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Original Article

Antioxidant activity, amino and fatty acid composition of aqueous and methanol extract of *Gardenia ternifolia* fruits

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ABSTRACT

Gardenia ternifolia (Yellow Gardenia) is an evergreen shrub native to tropical and southern Africa. It is a plant traditionally used in herbal medicine. However, it lacks comprehensive scientific data on its antioxidant activity, fatty acid and amino acid profiles of its fruit. This research aimed to evaluate and compare the antioxidant activity of its fruit extracts using DPPH (2,2-diphenyl-1-picrylhydrazyl (DPPH) and Beta-Carotene Bleaching Test (BCBT) assays, and to characterize its fatty acid and amino acid profiles using Gas Chromatography-Mass Spectrometry (GC-MS). Fruit extracts were prepared using aqueous and methanolic solvents. The antioxidant assays revealed that the methanolic extract exhibited higher antioxidant activity than the aqueous extract, with DPPH values of 85.95 $\pm$ 0.15 µg/mL at 500 mg/mL, and BTBC values of 18.93 $\pm$ 0.19 µg/mL at 100 mg/mL, compared to the aqueous extract that showed moderate activity, with DPPH values of 60.98 $\pm$ 0.11 µg/mL at 500 mg/mL and BTBC assay of 26.14 $\pm$ 0.16 µg/mL at 100 mg/mL. GC-MS analysis identified a diverse range of saturated fatty acids (Octanoic acid 4.12%, n-Decanoic acid 8.23%, and Tetradecanoic acid 20.35%) and unsaturated fatty acids (9,12,15-Octadecatrienoic acid 16.24%, 9,12-Octadecadienoic acid 17.14%, and Oleic acid 2.52%). The amino acid profile revealed essential amino acids such as valine (6.73 mg/100g), leucine (6.39 mg/100g), and methionine (5.90 mg/100g), alongside non-essential amino acids including glycine (7.99 mg/100g), aspartic acid (8.85 mg/100g), and arginine (6.99 mg/100g). These results demonstrate that *Gardenia ternifolia* possesses strong antioxidant potential and nutritional value, suggesting its potential as a natural source of bioactive compounds with applications in food, nutraceutical, and pharmaceutical industries.

Keywords: *Gardenia ternifolia*, Antioxidant activity, Fatty acid profile, Amino acid profile, fruit extract.

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INTRODUCTION

Plants, especially fruits are natural antioxidant reservoirs. Antioxidants are a substance that delay or completely prevents oxidation of substrate [1]. Antioxidants are known for the protective effect against oxidative damage, converting free radicals into less harmful substances. Free radicals cause oxidative stress, leading to damage of tissue or even cell death. They are cause of many degenerative diseases like arthritis, cancer, cirrhosis, Alzheimer and cardiovascular diseases [2]. Due to probable toxicity of artificial antioxidant like butylated hydroxyanisole (BHA) and butylated hydrox-toluene (BHT) focus has shifted to natural alternatives from plant with therapeutic value [3].

Gardenia ternifolia Schumach. & *Thonn.* (*Rubiaceae*) is an evergreen flowering plant that grows up to 10 m tall with intertwined short thorny and hard twigs branches. It belongs to the *Rubiaceae* family and is found distributed in Pacific islands, Asia and the Tropics [4]. The leaves are obovate and grayish white in colour. It has whorls leaves of three, 7-11 cm wide. The *Gardenia ternifolia* fruit is a berry, with ellipsoid of 6.5 cm long and grey brown in colour but yellow at maturity [5]. In Nigeria, it is known as langbà by Nupes and gáuɗènkúúráá by the Hausas while the the *fufuldes* called it''*Djinalii gorki''* Popularly known as "Oroto" and "Ulimili" by the Yorubas and Igbos respectively [5,6]. Traditionally, it is used to treat several illnesses, the fruit decoction is used as remedy for malaria and eye treatment complaints. In west Africa, the soot is used as toothbrushes and leafy vegetables [7]. Maroyi (2020) and Jacob *et al.* (2016) identified and quantified numerous nutrients in the fruit pulp of *G. ternifolia* [7,8] The fruit contain health-promoting nutrients such as carbohydrates, proteins, crude fibre, calcium, copper, iron, magnesium, potassium, sodium, zinc and vitamin C. Phytochemicals detected from the aerial parts and roots of *G. ternifolia* include alkaloids, saponins, tannins, coumarins, flavonoids, phenols, anthocyanins, terpenoids, steroids and stereoisomeric neolignans. These bioactive compounds are responsible pharmacological activities of the species [8].

The leave extracts of *G. ternifolia* have been reported to have antioxidants, hepatoprotective and antidiabetic properties [9, 10]. Previous studies have confirmed the antibacterial and antiviral activities of the stem and root barks [11]. Immune boosting potential, aphrodiasiac activity and breast cancer treatment with root bark have also been documented [12,10,13]. Other biological activities such as anti-inflammatory, antileishmanial, antisickling and larvicidal properties have been reported [7]. The fruit of *G. ternifolia* is consumed by the Nupes in North central Nigeria. Despite the plant's widespread use and numerous pharmacological activities, there is a significant gap in the scientific literature regarding the detailed profiles of fruit fatty, amino acids composition and its antioxidant activity.

MATERIALS AND METHODS

Sample Collection

The fruits of yellow gardenia plant were collected from along Sunti Golden Sugar Road before Eppa community Mokwa, Niger State, Nigeria. A region characterized by a tropical savanna climate with distinct wet and dry seasons. Plant samples were collected during the flowering season to ensure optimal levels of bioactive compounds. The specimens were carefully identified and

authenticated by a botanist at Ibrahim Badamasi Babangida University, Lapai. The specimen was deposited at the herbarium of Ibrahim Badamasi Babangida University, Lapai with a voucher number IBBU2105398.

Sample Preparation

The fruits collected were washed, cut into pieces, dried at room temperature (30-40 ^{0}C), then pulverized into a fine powder, the sample was then stored in an airtight container to avoid contamination or spoilage at room temperature.

Reagents

All reagents used were of analytical grade. These include methanol, chlorofrm, sulfuric acid, ammonium hydroxide, hydrochloric acid, ascorbic acid, 2,2-Diphenyl-1-Picrylhydrazyl amino acid standards and fatty acid standards.

Extraction of Lipids

Pulverized sample weighing 50.00 g was added to 500 ml methanol in a flat bottom flask and heated for 4 h at 67 °C on a soxlet apparatus. After the extraction processes, the residue was exposed to the atmosphere and the solvent allowed to evaporate and the extract was then collected for GC–MS analysis [14].

Conversion of Fatty Acids to Derivatives (Methyl Esters)

The fatty acids were transesterification by reacting it with methanol with sulfuric acid as a catalyst. This is due to the fact that fatty acids are not volatile enough for direct GC-MS analysis, and therefore must be converted into fatty acid methyl esters (FAMEs) [14]. The corresponding methyl esters formed are more volatile and vaporized easily during gas chromatography.

Defatting sample

Methanol/ chloroform mixture (1:2) was used to remove the fat component in the sample. 1.0g of the sample was extracted in soxhlet extraction apparatus for 1 hour.

Extraction and Derivatization of Amino Acids

A gram of sand (fine quartz) mixed with 100.0 mg of the defatted sample were thoroughly ground in a thick ceramic dish with 5 ml distilled water (homogenisation). The marc was centrifuge for 5 min and 0.5 ml of the supernatant was slowly passed through a separating funnel filled with wools and eluted with 4M NH_4OH [15].

The esterification of the carboxylic functional group, by treating the sample with 200 ml butanol, HCl 3M, for 1h at 110^{0}C, followed by acetylation of the amine functional group using 100 ml trifluoroacetic anhydride, for 20min at 60^{0}C constitute the derivatization method. The excess solvents left after the derivatization were evaporated by nitrogen gas. 500 ml ethyl acetate was used to dissolve dried derivatives and analyzed by GC–MS [16].

Amino Acid Analysis

A Varian 3800/4000 gas chromatograph / mass spectrometer was used for the GC–MS analysis. The capacity of gas chromatograph on Agilent capillary column for sample injection was 30m x 0.25mm, 0.25µm film thickness using a temperature program from 70^{0}C, 2min, 5^{0}C/min to 110^{0}C, 10^{0}C/min to 290^{0}C, and 16^{0}C/min to 300^{0}C. The flow rate of the carrier gas (nitrogen) was 1mL/min. A TriPlus autosampler was used to inject 1 µL of each sample in a split mode. The following conditions were followed: transfer line temperature 250^{0}C, injector

temperature 200^{0}C; ion source temperature 250^{0}C, splitter: 10:1. 100 mA and 70eV was the emission current and electron energy respectively applied. The internal standard for quantitative determination was 15N-methionine. Verification of detected amino acids were done using amino acid standards, by injecting standard solutions of amino acids, following the above presented extraction and derivatization procedure. The concentration of each amino was determined using a linear regression curve produced using amino acid standards with known concentrations (0–100 µg/mL) and 50 µg/mL of an internal standard [17].

Sample extraction for DPPH and BCBT Assays

100g of the pulverized fruit sample (*Gardenia ternifolia*) were accurately weighed using Nanbei NBT-A200 weighing balance in to 500ml of distilled water and methanol in 1500ml flask respectively. The samples were allowed to stand for 72 hours before filtration. Filtration was done using cheese cloth first and then followed by a funnel and Whatman filter paper. The marc were concentrated on a rotary evaporator at 50°C to obtain a crude extract of both methanolic and aqueous extract of *Gardenia ternifolia*. The crude aqueous and methanolic extracts obtained were stored at 40°c prior to usage.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical Scavenging Assay

2,2-Diphenyl-1-Picrylhydrazyl radical scavenging antioxidant activity of the fruit extract was estimated as described by Baliyan *et al.*, 2022[18]. Briefly, stock solutions (1000 µg/ml) were prepared by dissolving 0.01g of the extracts and ascorbic acid, respectively in 10 mL of methanol. Different concentrations of extracts and ascorbic acid (62.50, 125, 250 and 500 µg/mL) were prepared.. Thereafter, 2 mL of 0.004% DPPH in methanol added to 1 ml. of various concentrations of plant extracts and ascorbic acid, respectively. The absorbance of each test mixture was read against blank at 517 nm on a double beam Shimadzu UV-1800 series spectrophotometer, after an incubation period of 30 minutes at 25°C. The experiment was performed in triplicates. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ Inhibition} = \frac{\text{Ablank-Asample}}{\text{Ablank}} \times 100$$

Beta-Carotene Bleaching Test (BCBT) Assay

The process involves measuring the loss of carotenes characteristic Yellow-Orange color due to oxidation or degradation. Carotene was extracted from the sample using a suitable hexane acetone.

A standard curve was plotted using known concentration of B-carotene. The extracted carotene solution was diluted to a suitable concentration, Hydrogen peroxide (a bleaching agent) was added to the carotene solution and mixed. The absorbance was taken and the mixture was incubated for 30minutes. The absorbance of the solution was measured before and after adding the bleaching agent at 450-460nm on a spectrophotometer [19].

Calculation:

Calculate the percentage of carotene bleaching using the formula.

$$\% \text{ Bleaching} = \frac{\text{IA-FA} \times 100}{\text{IA}}$$

Where:

IA=Initial absorbance, is the absorbance before bleaching. FA= Final absorbance, is

the absorbance after bleaching. The procedure was repeated in a triplicate and average the results was taken [19]

Data Analysis

Data were analyzed using Analysis of variance (ANOVA) of the statistical package Mini tab version 14.0. Results were expressed as mean ± standard error of mean of triplicate determinations.

RESULTS

Fatty Acid Composition

Table 1 presents the fatty acid composition of *G. ternifolia*. The results showed that the predominant fatty acids were Tetradecanoic acid (Myristic acid) (20.35%), 9,12-Octadecadienoic acid (linoleic acid) (17.14%), 9,12,15-Octadecatrienoic acid (Alpha-linolenic acid) (16.24%), n-Hexadecanoic acid (Palmitic acid) (12.07%) and Octadecanoic acid (Stearic acid) (10.81%) respectively. While the least fatty acids were Octanoic acid (Caprylic acid) (4.12%), Palmitoleic acid (2.86%), Dodecanoic acid (Lauric acid) (2.54%), Oleic Acid (2.52%), Eicosanoic acid (Arachidic acid) (1.76%) and 9-Octadecenoic acid (Oleic acid) (1.34%) respectively. Notably, saturated fatty acids (SFAs) detected were Octanoic acid, n-Decanoic acid, Dodecanoic acid, Tetradecanoic acid, n-Hexadecanoic acid and Eicosanoic acid (59.9 %). While unsaturated fatty acids (UFAs) were 9,12,15-Octadecatrienoic acid, 9,12-Octadecadienoic acid, Oleic Acid and Palmitoleic acid (40.1%).

Table 1: Fatty Acid Composition of Yellow Gardenia

Peak	Compound detected	Mol. Formula	Molecular Weight	% Weight composition
1	Octanoic acid	$C_8H_{16}O_2$	144	4.12
2	n-Decanoic acid	$C_{10}H_{20}O_2$	172	8.23
3	Octadecanoic acid	$C_{18}H_{36}O_2$	284	10.81
4	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	16.24
5	Dodecanoic acid	$C_{12}H_{24}O_2$	200	2.54
6	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	20.35
7	Palmitoleic acid	$C_{16}H_{30}O_2$	254	2.86
8	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	1.34
9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.07
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	17.14
11	Oleic Acid	$C_{18}H_{34}O_2$	282	2.52
12	Eicosanoic acid	$C_{20}H_{40}O_2$	312	1.76

Amino Acid Composition

Table 2 presents the amino acid composition of *G. ternifolia*. The gas chromatogram detected 16 peaks for 16 amino acids with different retention time. The primary amino acids in *G. ternifolia* were Aspartic acid (8.85 ± 0.09), Glycine (7.99 ± 0.17), Arginine (6.99 ± 0.17), Serine (6.96 ± 0.18), Proline (6.89 ± 0.93), Valine (6.73 ± 0.50), Leucine (6.39 ± 0.12). While the least dominant were Tryptophan (5.96 ± 0.56), Methionine (5.90 ± 0.17), Histidine (5.34 ± 0.27), Isoleucine (3.87 ± 0.92), Phenylalanine (3.45 ± 0.09), Alanine (3.04 ± 0.34), Threonine (2.96 ± 0.12), Tyrosine (2.62 ± 0.15) and Lysine (1.98 ± 0.12) and respectively. Notably, Essential amino acids (EAAs) detected were Valine, Leucine, Methionine, Isoleucine, Phenylalanine, Tryptophan, Histidine, Threonine and Lysine. While the non-essential amino acids (NEAAs) were

Glycine, Proline, Alanine, Aspartic Acid, Serine, Tyrosine, Arginine.

Table 2: Amino Acid composition of Yellow Gardenia

Peak	Compound detected	Comp (mg/100g)
1	Aspartic acid	8.85 ± 0.09
2	Threonine	2.96 ± 0.12
3	Serine	6.96 ± 0.18
4	Glycine	7.99 ± 0.17
5	Valine	6.73 ± 0.50
6	Phenylalanine	3.45 ± 0.09
7	Proline	6.89 ± 0.93
8	Leucine	6.39 ± 0.12
9	Methionine	5.90 ± 0.17
10	Isoleucine	3.87 ± 0.92
11	Tyrosine	2.62 ± 0.15
12	Tryptophan	5.96 ± 0.56
13	Lysine	1.98 ± 0.12
14	Histidine	5.34 ± 0.27
15	Arginine	6.99 ± 0.17
16	Alanine	3.04 ± 0.34

Beta-Carotene Bleaching Test (BCBT) Results

The BCBT assay results for different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/mL) is presented in Table 3 below. The results showed that the aqueous extract of *G. ternifolia* had values ranging from 26.14±0.16 mg/mL at 100 mg/mL concentration to 47.95±0.04 mg/mL at 12.5 mg/ml concentration indicating the highest inhibition potential. In contrast, the methanolic extract showed moderate inhibition potential, with values ranging from 18.93±0.19 mg/mL at 100 mg/mL to 33.99±0.01mg/mL at 12.5 mg/mL concentration. Ascorbic acid, a standard antioxidant, revealed the least inhibition across all concentrations, with values from 9.98±0.09 mg/mL at 100 mg/mL to 31±0.10 mg/mL at 12.5 mg/mL concentration.

Table 3 Beta-Carotene Bleaching Test (BCBT) Results (mg/mL)

Samples	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL
Aqueous extract	26.14 ± 0.16^{c}	34.03 ± 0.12^{c}	41.02 ± 0.05^{c}	47.95 ± 0.04^{c}
Methanolic extract	18.93 ± 0.19^{b}	24.96 ± 0.13^{b}	32.06 ± 0.16^{b}	33.99 ± 0.01^{b}
Ascorbic acid	9.98 ± 0.09^{a}	17.54 ± 0.30^{a}	24.02 ± 0.18^{a}	31.00 ± 0.10^{a}

Means along the column with different superscript are significantly different ($P < 0.05$)

DPPH Radical Scavenging Assay Results

Table 4 presents the DPPH assay results for various concentrations (500 mg/mL, 250 mg/mL, 125mg/mL and 62.5mg/mL). The DPPH assay results showed that ascorbic acid exhibited the highest radical scavenging activity, with values ranging from 95.96±0.05 mg/mL at 500 mg/ml concentration to 44.98±0.07 mg/mL at 62.5 mg/mL concentration. Similarly, the methanolic extract of *Gardenia ternifolia* showed a higher radical scavenging activity, 85.95±0.15 mg/mL at 500 mg/mL concentration compared to the aqueous extract 60.98±0.11 mg/mL.

Table 4 DPPH Radical Scavenging Assay Results (mg/mL)

Samples	500 mg/mL	250 mg/mL	125 mg/mL	62.5 mg/mL
Aqueous extract	60.98±0.11[a]	45.07±0.19[a]	33.18±0.19[a]	18.23±0.25[a]
Methanolic extract	85.95±0.15[b]	68.00±0.10[b]	51.96±0.25[b]	36.57±0.59[b]
Ascorbic acid	95.96±0.05[c]	79.03±0.14[c]	62.04±0.17[c]	44.98±0.07[c]

Means on the same column with different superscript are significantly different ($P < 0.05$)

DISCUSSION

The *G. ternifolia* fruit contains all essential amino acids with valine (6.73) being the most abundant. These amino acids require muscle repair, immune function and protein synthesis in humans. Lysine, in particular, is important for the synthesis of enzymes, hormones, and antibodies. Similarly, it also contains all non-essential amino acids (except asparagine, glutamic acid, glutamine, and cysteine that were not detected). This can be gotten from the diet; these amino acids are crucial for maintaining the acid-base balance in cells and supporting neurotransmitter function in humans [20, 21]. Amino acids are substrates for protein synthesis; however, nine amino acids cannot be synthesized de novo but must be supplied via dietary intake [22]. Amino acids are indispensable for the structure, function, and regulation of tissues and organs. There are 20 standard amino acids, and their sequence in a protein determines its structure and function. In plants, amino acids are involved in the synthesis of proteins, enzymes, and secondary metabolites, which are vital for plant growth, development, and defense mechanisms [23]. From this study, it can be deduced that *G. ternifolia* contains a broad range of amino acids, both essential and non-essential amino acids which may add to its nutritional value and supports its use in diets. Fatty acids and amino acids are fundamental components in biological systems, serving crucial roles in various metabolic processes.

Fatty acids, which are carboxylic acids with long hydrocarbon chains, are key constituents of lipids, which make up cell membranes and provide energy storage. They are involved in signaling pathways and the synthesis of essential molecules such as hormones. Saturated and unsaturated fatty acids, distinguished by the presence or absence of double bonds, play different roles in maintaining cellular function and organism health [24]. Twelve fatty acids were detected and quantified, with Tetradecanoic acid (20.35%) being the predominant. These fatty acids can generate energy via β–oxidation, act as storage molecules in the form of triacylglycerol, they can also elicit some pharmacological and physiological functions [25]. The value for Tetradecanoic acid (Myristic acid) (20.35%) is similar to Tao et al. (2014) who reported tetradecanoic acid to be 15.36% in supercritical fluid extract of whole fruit oil from *G. jasminoides*.[25] n-Hexadecanoic acid (Palmitic acid) (12.07%) of this study agrees with 13.70% reported for the same fatty acid in *G. jasminoides* fruit oil by Tao et al. (2014)[25], and 16.11-19.16% by Jin et al. (2023)[27]. Oleic acid (2.52%) does not align with the value of 32.93–35.08% reported by Jin et al. (2023). The value reported for Octadecanoic acid (10.81%) is higher than 3.1–4.28%, and 9, 12-Octadecandienoic acid composition of 17.14% is significantly lower than 37.02–43.81% reported by Jin et al. (2023) in *G. jasminoides* fruit oil. These might be due to specie variation or location.

Fatty acids modulate immune cell functions as they alter the constituent of cell membrane phospholipids and membrane fluidity. They are components involve in bioactive synthesis of lipid mediators and signal transcription factors [24,28]. *Gardenia ternifolia* fruit it is also rich in essential fatty acids (linoleic and linolenic acid). Linolenic acid is an omega-3 fatty acid and is a precussor for synthesis of eicosapentaenoic acids (EPA)and docosahexanoic acid (DHA) (ie other omega-3 fatty acids) [29]. Linoleic acid is an omega-6 fatty acid and also a precursor of other omega-6 fatty acids (arachidonic and gamma linolenic acids) biosynthesis [30]. Eicosapentaenoic acids can reduce swelling and pain associated with inflammation while functional brain growth and development in infant and normal brain function maintenance in adult is supported by docosahexanoic acid [29]. The findings in the present study indicate that *G. ternifolia* contains a balanced mix of both saturated fatty acids and unsaturated fatty acids, which are important for various biological functions. Fruits are excellent sources of natural antioxidant like vitamins C and E, flavonoids and carotenoids with protective effect against free radical within the biological system. Similarly, the body have internal antioxidant mechanisms such as glutathione peroxidase, superoxide dismutase, glutathione reductase and catalase [31]. During oxidative stress, oxygen radical like superoxide anion (O_2^-), hydroxyl radical (OH^-) and peroxyl radical (H_2O_2) are generated within the body [31]. These radicals can damage DNA, causing genetic mutations and chromosomal abnormalities. These free radical also oxidize cellular thiols and remove hydrogen from unsaturated fatty acids, initiating lipid peroxidation of cell membrane. ROS are generated during normal cellular oxidation in the mitochondria and diseased state. Reactive species such as superoxide anion (O^-_2) and nitric oxide (NO^-) are produce as part of a defense mechanism during invasion of foreign substance. Phargocytes such as monocytes, macrophages or neutrophile synthesis large amount of O_2 and NO against foreign substance [32].

Antioxidants prevent oxidation by scavenging free radicals, which are unstable molecules with unpaired electrons that can harm cells. Free radicals are classified into two; Reactive oxygen species (ROS) and Reactive nitrogen species (RNS), which both causes oxidative stress when their concentrations are above the body's capacity to control them [33]. Natural alternatives mitigate free radical induced damage more safely and effectively compared to the synthetic ones [34]. Beta-Carotene Bleaching Test measures the ability of the extracts to inhibit the oxidative degradation of beta-carotene, the findings in the present study revealed that the aqueous extract of *G. ternifolia* had the highest inhibition potential 47.95$\pm$0.04 mg/mL at 12.5 mg/ml concentration. This indicates that the aqueous extract has a strong capacity to prevent oxidative damage, particularly at lower concentrations. The trend in the test samples and standard showed that, as the concentration was decreasing the inhibition potential was increasing. The methanolic extract showed a lower activity with value of 33.99$\pm$0.01mg/mL at 12.5 mg/mL concentration. The lower activity in the methanolic extract compared to the aqueous extract may indicate that the water-soluble antioxidant components in *G. ternifolia* are more effective at protecting beta-carotene from oxidation. Methanol extracts have been

reported to have greater antioxidant activity compared to ethanol extract of the same plant [1]. These means that methanol, which is more polar compared to ethanol is able to extract more polar compounds from the fruit. Although ascorbic acid is a known potent antioxidant, the higher activity observed in *G. ternifolia* extracts indicate that the plant contains a complex mixture of antioxidant compounds that work synergistically to enhance their protective effects against oxidative degradation. *G. ternifolia* is recognized for its diverse range of antioxidant compounds, flavonoids, phenolic compounds, quinones, tannins, and terpenoids which contribute to its therapeutic potential with each playing a role in neutralizing free radicals and reducing oxidative stress [8].

The DPPH assay measures the ability of the extracts to donate hydrogen atoms or electrons to neutralize DPPH free radicals. The result showed that ascorbic acid had the highest free radical scavenging activity. The trend in the two samples showed a dose-dependent increase in radical scavenging activity. Methanolic extract of *G. ternifolia* (85.95±0.15 mg/mL) had a higher radical scavenging activity than the aqueous extract. This indicate that methanol may be more effective in extracting compounds that are capable of donating hydrogen atoms or electrons to neutralize free radicals generated from oxidative stress and agrees with Saravanakuma et al. (2021) [35] and Sayd et al. (2010) who also found higher radical scavenging activity in methanolic extract of *G. jasminoides* than the aqueous extract in a similar manner [36]. The lower DPPH radical scavenging activity observed in the aqueous extract indicate that while it is effective in the BCBT, it may have a more selective range of antioxidant activity compared to the methanolic extract. This difference could be due to the nature of the antioxidant compounds extracted by each solvent, with methanol potentially extracting more phenolic and polyphenolic compounds known for their strong DPPH scavenging ability. Several studies substantiate the positive correlation of quality of phenolics compounds with the DPPH free radical scavenging effect [37,38 and 39]. *Gardenia ternifolia* contain bioactive compounds capable of donating hydrogen atom/proton to free radicals and thereby neutralizing the unpaired electrons responsible for the activity.

This antioxidant activity is in tendem with the report of Onyekachukwu *et al.*, (2023), that leaf extract of *G. ternifolia* ameliorated the effect of dichlovos (sniper) toxicity, restored the antioxidant and liver enzyme levels [9]. The aqueous and methanolic extracts of *G. ternifolia* have all been established as a reservoir of natural antioxidants and could mitigate negative impact of free radicals in the biological system ranging from damage to protein, lipids, DNA or nucleic acids and loss of enzyme activity [40]. Thus, these antioxidants could delay lipid peroxidation, thereby prevent the deterioration of the fruit and extend its shelf life and that of its products.

CONCLUSION

Gardenia ternifolia have health and nutritional value, neutralizing free radical. The fruit of *G. ternifolia* contains essential and non-essential amino acids in abundance, particularly valine. Linoleic and linolenic acids and needed for growth and development, could be harnessed from this fruit. *G.ternifolia* fruit can donate proton to DPPH in a concentration

dependent, thereby reducing the risk of free radical damage The fruit have antioxidant capacity and can serve as alternative source of natural bioactive compounds with potential application in nutraceutical, diet, pharmaceutical and functional food industries.

Authors Contribution

SSR conceptualized the study. SSR, AOA and BUM designed the study. SSR, AOA, BUM, NM, MR and AAH participated in fieldwork and data collection. SSR, AOA and BUM performed the data analysis; SSR, AOA and BUM interpreted the data. NM, MR, AAH prepared the first draft of the manuscript, reviewed by SSR, AOA and BUM. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

None

Ethics Approval and Informed Consent

This study did not use human or animal subjects. Therefore, ethical consideration was not applicable.

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