and recorded. The tests were carried out in duplicate (Wikler et al., 2011).

Table 2, showed the antimicrobial activity of the different plants extracts, the results of the sensitivity test of different organisms with the concentrated extracts using the paper disc diffusion method.

**Table 2** Antimicrobial activitities of various extracts of *Azadirachta indica* and *Ocimum grattisimum*

| ***Azadirachta indica*** | | | ***Ocimum gratissimum*** | |
|---|---|---|---|---|
| Microorganisms | Ethanol | Aqueous | Ethanol | Equeous |
| *S.aureus* | ++ | + | ++ | + |
| *P. auruginosa* | ++ | + | ++ | + |
| *E. coli* | ++ | ++ | ++ | + |
| *C. albicans* | + | + | + | _ |

Key: + means inhibition < (3.0mm) diameter, ++ means highly susceptible, + means susceptible, - means Trace.

Aqueous extracts of *Azadirachta indica* and *Ocimum gratissimum* showed little zones of inhibition on *Escherichia coli, Staphylococcus* aureus and *Pseudomonas aeruginosa*, but no zone of inhibition was found for *Candida albicans*. In contrast, both ethanol extracts of *Azadirachta indica* and *Ocimum gratissimum* showed significant zones of inhibition greater than 5.0 mm diameter for bacterial isolates but less zones of inhibition for *Candida albicans*. On the other hand, 2mg/ml of gentamycin showed wider zones of inhibition on bacterial isolates with minimal clear zone for *Candida albicans,* as shown in table 3 below.

**Table 3** Zones of inhibition (mm) produced by the Aqueous and Ethanol extracts of *Azadirachta indica* and *Ocimum gratissimum*

| | | | **Diameter Zone(mm)** | | |
|---|---|---|---|---|---|
| Organism | *Azadirachta indica* | | *Ocimum gratissimum* | | |
| | Ethanol | Aqueous | Ethanol | Aqueous | Gentamycin |
| *E. coli* | 6.5 | 3.0 | 7.0 | 4.0 | 10.0 |
| *S.aureus* | 7.5 | 3.0 | 6.5 | 3.5 | 9.0 |
| *P. aeruginosa* | 5.0 | 3.0 | 4.0 | 2.0 | 9.0 |
| *C. albicans* | 4.5 | 0.0 | 3.5 | 0.0 | 7.0 |

## 3. Discussion

The antimicrobial properties of the extracts expressed in the form of diameter zones of inhibition are given in table 3. At a concentration of 400mg/ml, the extracts, though less effective than gentamycin, inhibited the growth of the pathogens. *Psuedomonas aeruginosa* and *Candida albicans* were less susceptible, which was in tandem with the findings of Ajaiyeoba (2002); Faiza, et al., (2009); Batool, et al., (2018); Vaou et al., (2021). The lack of susceptibility of *Pseudomonas aeruginosa* and *Candida albicans* to the extracts could be attributed to the fact that these pathogens are inherently resistant to many antibiotics and non-antibiotics antimicrobial agents due to the permeability barrier afforded by their outer membranes (Lino and Deogracious, 2006). The aqueous extracts of the plants displayed similar zones of inhibitions compared to ethanol extract.

## 4. Conclusion

Water and ethanolic extracts of *Azadirachta indica* and *Ocimum gratissimum* were assessed in this study. The results seem to justify their continued use in the treatment of microbial infections. The inhibition of growth of the test

organisms that are known to cause nosocomial infections and displaying multidrug resistance to most antibiotics and non-antibiotic microbial agents justified the continued use of these plants in folk and traditional medical practice. Studies should therefore be done in order to identify the phytochemical constituents and evaluate their effectiveness invitro, so that they can be synthesize and produced commercially.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No competing interests are disclosed by the writers.

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