

Antimicrobial Potential and Biochemical Profile of Methanolic Extracts of Common *Solanum* Species in Nigeria

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ABSTRACT: Due to the rise in microbial resistance to orthodox medicines, there is need for investigation towards the discovery of plants with potential pharmacological effects. Methanolic extracts of the leaves of four *Solanum* species were screened for their antimicrobial properties and for various biochemical analyses, using standard methods. *S. melongena* showed highest antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. The antifungal activity of *S. gilo* extract was the highest at 5 mg/ml against *Aspergillus flavus* and *Candida albicans*. Antifungal activity of *S. macrocarpon* extract was observed as the highest at 25 mg/ml against *Candida albicans*. The promising antimicrobial potential of the investigated plants can be attributed to the presence of specific biochemical compounds detected in them by means of GC-MS profiling and other biochemical analyses. The study revealed that the four species of African eggplant are nutritionally and therapeutically valuable and can be further developed into medicinal products.

Keywords: *Solanum*, Phytochemicals, GC-MS, Antimicrobial, Nutritional, Medicinal

INTRODUCTION

There is a current increase in the prevalence of multiple pathogenic microorganisms' resistance to drugs. This is as a result of long-term use of industry-manufactured drugs which are commonly utilized in the treatment of infectious diseases.¹ The efficiency of several antibiotics had significantly decreased as a result, and new, multi-resistant bacterial strains had begun to appear.² As a result, it is increasingly important to produce novel antibacterial substances for resistant species.^{3,4} Herbal medicine has long been used as a complementary type of treatment. Higher plants are a potential source of novel antibiotic prototypes, according to a screening of selected plant extracts and other plant products for phytomedicinal and antibacterial potentials.⁵

A study conducted by Sukprasansap *et al.* (2019)⁶ showed that the extracts from eggplants may be able to prevent DNA damage and that they appear to be safe for consumption. Eggplants can also offer health benefits and prevent or lower the chance of acquiring chronic diseases like cancer. Furthermore, by chelating ferrous iron, extracts from the skin of the eggplant fruit have a great potential for scavenging superoxide free radicals and preventing the generation of hydroxyl radicals.⁷ Alkaloids, saponins, steroids, tannins/phenolics, flavonoids, proteins, and carbohydrates were found in the methanolic and aqueous extracts of the fruit and crown of *Solanum melongena*, according to a phytochemical analysis.^{8,9} The extracts of *Solanum* spp. have been shown to include dietary fibre, potassium, manganese, copper, thiamin (Vitamin B1), Vitamin B6, folate, magnesium, and niacin.¹⁰ Antimicrobial substances like nasunin and delphinidin are said to be abundant in the peel of the purple species.¹¹ Due to the continuous development

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of bacterial resistance to the widely used antibiotics, there is a constant need for the discovery of new medications. This study sought to determine how certain test bacteria responded to substances identified in phytochemical, proximate, organic, and mineral studies as antimicrobials.

MATERIALS AND METHODS

Plant collection and preparation. Fresh leaves from the four species of *Solanum*-*S. melongena*, *S. gilo*, *S. aethiopicum*, and *S. macrocarpon* were collected into distinct sterile polythene containers. The Ladoke Akintola University of Technology (LAUTECH) Herbarium, Department of Pure and Applied Biology, Ogbomosho, Nigeria, verified the species identities.

Preparation of extracts. Fresh leaves of the four species were properly cleaned to remove any debris, and were air-dried for one week on initially-sterilized laboratory slabs. They were turned into powder with the use of a porcelain mortar and pestle and stored in different airtight containers until they were required for additional research. A container containing 100 ml of methanol and 10 g of powder from each of the four species was covered for one week after. The mixture was then sieved through a clean sieve to remove the shaft, and filter paper was used to collect the residual liquid. The concentrated filtrates of each extract were then put on a rotary evaporator to remove excessive moisture. The extracts were stored in airtight glass bottles with corks at 4°C before conducting additional experimental studies.¹²

Chemicals and reagents. Labtrade Nigeria Ltd, located in Kwara State, Nigeria, provided all the chemicals and reagents used and they met the analytical requirements.

Phytochemical, proximate and mineral Elements Analyses. Different phytochemicals were analyzed in the four extracts from *Solanum* species. The spectrophotometric method of Brunner (1984)¹³ was used to determine the saponin content, and Harborne's flavonoid measurements were adopted. The alkaline precipitation gravimetric technique was

used to measure alkaloids (Harborne, 1984).¹⁴ The spectrophotometric approach of Makkar *et al.* (1993)¹⁵ was used for tannins analysis. According to Mahadevan and Sridhar (1982)¹⁶ and El-Olemy *et al.* (1994),¹⁷ respectively, the total phenols and cardiac glycosides were measured using Buljet's reagent. The amounts of moisture, ash, crude fibre, crude protein, fat, and carbohydrates were measured in the leaves of the four species under investigation. Moisture content (AOAC, 1995),¹⁸ crude fibre (James, 1995),¹⁹ protein (Pearson, 1976),²⁰ fat (Onwuka, 2005),²¹ and carbohydrate (Arithmetic Difference Method, i.e., % CHO = 100 - (% fat + % ash + % fibre + % protein)) are all measurements of different types of food. Using a NOVA 400 atomic absorption spectrometry (ANALYTIK JENA AG, Jena, Germany) with hollow cathode lamps and an acetylene/air flame to measure absorbance, mineral elements in the four species of *Solanum* were examined. Mn, Zn, Cu, Na, K, Mg, Ca, Fe, and P were computed and their amounts were represented in ppm utilizing slits, wavelengths, and lamp current.

Quantification of organic compounds. The organic chemical contents of the leaf extracts were determined using an Agilent 19091S Gas Chromatograph (GC) connected to a mass spectrometer (433HP-5MS) under the following conditions: a silica capillary column fused with 100% phenyl methyl silox, measuring 30 m 250 m in length and 0.25 m in film thickness. An electron ionization device with an ionization energy of 70 eV was activated for GC-MS detection. The injector temperature was 300°C, the split ratio was 50:1, and the average velocity was 45.67 cm/s. Helium (99.999%) was utilized as the carrier gas at a steady flow rate of 1.5 ml/min. The oven's temperature was programmed to rise from 100°C (isothermal for 4 min) to 240°C at a rate of 4°C per minute. 49 minutes in total were spent on the GC. By comparing each component's average peak area to the sum of all areas, the relative percentage quantity of each component was computed. NIST Ver. 2.0, 2009 library29 was used for the detection step, and Turbomass software was used to manage the mass spectra and chromatograms. Using their spectra, the

components were identified following the GCMS performance.¹²

Test organisms for antimicrobial studies. The bacteria, fungi and yeast isolates were obtained from the biotechnology Laboratory, Pure and Applied Biology Department, Ladoko Akintola University of Technology, Ogbomoso, Nigeria. Bacterial cultures of different strains including *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus cereus*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were maintained at 37°C. *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* fungal cultures were maintained at 25°C. Yeast culture of *Saccharomyces cerevisiae* was maintained at 25°C.

Preparation of inoculum. In a clean beaker, 0.2 g of yeast extract, 1 g of sucrose, and 100 ml of water were combined to create the fungal inoculums. To sterilize the media, 5 ml of the mixture was pipetted into each test tube, sealed with a cap, and autoclaved for 15 min at 121°C. After allowing the media to cool, two loopfuls of the test fungi were inoculated into each bottle, and they were then incubated. After two and a half hours, there was a noticeable fungus growth.

In the preparation of bacterial inoculum, 5 ml of nutrient broth was pipetted into each test tube, corked, autoclaved and allowed to cool. The test tubes were arranged on slide rack and were labelled. Two loopful of each of the test bacteria was inoculated in each test tube, mixed very well and then transferred into an incubator for 2 h at 37°C. Visible growth of the organisms was noticed thereafter.

Antimicrobial activity. For the antibacterial and antifungal activity, disc-diffusion techniques were used. For bacteria, petri dishes were prepared with 20 ml of sterile nutrient agar, and for fungus, potato dextrose agar (PDA). 5 ml of nutrient broth were added to each test tube, which was after which it was corked, autoclaved, and allowed to cool before being used to make bacterial inoculum. The test tubes were arranged and labeled on a slide rack. Two loopfuls of each of the test microorganisms were inoculated into each test tube, which was then properly mixed before

being incubated for two hours at 37°C. After then, the organisms' growth could be observed. Each plate's solidified agar was separated into neatly spaced segments for concentrations of 5, 25, 50, 100, and 250, with the control in the middle. Each plate had the name of the organism clearly stated in capital letters. The test culture was swabbed on top of the solid agar media, and the perforated filter paper (4 mm) was added to the extract at several concentrations (0.0025, 0.005, 0.25, 0.1) and placed into the marked, labelled, and ready region, while the control disc contained just methanol. Fungi were cultivated for 48 h at 28°C and bacteria for 24 h at 37°C. Broad spectrum antibiotics and solvents were used to prepare the negative and positive controls, respectively. For bacteria, the plates were incubated for 24 hours at 37°C, and for fungi, for 48 hours at 28°C. Inhibitory zones were measured in millimetres.

Statistical analysis. Using One-Way Analysis of Variance (ANOVA), statistical analysis was performed. The results were shown as the mean and standard deviation (or SEM) of three independent measurements. Statistical significance of the mean value was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

The quantitative phytochemical screening conducted on four extracts of *Solanum* species showed the presence of alkaloid, saponin, phenol, tannin, flavonoid, terpenoid, cardiac glycoside and phlobatannin in all extracts (Table 1). MACG had the highest quantity of saponin (0.80 ppm) and alkaloid (0.71 ppm) which were greater than the quantities of other extracts. MELO had the highest quantity of tannin (0.44 ppm), which was more than the quantity of tannin of other extracts. GILO had the highest quantity of terpenoid (0.94 ppm) among all the extracts

Table 2 reveals the result of percentage proximate content of the four *Solanum* species. Based on the results obtained, the percentage crude fibre and percentage moisture content of MACG, AETK, GILO and MELO extracts were not significantly different between the various plants. MELO had the

least percentage crude carbohydrate (6.16 %), which is of significant difference from those of other extracts.

Table 3 shows the result of mineral element compositions (in ppm) of the four *Solanum* species.

The quantity of the mineral elements obtained from

Table 1. Mean quantities of some secondary metabolites (ppm) in the leaves of *Solanum* species studied.

Taxa	Alkaloid	Saponin	Phenol	Tannin	Flavonoid	Terpenoid	Cardiac glycoside	Phlobatannin
MACG	0.71 ^a	0.80 ^a	0.13 ^b	0.37 ^{ab}	0.55 ^a	0.78 ^b	0.84 ^a	0.30 ^c
AETK	0.64 ^b	0.34 ^c	0.21 ^a	0.31 ^b	0.60 ^a	0.73 ^b	0.90 ^a	0.36 ^b
<i>GILO</i>	0.63 ^b	0.53 ^b	0.10 ^b	0.38 ^a	0.51 ^b	0.94 ^a	0.63 ^b	0.38 ^b
MELO	0.61 ^b	0.78 ^a	0.12 ^b	0.44 ^a	0.45 ^b	0.86 ^a	0.93 ^a	0.45 ^a

(MACG = *S. macrocarpon* Glabrous cultivar, AETK = *S. aethiopicum* kumba variety, *GILO* = *S. gilo*, MELO = *S. Melongena*) Values with varying alphabetical superscripts indicate significantly different means at $P \leq 0.05$ while the ones with the same alphabets are not of significant difference at $P \leq 0.05$.

Table 2. Percentage proximate composition of the species of *solanum* studied.

Taxa	% Cr. Fibre	% Cr. CHO	% Dry matter	% Cr. Fat	% Moisture content	% Cr. Protein	% Total ash
MACG	16.21 ^a	7.31 ^a	2.450 ^a	12.262 ^a	35.58 ^{bc}	33.60 ^a	15.206 ^b
AETK	16.01 ^a	7.34 ^a	2.150 ^b	12.331 ^a	36.91 ^a	33.00 ^a	15.281 ^b
<i>GILO</i>	16.31 ^a	7.11 ^a	2.721 ^a	12.304 ^a	36.00 ^b	32.92 ^a	16.841 ^a
MELO	16.30 ^a	6.16 ^b	2.220 ^b	12.171 ^a	36.11 ^b	31.57 ^b	16.205 ^a

(MACG = *S. macrocarpon* Glabrous cultivar, AETK = *S. aethiopicum* kumba variety, *GILO* = *S. gilo*, MELO = *S. Melongena*) Values with varying alphabetical superscripts indicate significantly different means at $P \leq 0.05$ while the ones with the same alphabets are not of significant difference at $P \leq 0.05$.

Table 3. Mineral element content of the species of *Solanum* plants studied (ppm).

Taxa	Mn	Zn	Cu	Na	K	Mg	Ca	Fe	P
MACG	0.309	0.555	0.488	1.728	27.481	13.222	27.000	0.359	0.327
AETK	0.300	0.544	0.412	1.720	27.300	13.126	27.306	0.354	0.311
<i>GILO</i>	0.306	0.501	0.422	1.701	27.616	13.312	27.011	0.300	0.806
MELO	0.333	0.577	0.481	1.707	27.541	13.288	27.003	0.355	0.327

(MACG = *S. macrocarpon* Glabrous cultivar, AETK = *S. aethiopicum* kumba variety, *GILO* = *S. gilo*, MELO = *S. melongena*).

Antimicrobial activities of methanolic extracts of the four *solanum* species. The antimicrobial potentials of foliar extracts of *Solanum* species as revealed by zones of inhibition in millimeters (mm) on selected bacteria, fungi and yeast are presented in Figures 1 to 4.

In Figure 1, the antifungal activity was seen to be highest at 25 mg/ml above those of *A. niger* and *A. flavus* with a zone of inhibition of 12 mm and 16

the four extracts were not significantly different ($P \leq 0.05$) with respect to each element except for the quantity of Phosphorus in *GILO* that was statistically higher than that of the other extracts.

mm, respectively for *S. melongena*. Moreover, 5 mg/ml concentration resulted into zones of inhibition of 12 mm and 13 mm against *C. albicans* and *S. cerevisiae*, respectively. The antifungal activity of the *S. gilo* extract was the highest at 5 mg/ml against *A. flavus* which produced highest inhibition zone of 15 mm and against *C. albicans* which gave an inhibition zone of 12 mm (Figure 2).

The antifungal ability of the extract was found to be highest at 5 mg/ml against *A. flavus* which produced an inhibition zone of 17 mm for *S. aethiopicum* and also, 5 mg/ml produced highest zone of inhibition of 13 mm for *S. aethiopicum*

against the yeast test organism, *S. cerevisiae* (Figure 3). The antifungal effects of the *S. macrocarpon* extract were the highest at 25 mg/ml against *A. flavus* and *C. albicans* with a zone of inhibition of 13 mm and 12 mm, respectively (Figure 4).

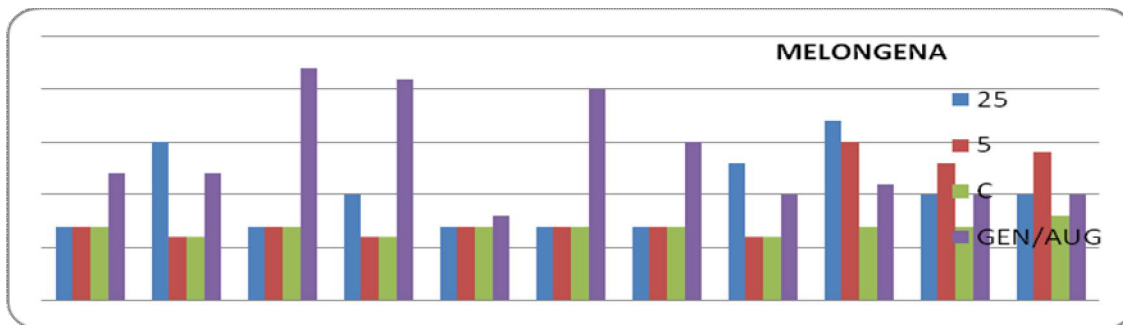


Figure 1. Effects of *Solanum melongena* on the test organisms. (PA= *P. aureginosa*, *P. putida*, *B. cereus*, *K. pneumonia*, *B. subtilis*, *E. coli*, *S. aureus*, *A. niger*, *A. flavus*, *C. albicans*, *S. cerevisiae*. AUG – Augmentin GEN – Gentamicin, C= control)

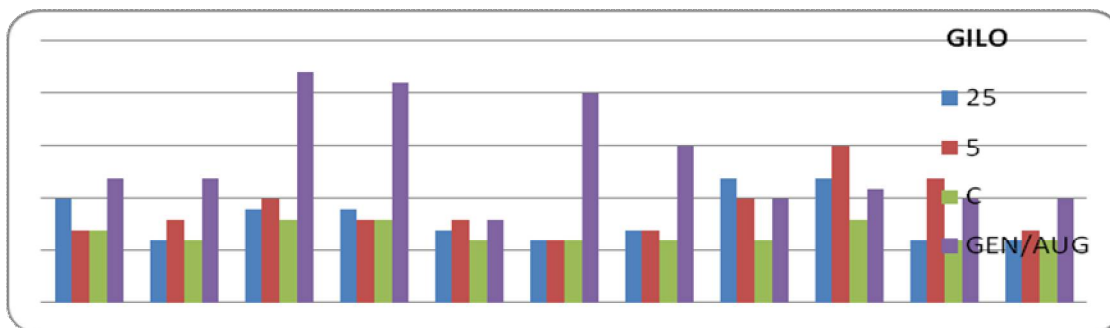


Figure 2. Effects of *Solanum gilo* on the test organisms. (PA= *P. aureginosa*, *P. putida*, *B. cereus*, *K. pneumonia*, *B. subtilis*, *E. coli*, *S. aureus*, *A. niger*, *A. flavus*, *C. albicans*, *S. cerevisiae*. AUG – Augmentin GEN – Gentamicin, C= control)

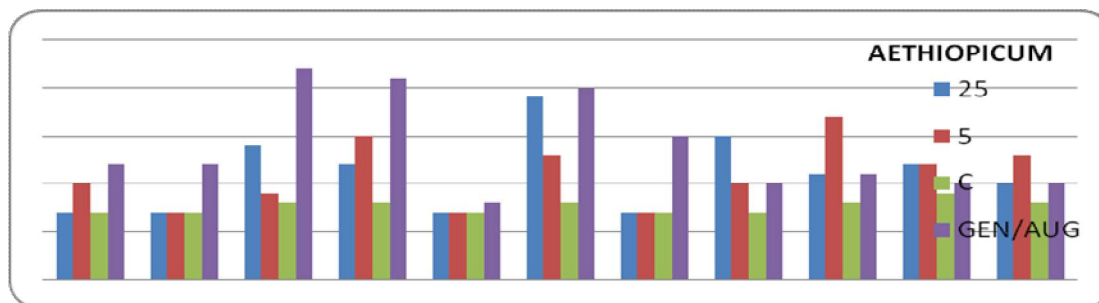


Figure 3. Effects of *Solanum aethiopicum* on the test organisms. (PA= *P. aureginosa*, *P. putida*, *B. cereus*, *K. pneumonia*, *B. subtilis*, *E. coli*, *S. aureus*, *A. niger*, *A. flavus*, *C. albicans*, *S. cerevisiae*. AUG – Augmentin GEN – Gentamicin, C= control)

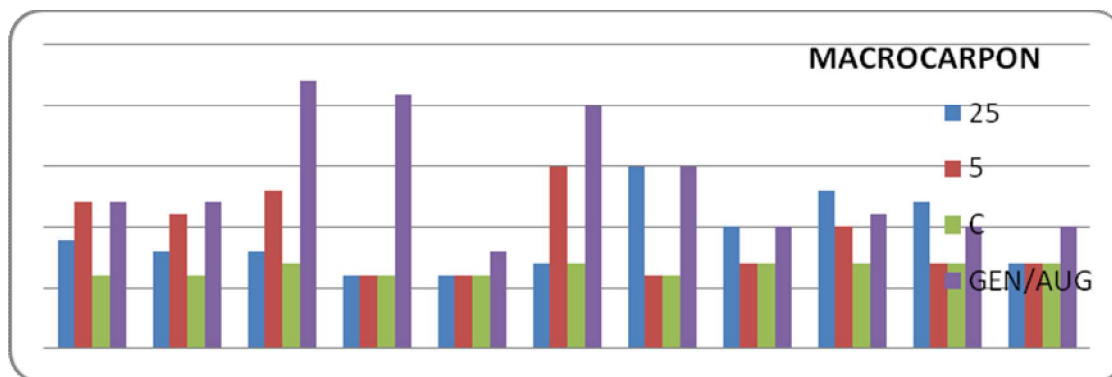


Figure 4. Effects of *Solanum macrocarpon* on the test organisms. (PA= *P. aureginosa*, *P. putida*, *B. cereus*, *K. pneumonia*, *B. subtilis*, *E. coli*, *S. aureus*, *A. niger*, *A. flavus*, *C. albicans*, *S. cerevisiae*. AUG – Augmentin GEN – Gentamicin, C= control)

Table 4. Percentage peak areas of some organic compounds detected in the leaves of some Nigerian species of *Solanum*.

Name of Compound	Functional group	% Peak Area of the Organic Compounds			
		<i>S. macrocarpon</i> (G)	<i>S. aethiopicum</i>	<i>S. gilo</i>	<i>S. melongena</i>
Undecanoic acid, 10-methyl, methyl ester	Ester	1.19	1.73	1.03	4.52
2-pentadecanone, 6, 10, 14-trimethyl	Alkanone	6.83	4.15	6.12	0
Hexadecanoic acid methyl ester	Alkanoic acid	37.66	41.57	40.57	30.7
Methyl 10-trans, 12-cis-octadecadienoate	Alkanoate	0	6.55	6.05	0
10-octadecenoic acid methyl ester	Ester	9.18	10.17	10.17	0
Methyl stearate	Alkanoate	13.8	15.21	13.11	0
Phytol	Alcohol	21.96	18.35	20.11	31.25
Eicosanoic acid methyl ester	Ester	3.01	0	0	0
13-Octadecenoic acid, methyl ester	Ester	0	0	0	24.25

From Table 4, it is observable that undecanoic acid, 10-methyl, methyl ester was present in all the samples, with the highest %peak area (4.52) found in *S. melongena* while the lowest (1.03) was found in *S. gilo*. There was no trace of 2-pentadecanone, 6, 10, 14-trimethyl in *S. melongena* but the highest %peak area (6.83) was recorded in *S. macrocarpon*. Hexadecanoic acid methyl ester among other compounds was found to have the highest occurrence ever with the highest %peak area (41.57) reported in *S. aethiopicum* and the lowest (30.7) reported in *S. melongena*. The presence of Methyl 10-trans, 12-cis-octadecadienoate was found exclusively in *S. aethiopicum* and *S. gilo* and in close levels as well, while phytol was found in all the samples. The highest %peak area (31.25) was recorded in *S. melongena* and the lowest (18.35) was recorded in *S. aethiopicum*. Methyl stearate was absent in *S. melongena*, Eicosanoic acid methyl ester and 13-

Octadecenoic acid, methyl ester were found only in *S. macrocarpon* (3.01) and *S. melongena* (24.25) respectively.

The discovery that plant extracts from medicinal plants contain minerals and a variety of secondary metabolites with therapeutic benefits is a result of the usage of these plants by humans.^{22,23} Plants' chemical composition, both in terms of quality and quantity, determines their medicinal and bioactive characteristics.²⁴ The existence of these phytochemicals determines the medicinal and therapeutic benefits of plant products.²⁵ Several *Solanum* species are renowned for their cytotoxic and antimicrobial phytochemicals, particularly their steroid, alkaloid, and saponin species.²⁶

In this study, the antibacterial efficacies of four different *Solanum* species were examined in relation to the compounds found in their extracts. Many

studies have demonstrated that these plants contain a range of chemicals, including flavonoids, phenolic acids, alkaloids, saponins, and others which are responsible for the treatment of a range of human diseases.²⁷ As a result, the antimicrobial properties of the extracts demonstrated in this study are justified. The finding in Table 1 demonstrates that the phenol in the AETK extract was substantially different ($P < 0.05$) from the other extracts. The extracts with the highest concentrations of phenol were AETK, MACG, MELO, and GILO. In addition to a variety of anti-inflammatory, anticancer, anti-aging, antibacterial, and antiviral actions, plant extracts and phenolic compounds have protective benefits against oxidative stress and inflammation.²⁸

The highest concentration of flavonoids was found in AETK extract, which was followed by MACG, GILO, and lastly MELO extracts. By stopping and chelating radical intermediate molecules, flavonoids are known to exhibit antioxidant properties and diminish free radicals.²⁹ According to Singh *et al.* (2019),³⁰ flavonoids obtained from *S. melongena* showed strong antioxidant action against chromosomal abnormalities brought on by doxorubicin. MACG has the most saponin, with the least amount being found in MELO, GILO, and AETK. Saponins are crucial nutritional supplements and nutraceuticals; they have antibacterial properties that aid in shielding their users against microbial infections.³¹ Because eggplants contain saponins, their use in traditional medicine is justified. Glycosides are sugars that can resemble carbohydrates structurally and are a substrate of α -glucosidase.³² Additionally, greater phenolic and flavonoid content may be the cause of the α -glucosidase inhibitory properties.³³ The mean amount of glycoside in MELO extract was 0.93 ppm, which was substantially greater ($P > 0.05$) than that in other extracts. AETK was second with 0.90 ppm. The least amount of glycoside was found in GILO, with a value of 0.63 ppm.

The results of this study also showed that all of the extracts had crude protein, crude ash, crude CHO, dry matter, crude fat, moisture content, and crude

fibre in the proximate analysis. Each of the four species had a different amount of crude fibre, although there was little difference between them. The high crude fibre content of these fruits may help to avoid atherosclerosis, diverticulitis, colon and rectum cancer as well as constipation.³⁴ The effect of African eggplants on weight loss may also be attributable to the presence of crude fibre in them.³⁴ The low carbohydrate and high fibre content of eggplant fruits is advantageous for the management of type 2 diabetes.³⁵ Total ash content represents a measure of the total amount of minerals present which makes the four *Solanum* species good sources of minerals with *S. gilo* yielding higher level of minerals.

Minerals are essential components for healthy development, muscle function and skeletal growth. Iron is necessary for oxygen transport, copper for cellular activity, magnesium for many biochemical reactions and absorption through the intestines, while sodium and potassium are necessary for fluid balance and nerve transmission. Manganese also plays a significant role in energy production and immune system support.³⁶ Lack of essential nutrients and minerals has an impact on both human and animal performance and health.³⁷ These species are good sources of raw material for the food industry because they contain a good amount of carbohydrates, crude fibre, and crude proteins.^{27,38}

The combined effects of chemicals found through proximate, organic, mineral, and phytochemical investigations of the foliar extracts of *S. melongena*, *S. gilo*, *S. aethiopicum*, and *S. macrocarpon* are likely the cause of the antimicrobial actions of the methanolic extracts of these plants. According to Sofowora (1993),³⁹ plant extracts' antimicrobial activity has been demonstrated to be a function of their phytochemicals, which lends validity to this assertion. The solanine concentration and associated glycoalkaloids in the plant's fruits and leaves are thought to be responsible for its antibacterial capabilities.²⁴ Previous studies have suggested that the chemical compounds discovered

through GC-MS analysis in the *Solanum* samples with references to the NIST library have significant.

The chemical compounds discovered in *Solanum* plants have similarities to those found in the Capsicum plants described by Adepoju *et al.* (2019, 2020)^{40,41} and the *Solanum* sect. *Melongena* reported by Adepoju *et al.* (2021).¹² Hexadecanoic acid has been described as hemolytic, pesticidal, flavoring, antioxidant, antibacterial, and antifungal by Femi-Adepoju *et al.* (2021)⁴ and Mensah-Agyei *et al.* (2020).⁴² Undecanoic acid, 10-methyl, methyl ester was determined to be a promising chemical molecule by Narra *et al.* (2017)⁴³ who conducted research on its antioxidant and anticancer potentials. Moreover, Nazarudin *et al.* (2021)⁴⁴ discovered the antioxidant activity of 2-Pentadecanone 6,10,14-trimethyl. 13-Octadecenoic acid, methyl ester is a striking antimicrobial compound while Phytol on the other hand has diuretic, antitumor, chemo-preventive, anticancer, antioxidant, anti-inflammatory, antimicrobial and vaccine formulation properties. These compounds were found to be present in the *Solanum* plants investigated at varying proportions. Belakhdar *et al.* (2015)⁴⁵ has reported that 10, octadecenoic acid methyl ester is antibacterial, antifungal, antioxidant and has the ability to decrease blood cholesterol.

CONCLUSION

The study concluded that the extracts of four *Solanum* species are rich in nutrients, bioactive compounds and possessed antimicrobial properties. The leaf contained significantly, the highest composition of bioactive compounds and showed significantly, the highest inhibitory activity against the tested pathogens. Thus, this justifies the use of the plants as food and could also be included as ingredients for production of drugs which are formulated for use in the treatment of microbial infections.

DECLARATION

We declare that there is no conflict of interest in this study.

AUTHOR CONTRIBUTION

Adepoju A. Olufemi: Developed the experiments' concept and design, carried them out, provided materials and reagents, and wrote the report.

Femi-Adepoju A. Grace: Edited the article, ran the experiment, and analyzed and interpreted the findings.

Oni S. Oluwasunmibare: Performed data analysis and interpretation, provided resources for data and analysis tools, and produced the report.

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