

***In vitro* Antioxidant and Antimicrobial Activity of Ethanolic Extract of Egg Plant (*Solanum melongena* Linn) Fruit**

ABSTRACT

Ethno-medicinal use of plant has been effective in the amelioration of oxidative stress and in the inhibition of pathogenic microorganism. The ethanolic extract of *Solanum melongena* Linn fruit as a potent *in vitro* antioxidant and antimicrobial agent was investigated using standard protocols. The results showed that the ethanolic extract of *Solanum melongena* Linn fruit scavenged 1,1-diphenylpicrylhydrazyl (DPPH) and nitric oxide (NO), and inhibited lipid peroxidation (LPO) compared to standard (vitamin C). It also reduced ferric ion (as measured through ferric reducing/antioxidant potential, FRAP) compared to the standard, diethyltetraacetic acid (EDTA). The observed *in vitro* antioxidant activity of the sample was dose dependent. At a minimum inhibition concentration (MIC) of 50 mg/ml, the ethanolic extract of *Solanum melongena* Linn fruit showed the highest activity against the bacterium, *Staphylococcus aureus* (16.33 ± 2.69 mm) and the fungi, *Aspergillus flavus* (3.33 ± 0.38 mm at 70mg/ml). The bacteria, *Escherichia coli* and *Salmonella typhi* and the fungus, *Rhizopus stolonifer* were not susceptible to the ethanolic extract at the tested concentrations. The ethanolic extract at 10 mg/ml and 30 mg/ml could not inhibit any of the studied pathogens. In conclusion, the ethanolic extract of *Solanum melongena* Linn fruit exhibited a varied degree of appreciable *In vitro* antioxidant activities compared with the tested methods and standard. Its activity against the tested bacterial and fungal strains underscores its apparent pharmacological potential, warranting further studies.

Keywords: *Ethnomedicinal, Solanum melongena* Linn fruit, antioxidant, susceptibility and pathogens.

INTRODUCTION

The use of plant for their ethnomedicinal potentials has long been applicable in the search to overcome drug resistance conditions [1]. And, plant phytochemicals, including phenol, flavonoids, saponins and tannins, ensure the protection of plants against pathogenic microorganism [2]. Antioxidant studies are of great importance in plant studies. This is because; they are useful in the verification of the antioxidant potentials of phytoconstituents [3]. Antioxidants are biological defense system against free radical attack. Free radicals are unpaired electrons that are very reactive [2] and are constantly generated in the living system resulting to diseased conditions. Antioxidants neutralize the free radical effects, thus rendering them inactive [4], hence antioxidant may be useful in combating diseased states. Thus, there is increasing need to screen and exploit plants and plant parts for the possible antioxidant potentials. Moreover, plant antioxidants show relatively minimal adverse effect.

Solanum melongena is one of the best known egg plant species. The name egg plant was derived from the egg like shape. It is the third most important crop in the family of the solanaceas after potato and tomato [5], belonging to the genus *solanum* with lots of species. These species including *Solanum incanum*, *Solanum nigrum* and *Solanum melongena* are all cultivated in Africa: as an animal plant. *Solanum melongena* bears different colours, shapes, sizes and fruits [6]. The fruit is pendent, fleshly berry and the colour ranges from shinning purple to white, green, yellow and black often with strips and patches on the skin. This fruit shape varies from long cylindrical to round, oblong and oval shape and it undergoes self-pollination [7]. Egg plant has found indigenous medicinal uses including the management of diabetic [8], could reduce body weight, possess nutraceutical properties like analgesic and antipyretic potentials [9], antioxidant [10], antimicrobial [11]. These warranted the study aimed at assessing the *in vitro* antioxidant and antimicrobial activity of the ethanolic extract of *Solanum melongena* Linn fruit.

MATERIALS AND METHODS

Plant materials and preparations

Matured egg plant fruits were bought from a local market, *Ehere* market, in Aba, Abia State during the fruiting season of May, 2016. The fruit was identified as *Solanum melongena* Linn in the Plant Science Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The fruits were washed with clean tap water, crushed into smaller pieces using a knife and were air-dried for two weeks. The air-dried fruits were milled into powder using a laboratory miller and stored in an air tight container.

Extraction and concentration

200g of the Egg plant fruit, powder were taken in separate containers. To this, 1000 mls of ethanol was added and kept for 72 hours with interval shaking. The extract was filtered with No 1 Whatman filter paper. The filtrate was concentrated using water bath at 60°C and was further dried in an oven set at 50 °C. The extract was placed into a sample bottle and stored in a refrigerator at about 4°C until it was required for experiment.

Test Organisms

The bacterial and fungal strains were obtained from New Era Specialist Hospital, Laboratory section and from Hyon Foods Limited Microbiological Laboratory, both in Aba Abia State, Nigeria. In particular, *Pseudomonas aeruginosa*, *Candida albican* and *Staphylococcus aerues* were obtained from New era specialist while the other tested strains viz: *Eschericha coli*, *Shigella dystenteriae*, *Salmonella typhi*, *Rhizopus stolonifer* and *Aspergillus flavus* were obtained from Hyon Foods Laboratory. The bacteria biochemical characterization was done at Hyon foods Limited.

Media preparation and sterilization

Nutrient agar (NA) and Potato dextrose Agar (PDA) were used for bacteria and fungi inoculation respectively. They were prepared in accordance with the direction for use and autoclaved at 121°C for 20 minutes and then separately poured into the Petri dishes with different bacteria and fungi species.

Antimicrobial activity of the ethanolic extract of *Solanum melongena* Linn fruit

The disc diffusion method was used Nostro *et al.*, [12]. Stock solution (600 mg/ml) of extract was prepared using the diluent. Discs (6 mm) in diameter was made from Whatmann No.1 grade filter paper with the aid of a mechanical perforator and sterilized by autoclaving at 121°C and were impregnated with 10µl of different concentrations (10 mg/ml, 30 mg/ml, 50 mg/ml and 70 mg/ml) of the extract were inoculated and investigated. Each of the discs was placed at the center of the petri dish either for bacteria strains on NA or fungi strains on PDA. The plates were incubated at 37 °C for 20 - 24hours for bacterial studies and 25°C-48-seven two hours for fungal studies. Ciprofloxacin (25 µg/disc) and Fluconazole (10 µg/disc) were used as control. All tests were performed in triplicate and the antibacterial and antifungal activities were expressed as the mean diameter of inhibition zones (mm) produced by the ethanolic extract of *Solanum melongena* Linn fruit.

3.12 Antioxidant capacity of the ethanolic extract of *Solanum melongena* Linn fruit

Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was carried by the method described by Mensor *et al.* [13]. This method is based on the principle that when DPPH reacts with an antioxidant compound, which can donate hydrogen, itself is reduced. The change in colour from purple to light yellow shows the antioxidant activities of the extract and this was measured spectrophotometrically at 520 nm.

Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power was carried out as described by Benzie and Strain [14]. This method is based on the principle that ferric iron (Fe^{3+}) is initially reduced by electron-donating antioxidants present within the sample to its ferrous form (Fe^{2+}). The iron-colorimetric probe complex develops a dark blue color product upon reduction which can be measured at 550nm. Samples were compared to the EDTA as a standard for determining antioxidant potentials.

Determination of lipid peroxidation inhibition assay

A modified thiobarbituric acid-reactive species (TBARS) assay [15] was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media [16]. This method is based on the principle that malondialdehyde (MDA) in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct can be easily quantified spectrophotometrically at 540nm.

Determination of nitric oxide radical ($\text{NO} \cdot$) radical scavenging assay

Nitric oxide radical scavenging assay was determined using Griess reaction with minor changes as described by Marcocci *et al.* [17]. This method is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which were then determined by the use of Griess IIIosvoy reaction. This absorbance for this reaction was read spectrophotometrically at 540 nm.

Statistical Analysis

Collected data were subjected to statistical analysis using statistical package for the social sciences (SPSS) for Windows version 22.0. Results were expressed as Mean \pm standard error of the mean (SEM).

RESULTS AND DISCUSSION

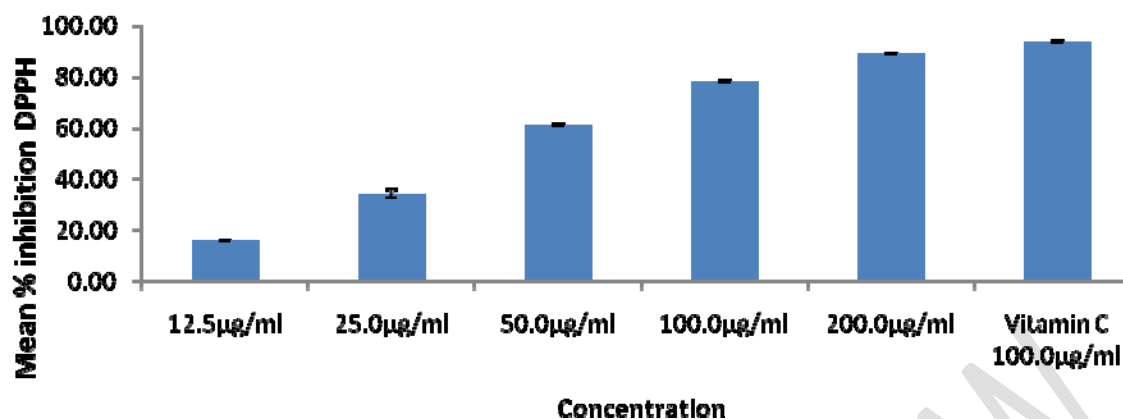


Figure 1: DPPH radical scavenging activity of the ethanol extract of *Solanum melongena* Linn fruit at different concentration compared to vitamin c at 100.0 µg/ml.

Figure 1 above showed a progressive increase in the DPPH radical scavenging ability of ethanol extract of *Solanum melongena* Linn fruit at different concentrations compared to vitamin C (a standard) at 100 µg/ml. The highest scavenging ability for the ethanol extract was observed at 200 µg/ml (89.11%) compared to vitamin c at 100 µg/ml (93.70%). The figure further showed concentration dependent scavenging ability of the plant extract. The plotted values are means \pm SEM of triplicate determination.

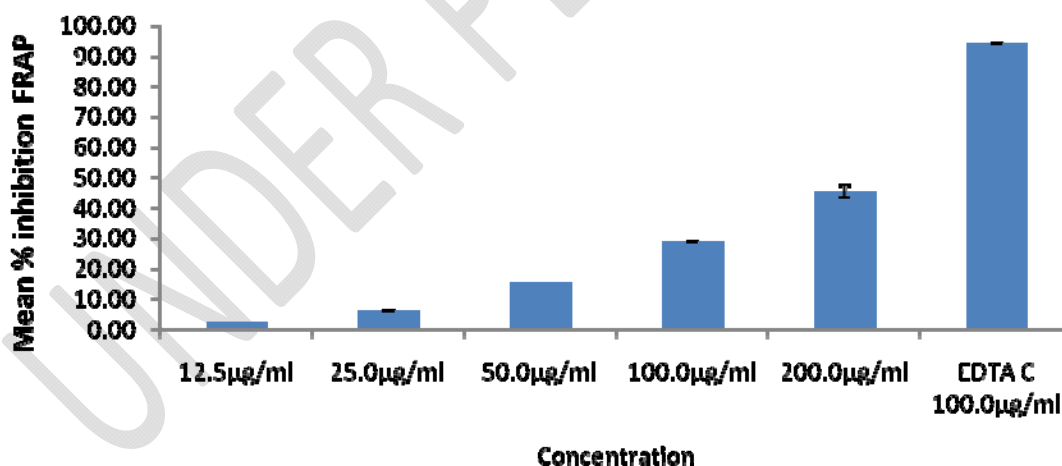


Figure 2: Ferric reducing antioxidant power of ethanol extract of *Solanum melongena* Linn fruit at different concentration compared to EDTA at 100.0 µg/ml.

Figure 2 above showed the iron binding capacities in terms of percent inhibition of the ethanol extract of *Solanum melongena* Linn fruit at different concentration (12.5 µg/ml through 200 µg/ml). The extract scavenging ability is not

comparable at 200 $\mu\text{g/ml}$ (45.33%) compared to EDTA (a standard) at 100 $\mu\text{g/ml}$ (93.95%). The plotted values are means \pm SEM of triplicate determination.

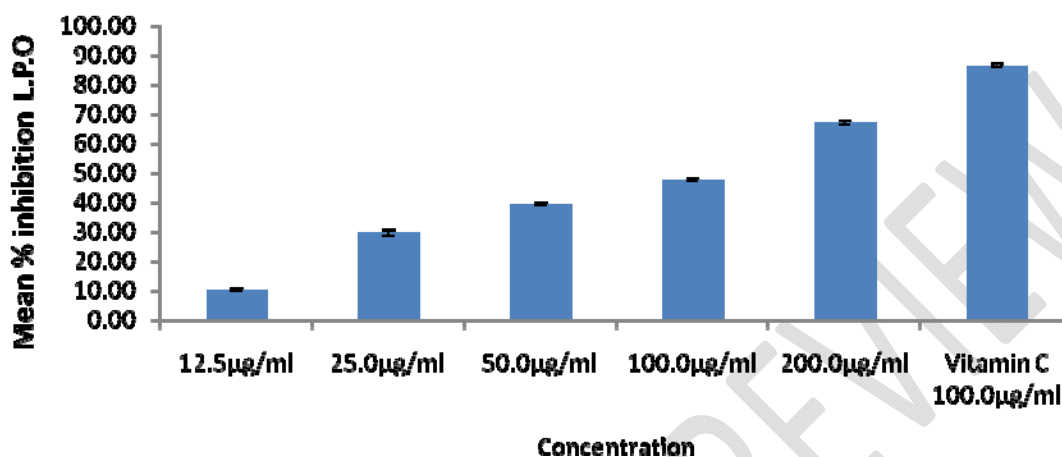


Figure 3: Lipid peroxidation inhibition activity of the ethanol extract of *Solanum melongena* Linn fruit at different concentration compared to vitamin C at 100.0 $\mu\text{g/ml}$.

Figure 3 above showed the inhibition effect of ethanolic extract of *Solanum melongena* Linn fruit and vitamin c (a standard) on lipid peroxidation inhibition ability. The inhibitory effect is concentration dependent. The plotted values are means \pm SEM of triplicate determination.

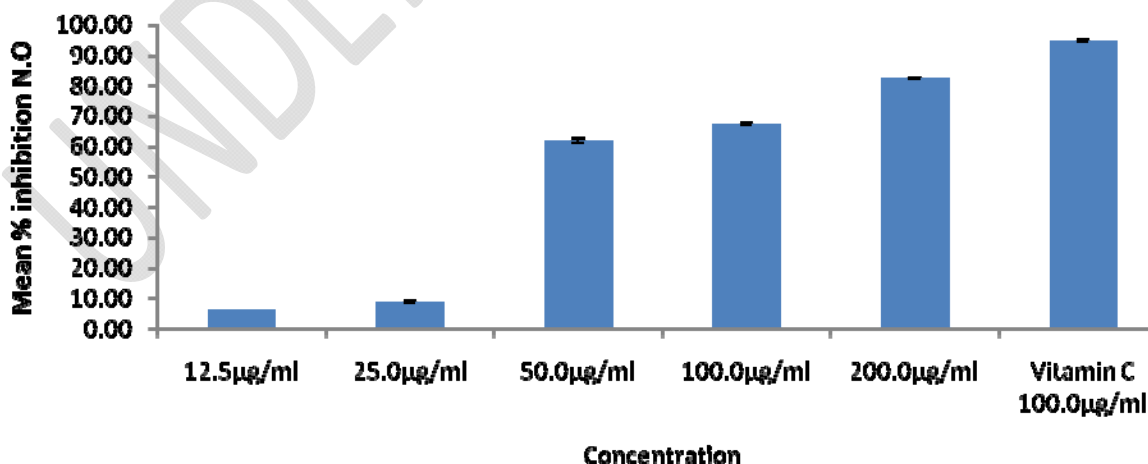


Figure 4: Nitric oxide radical scavenging activity of the ethanolic extract of *Solanum melongena* Linn fruit at

Parameter	<i>Salmonella spp.</i>	<i>E.coli</i>	<i>S.aerus</i>	<i>P.aeruginosa</i>	<i>S.dysenteriae</i>
Grams	Gram (-ve)	Gram (-ve)	Gram (+ve)	Gram (-ve)	Gram (-ve)
Motility	(+ve)	(+ve)	(-ve)	(+ve)	(-ve)
Catalase	(+ve)	(+ve)	(+ve)	(+ve)	(-ve)
Coagulase	(-ve)	(-ve)	(+ve)	(-ve)	n/a
Indole	(-ve)	(+ve)	(+ve)	(+ve)	(-ve)
Urease	(-ve)	(-ve)	(-ve)	n/a	(-ve)
Citrate	(-ve)	(-ve)	(-ve)	(+ve)	(-ve)
Methyl red	(+ve)	(-ve)	(+ve)	(-ve)	(+ve)
Voges Proskauer	(-ve)	(-ve)	(-ve)	(-ve)	(-ve)
Glucose	(+ve)	(+ve)	(+ve)	(-ve)	(+ve)
Lactose	(-ve)	(+ve)	(+ve)	(-ve)	(-ve)
Maltose	(+ve)	(+ve)	(+ve)	(-ve)	n/a
Mannitol	(+ve)	(+ve)	(+ve)	(+ve)	n/a
Oxidase	(-ve)	(-ve)	(-ve)	(+ve)	(-ve)

different concentration compared to vitamin C at 100.0µg/ml.

Figure 4 above showed the percentage inhibition of nitric oxide generated by ethanol extract of *Solanum melongena* Linn fruit and vitamin c (a standard). The extract scavenging ability is concentration dependent. The concentration at 200µg/ml (82.08%) is comparable to 100µg/ml (94.53%) for vitamin c (a standard). The plotted values are means \pm SEM of triplicate determination.

Table 1: Biochemical characterization of test organisms

Note: -ve and +ve signifies negative and positive respectively.

Table 2: Antimicrobial susceptibility test results for ethanol extract of *Solanum melongena* Linn fruit on some selected microorganisms.

Pathogens	Extract conc. (mg/ml)	Zone of inhibition (mm)	MIC (mg/ml)	Standard drugs Ciprofloxacin Zone of inhibition (mm)
<i>Escherichia coli</i>	10	-	-	21
	30	-		
	50	-		
	70	-		
<i>Staphylococcus aureus</i>	10	-	50	22
	30	-		
	50	15.33 \pm 1.83		
	70	16.33 \pm 2.69		
<i>Pseudomonas aeruginosa</i>	10	-	70	18
	30	-		

	50	-		
	70	13.67 ± 0.38		
<i>Shigella dysenteriae</i>	10	-	70	22
	30	-		
	50	-		
	70	12.83 ± 1.25		
<i>Salmonella typhi</i>	10	-	-	24
	30	-		
	50	-		
	70	-		
For some fungi				Fluconazole
<i>Candida albican</i>	10	-	50	20
	30	-		
	50	1 ± 0.00		
	70	2 ± 0.00		
<i>Rhizopus stolonifer</i>	10	-	-	-
	30	-		
	50	-		
	70	-		
<i>Aspergillus flavus</i>	10	-	50	24
	30	-		
	50	0.67 ± 0.38		
	70	3.33 ± 0.38		

Table 2 above showed the inhibitory effect of ethanol extract of *Solanum melongena* Linn fruit with minimum inhibition concentrations (MIC), the standard antibacterial drug (Ciprofloxacin) and antifungal standard drug (Fluconazole) against some selected microorganisms. *Staphylococcus aureus* recorded the highest MIC at 50mg/dl showing 15.3mm inhibition zone, *Pseudomonas aeroginesa* and *Shigella dysenteriae* had MIC of 70mg/dl with 13.7mm and 12.8mm for their respective inhibition zones compared to the standard drug (Ciprofloxacin). However, *Salmonella typhi* and *Escherichia coli* were not inhibited at the tested concentration. Furthermore, *Candida aalbican* and *Aspergillus flavus* both showed a MIC of 50mg/dl and inhibition zone of 1mm and 0.7mm respectively against fluconazole, which showed inhibition zones of 20mg/dl and 24mg/dl respectively. *Rhizopus stolonifer* was not inhibited by the ethanolic extract of *Solanum melongena* Linn fruit.

Discussion

Figure 1, 2, 3 and 4 showed the *in-vitro* antioxidant activity of ethanol extract of *S. Melongena* on DPPH radical scavenging activity, lipid peroxidation inhibition assay (LPO) and Nitric oxide radical scavenging assay against Vitamin C as a standard and FRAP activity against ethylenediaminetetraacetic acid (EDTA). The results revealed that the extract exhibited antioxidant potentials.

DPPH is a stable free radical widely used to evaluate scavenging ability of different antioxidants. The result of this study showed that as the concentration increases, the scavenging activity increases. This reveals that *S. melongena* could be a potent *in-vitro* antioxidant. Gradiappan and Rangasamy, [18], reported a similar finding on antioxidant activity of different species of solanaceae. This suggests that *Solanum melongena* Linn fruit is capable of donating hydrogen to a free radical in order to remove odd electrons that lead to free radical reactivity. This is the main mechanism of determining DPPH radical scavenging activity as noticed by the change of colour that was measured spectrophotometrically. This could imply that pathological health challenges could be managed with ethanolic

extract of *Solanum melongena* Linn fruit at high concentration [19]. The highest scavenging ability for the ethanolic extract was observed at 200 µg/ml (89.11%) compared to vitamin c at 100µg/ml (93.70%).

Generally, plant acts as antioxidant via metal chelating, as a reducing agent and/or free radical scavenger [3]. Ethanolic extract of *Solanum melongena* Linn fruit FRAP assay was done to determine its ability to reduced Fe^{3+} . FRAP study uses these three mechanisms at the same time to achieve antioxidant potentials. Free radicals chains are broken by donating hydrogen, which is a reducing activity. The ethanolic extract of *Solanum melongena* Linn fruit could donate electrons to stabilize radical activity. This makes it a good scavenger. This plant extracts reduced ferric iron as an antioxidant when compared to a standard EDTA though its inhibitory effect was minimal. Furthermore, the reducing activity increased as the concentration increases. This points that the extracts could play pivotal roles to protect against oxidative damage.

In this study, the lipid peroxidation inhibition activity was determined using the TBARS assay. TBA reacts specifically with MDA, a secondary product of lipid peroxidation to give a red chromogen, which may then be determined spectrophotometrically [15]. The present study also showed concentration dependent for the antioxidant activity. This study further revealed that the ethanolic extract of *Solanum melongena* Linn fruit could stand against reactive radical species from producing adducts from lipids, proteins, and DNA. This suggests that oxidation of unsaturated fatty acid in biological membranes could be ameliorated through ingestion of ethanolic extract of *Solanum melongena* Linn fruit at high concentration.

Nitric oxide (NO), a synthetic product of L-arginine, could play physiological roles in the endothelial relaxation of the smooth muscles [20]. However, in the cells, when the antioxidant level is low, there is a concomitant production of peroxynitrite (ONOO.). This is the chief free radical produced from nitric oxide. Free radicals have been implicated in the etiology of inflammations and aging related disease conditions. Hence, there is a need for the system to constantly maintain its antioxidant defenses. Ethanolic extract of *Solanum melongena* Linn fruit inhibited NO[•] oxidation in this *In vitro* study. NO scavenging activity showed a similar pattern of increasing activity with increasing concentration [21; 22].

The ethanolic extract of *S. melongena* Linn fruit exhibited varying degree of antibacterial and antifungal activities on most of the tested pathogens (*Escherichia coli*, *Staphalococcus auerus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi*, *Candida albican*, *Rhizopus stolonifer* and *Aspergillus flavus*) as shown in the results (Table 1). The ethanolic extract of *Solanum melongena* Linn fruit showed the most inhibitory effect against *Staphalococcus auerus*, with inhibition zone of 15.33mm at 50mg/ml and a MIC of 50mg/ml compared to *Shigella dysenteriae* (12.83mm at 70mg/ml) and *Pseudomonas aeruginosa* (13.67mm at 70mg/ml). This is an indication that as the concentration of the extract increases the inhibition of these microbes would increase. The extract exhibited antibacterial activity against the tested bacterial compared to a used standard drug (Ciprofloxacin at 25µg/disk). This could indicate its broad spectrum activities as an antimicrobial agent and with a potent pharmacological activity. These results are comparable with the report by Oranusi *et.al.* [23], on antimicrobial activity of *chrysophyllum cainito* and Ajiboye *et.al.* [24], for antimicrobial activity of fruit pulp on *Dialium guineese*. Further to this, the antifungal activities of the extract appear to be more potent on the fungus (*Asperigulls flavus*) with 0.70mm and 3.33mm for 50mg/ml and 70mg/ml respectively and a MIC of 50mg/ml than the other tested fungi compared to standard antibiotics (Fluconazole at 10µg/disk). The extract did not inhibit the activity of the fungi *Rhizopus stolonifer*. The spectra of antimicrobial activity shown by the extract could be as a result of the presence of flavonoid, tannins and saponin.

CONCLUSION

The ethanolic extract of *Solanum melongena* Linn fruit exhibited a varied degree of appreciable *In vitro* antioxidant activities compared with the tested methods and standard. Its activity against the tested bacterial and fungal strains underscores its apparent pharmacological potential, warranting further studies.

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