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# Detection of antimicrobial and antimycotic activities of African garden egg fruits (*S.melongena L.*) against pathogenic organisms in solvents of varying polarities

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**ABSTRACT**

*The present study was carried out to investigate the antibacterial and antifungal activity of garden egg plant (Solanum Melongena L). Acetone, Methanol and distilled water extracts of the plant was tested against antibiotic resistant bacteria (Staphylococcus Spp, Eschericha coli, and Proteus spp.) and fungus (Candida Spp). The result obtained revealed antibacterial effect of acetone and methanol extract on Escherichia coli and proteus Spp at 20-40g and 80g respectively but was not effective with water as extractant, as a result of these solvents ability to solubilize the active agent in garden egg unlike water. This means garden egg extract can be utilized as Nigerian genetic resource to obtain cheap antimicrobial drugs in addition to their nutritional value. However, no extract revealed any antimycotic effect on candida spp, meaning the extracts cannot substitute any of the existing antifungal drugs.*

**Key words:** garden egg, solvents, polarities and antimicrobial activity.

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## INTRODUCTION

Literatures have shown that the organic and aqueous extract of plants possesses potent and selective antimicrobial activity compared with standard antibiotics such as penicillin, nystatin and ampicillin [1] and [2].

Research in the design and synthesis of antimicrobials and antimycotic will be everlasting endeavors on our planet considering the fact that bacteria and fungus developed resistance to antimicrobials over a period of time. This is primarily attributed to indiscriminate use of commercial antimicrobial drugs used for the treatment of infectious diseases. Antibiotic resistance has now become a global concern. This has led to the search for new antimicrobials, both herbal and synthetic. Be that as it may, synthetic drugs/medicine has been reported [3].

[4], reported that synthetic drugs/medicine has several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavor. Therefore, there is need to access the medicine values of plant used as food source. These efforts are being intensified for the production of food crops in the agro-industry that have antimicrobial properties in addition to their nutritional properties.

*Solanum Melongena L* (Garden egg plants) have been exploited severally in different solvents to determine its potentials in alternative to conventional antibiotics [5]. According to them, phytochemical examination of *Solanum Melongena L* in methanol and aqueous extracts of the fruit and crown showed the presence of alkaloids, saponins, steroids, tannins/phenols, flavonoids, proteins and carbohydrates.

Furthermore, various parts of *S. Melongena L* are useful in the treatment of inflammatory conditions, cardiac debility, and neuralgia, ulcers of nose, cholera, bronchitis and asthma. The fruit is a highly valuable vegetable all over the world because of its taste and higher percentage of vitamin B2. The fruit is also used in the treatment of diabetes [4].

This work therefore takes a look at testing crude extracts of *S. Melongena L* in Methanol, distilled water and Acetone against isolated pathogens: Staphylococcus Spp, Proteus Spp , Escherichia Coli and Candida Spp.

## MATERIALS AND METHODS

*2.1 Source of Garden Egg Plant.*
Fresh fruits of *Solanum Melongena L.* were randomly bought or collected from mile III market Port Harcourt, Rivers State of Nigeria.

*2.3 Preparation of Crude Garden egg plant Powder.*
They were thoroughly washed with distilled water sliced into equal pieces air-dried in shade, and then subjected to drying oven at $40^0$C to constant weight. The dried material was powdered and kept in plastic bags or air tight containers until when needed.

*2.4Preparation of Crude S. Melongena L Extract*
20gm of the dried powder was transferred into a beaker containing 200ml of distilled water and stirred vigorously to form a slurry, from which *S. melongena L.* was extracted in acetone, methanol and distilled water respectively. Three sets of five beakers were used to prepare crude extracts of *S. melongena L.* 10g, 20g, 40g, 60g and 80g of the slurry formed was transferred into each of the five beakers, followed by the addition of 100ml of acetone, methanol and distilled water respectively. It was stirred vigorously or shaken for 10minutes, allowed to settle for one hour, filtered through a whatman filter paper no 42 and the filtrate used immediately for the experiment.

*2.5 Media Preparation*
Agar media was prepared by dissolving accurately weighed 28g of nutrient agar in 1000ml of distilled water, melted and sterilized at $121^0$C for 15 minutes in an autoclave, model: YX-280B made by Ocean Med. The media was distributed in several glass petri dishes and allowed to set for hours. They were properly labeled, each plate was divided into three (3) parts by a marker pen, representing each of the *S. melongena L.* extracts (solution) at different concentrations ranging from 10-80g in acetone, methanol and distilled water respectively.

*2.6 Antibacterial Activity of Crude Extracts*
Petri plates containing 20ml of nutrient agar medium were seeded in a 24 hr culture of the bacteria strains Staphylococcus Spp, Escherichia Coli, Proteus sp) and candida Spp (fungus). Wells of 5mm diameter each were cut into the nutrient agar. To each well 50µl (concentration of 100mg/ml) of the investigated garden egg plant (*S. melongena L.*) were added. The inocula size was adjusted so as to deliver final innocula approximately 108 colony forming units (CFU/mol). Incubation was performed at $37^0$C for 24-48hrs. The assessment of antibacterial activity was based on observation and detection of the inhibition zone around the well after 48 hours and reported as susceptible (+), very susceptible (++) or resistance(R). The test for antimicrobial activity was plated out in an extra plate which served as control.

A total of three (3) isolates of Staphylococcus Spp, Escherichia coli and Proteus spp were obtained from Braithe Waite Memorial Specialist Hospital, in Port Harcourt city, Rivers State Nigeria. The test organisms were sub-cultured at $37^0$C and maintained on nutrient agar media.

The bacteria isolates were tested for its bacterial resistance using disc diffusion method. Antibiotic disc (oxoid) used for gram positive disc with E. coli and Proteues spp were cefixime, ofloxacin, augmentin, Nitrofuratoin, Ciprofloxacin, Ceftaxidine, Cefuroxime, Gentamycin, Septrin and Streptomycin, while for gram negative disc: Norbactin, Chloramphenicol, Ciprofloxacin, Erythromycin, Levofloxacin, Gentamycin, Ampiclox, Rifampicin, Anoxicillines, Streptomycin, Ofloxacin, Nalidixic acid, pefloxin, Augmentin, septrin and cefpodoxine were used.

The inhibition zones were observed and detected according to [6], and isolates were categorized as susceptible and resistance, after replications and presented in a table. Similar method was used for the fungus candida Spp.

## RESULTS

**Table 1: Antimicrobial Activity of Crude S. *melongena L.* in Different Solvents ($CH_3OCH_3$, $CH_3OH$ and $H_2O$)**

| S. Melongena L. Slurry | Resistance and susceptibility of organism in acetone extract | | | | Resistance and susceptibility of organism in methanol extract | | | | Resistance and susceptibility of organism in water extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. (g) | *E. coli* | *Stapp Spp* | *Proteus Spp.* | *Candida Spp* | *E. coli* | *Stapp Spp* | *Proteus Spp.* | *Candia* | *E. coli* | *StappSpp* | *Proteus Spp.* | *Candida Spp* |
| 10g | R | R | R | R | R | R | R | R | R | R | R | R |
| 20g | R | R | + | R | R | R | R | R | R | R | R | R |
| 40g | R | R | ++ | R | R | R | R | R | R | R | R | R |
| 60g | R | R | R | R | R | R | R | R | R | R | R | R |
| 80g | + | R | R | R | + | R | R | R | R | R | R | R |

**Table 2: The used antibiotics in gram positive (+ve) disc with E Coli and Proteus Spp in zones of inhibition**

| Antibiotic/Class | Antibiotic Name | Symbol | Disc Conc. | Zones of inhibition in | | | |
|---|---|---|---|---|---|---|---|
| | | | | *E. coli* | | *Proteus Spp.* | |
| | | | | R | S | R | S |
| B-lactam antibiotic | Cefixime | CXM | 20mcg | R | | R | |
| Quinolone | Ofloxacin | OFX | 10mcg | | ++ | R | |
| Beta-Lactamase Inhibitor | Augmentin | Aug | 30mcg | | + | R | |
| Quinolone | Nitrofurantin | NIT | 20mcg | | +++ | | ++ |
| Quinolones/Macrolides | Ciprofloxacin | CPR | 10mcg | R | | | + |
| 2nd Generation Antibiotic | Ceftaxidine | CAZ | 20mcg | R | | R | |
| 2nd Generation Antibiotic | Cefuroxine | CRX | 20mcg | R | | R | |
| Aminoglycosides | Gentamycin | GN | 10mcg | R | | R | |
| Sulphanamides | Septrin | SXT | 30mcg | R | | R | |
| Aminoglycosides | Streptomycin | ST | 30mcg | | + | | |

*Resistance (**R**), Sensitive or Susceptible (**S**)*

**Table 3: The used antibiotics in gram negative (-ve) disc with staphylococcus Spp. and proteus Spp. in zones of inhibition**

| Antibiotic/Class | Antibiotic Name | Symbol | Disc. Conc. | Zones of inhibition in | | | |
|---|---|---|---|---|---|---|---|
| | | | | Staphylococcus Spp. | | Proteus Spp. | |
| | | | | R | S | R | S |
| Norfloxacin | Norbactin | NB | 30mcg | | +++ | | - |
| Nitrobenzene | Chloramphinicol | CRL | 30mcg | | +++ | | - |
| Quinolone/Microlides | Ciprofloxacin | CPX | 10mcg | | + | R | |
| Macrolides | Erythromycin | E | 30mcg | | + | | - |
| Quinolones | Levofloxacin | LEV | 20mcg | | +++ | | - |
| Aminoglycosides | Gentamycin | GN | 10mcg | | + | | + |
| Penicilline | Ampiclox | APX | 20mcg | | + | | - |
| Rifamycin | Rifampicine | RD | 20mcg | | ++ | | - |
| Aminopenicillines | Amoxicilline | AMX | 20mcg | | + | | - |
| Aminoglycosides | Streptomycin | S | 30mcg | R | | | + |
| Quinolones | Ofloxacin | OFX | 10mcg | | - | | + |
| Quinolones | Nalidixic acid | NA | 30mcg | | - | | + |
| Aminoglycosides | Pefloxin | PEF | 10mcg | | - | | + |
| Protein synthesis of DNA blockage | Augmentin | Aug | 30mcg | | - | R | |
| Sulphanamides | Septrin | SXT | 30mcg | | - | R | |
| 3rd Generation antibiotic | Cefpodoxine | CEP | 10mcg | | - | R | |

*Resistance (**R**), Sensitive or Susceptible (**S**)*

**Table 4: The used antibiotic in single disc with proteus spp.**

| Antibiotic/Class | Antibiotic Name | Symbol | Disc. Conc. | Zones of inhibition in proteus | |
|---|---|---|---|---|---|
| | | | | R | S |
| Quinolones | Oflaxacin | OFX | 10mcg | | + |
| Carbapelen antibiotic | Meromem | MEM | 20mcg | R | |
| Piperacilline + Tazodacten | Revotaz | TZP | 20mcg | | +++ |

## DISCUSSION

The studied crude extracts of *S. melongena L.* fruits showed no antibacterial activity against Staphylococcus Spp and antimycotic effect on the fungus (Candida Spp) in all the extractants. However, antibacterial activity was observed against two bacterial isolates of antibiotic resistant strains identified as Escherichia coli and Proteus spp (table 1).

This result corroborated the work of Bhaltacharjee [7], who reported that fatty acids such as Palmitic, stearic and oleic acids which are major constituents of cestrum diurnum (Solanceal) showed antibacterial activity against the pathogenic strains of Staphylococcus Spp, Escherichia coli and Pseudomonas aeruginosa.

Literature have it that *S. melongena L.* contained lipid materials comprising of 16 fatty acids of which hexadecanoic acid (25.5%) and linolenic acid (23.0%) are the most predominant; fifteen (15) hydrocarbons of which the predominanat ones are penance (18%) and Dodecan-2-one (20%) and two sterols: 10-Demethyl syalene (2.5%) and 24-Beta-Ethyl-5-delta-cholesten-3-beta-ol (6%) as evident in gas chromatography/Mass spectroscopy [8]. The above mentioned fatty acids, hydrocarbons and sterols were implicated by them.

Again, it could be attributed to the biological activity of the long chain fatty acids (hexadecanoic acid and linolenic acid) present in *S. melongena L.* (garden egg plant).This idea was established by [9], who reported that antibacterial actions of fatty acids are usually attributed to long chain unsaturated fatty acids. This view was supported by [10], and [11], who reported that Lauric, Palmitic, Linolenic, Linoleic, Oleic, Stearic and Myristic acids are known to have potential antibacterial and antifungal agents.

Furthermore, the ability of acetone and methanol extracts to solubilize the active ingredients (nasunin) is another factor unlike water. Solubilization is a function of dielectric constant and plays a big role in drug solubility, both in organic and inorganic solvents, and affect many pharmaceutical processes such as synthesis, extraction, and formulation etc. Non electrolyte solutes are more soluble in organic solvents than in water because they are not ionizable. Changes in dielectric constant of the medium have a dominant effect on the solubility of ionizable solute in which higher dielectric constant can cause more ionization of the solute and result in more solubilization [12].

Since the active ingredent in *S. Melongena L* is not ionizable, its dissociation in water may be very small and cannot be significantly solubilised. Hence, the obtained results in water had no effect unlike acetone and ethanol [13].

On the other hand, methanol (ɛ34) and acetone (ɛ21) have dielectric constants lower than that of water (ɛ88).The lower the dielectric constants, the higher the ability of the solvent to extract more of the active ingredient from solution. Hence, acetone extracted more than methanol and water. Therefore, dielectric constant is a major property of the degree of extraction of active agents in acetone and methanol. And the summary is that acetone extract more than methanol and methanol more than water. i.e. acetone › methanol › water.

Considering hydrophobic or lipophilic properties of the plant extracts and their components, it enables them to partition the lipids of the bacterial cell membrane and mitochondria disturbing the cell structure and thus render them more permeable [14]. When this happens, the extensive leakage from bacterial cell or the exit of critical molecule and ions will lead to death [15].

Furthermore, the mechanism of action of the major constituents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis due to the presence of alkaloids, phenols, tanning and flavanoides in garden egg plant [16].

Finally, the concentration of the slurry used can also be attributed to the obtained results in methanol and acetone as depicted by table 1. The antibacterial effect is noticed as the concentration increased from 20-40g against proteus spp. and 80g against E. coli respectively. This is because more of the surface area of the active agents are being exposed to the isolated bacteria, hence their susceptibility.

## CONCLUSION

This method employed was simple and showed that antibacterial activity against two isolated bacteria was effective and that *S. Melongena L.* can compete favorably with drug resistance bacteria. However, it can be used in place of most bacterial resistant drugs such as nitrofuradantoin, aminoglycosides, quinolones etc.

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