INTERNATIONAL JOURNAL OF RESEARCH AND TECHNOPRENEURIAL INNOVATIONS (IJRTI)

Volume 1 Issue 1 (April 2024)

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Phytochemical Constituents, Antioxidant Activities and GC-MS Analysis of the Methanol Fruit Extract of *Thespian Garckeana* (Malvaceae)

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To cite this article:
Ugorji, C.O., Okoye, C.J., Onyesife, C.O., Berebon, D.P., Ugwu, G.N., Okonkwo, V.I., Orjiocha, S.I. Phytochemical Constituents, Antioxidant Activities and GC-MS Analysis of the Methanol Fruit Extract of *Thespian Garckeana* (Malvaceae). *International Journal of Research and Technopreneurial Innovations* 2024; 1(1): 21-35

Keywords:
Phytochemical, Thespian garckeana, Antioxidant models, GC-MS Analysis.

ABSTRACT

Africa is home to the tropical fruit plant known as *Thespian garckeana*. Its seeds are thrown, but its edible fruit is used as food or as herbal remedy. One kilogram of the powdered material was utilized, and a Soxhlet extraction method was performed to obtain the plant material's methanol crude extract. Alkaloid was not detected in the methanol extract of *T. garckeana,* despite screening for the presence of secondary metabolites in varied amounts. Folin-Ciocalteu, Aluminum Chloride colorimetric tests, and Folin's Phenol reagent were used to determine the total phenolics, total flavonoids, and total tannin content (TPC, TFC, and TTC). For total phenolics, total flavonoids, and total tannins, the internal standards were gallic acid, quercetin, and tannic acid. TPC was found at the highest concentration in the methanol extract of *T. garckeana* (54.00 ± 0.02 (mgGAE/g)), followed by TTC (24.67 ± 0.01 (mgTAE/g)) and TFC (11.99 ± 0.01 (mgQE/g)) in the quantitative study.The fruit (*T. garckeana*) demonstrated good antioxidant activity because the percentage inhibition increased as the concentration increased. The results of the three antioxidant models were reported as follows: (78.50 ± 0.01) for DPPH scavenging activity, (100.56 ± 0.03) for total antioxidant capacity, and (82.68 ± 0.01) for nitric oxide. The Gas Chromatography-Mass Spectrometry (GC-MS) was done and fifteen peaks were detected from the GC-MS spectra, corresponding to fifteen bioactive chemicals and the majority of the chemicals that were found were esters and carboxylic acids. According to this study, *T. garckeana* fruit possesses a substantial abundance of phenolic compounds and strong antioxidant properties *in vitro*. The fruit may potentially be used as a source of traditional medicine because the bioactive chemicals have key biological properties.

1. INTRODUCTION

Antioxidants are substances that eliminate or fix damaged molecules and function as a defensive mechanism against oxidative damage. It can stop or slow down the oxidation brought on by free radicals, and eating enough antioxidants is thought to guard against illness [1]. In addition to being created by the body naturally after a stress or breathing event, free radicals have also been linked to radiation, bacterial and viral toxins, alcohol, smoking, and psychological or emotional stress. Numerous antioxidant enzymes produced by the body, including glutathione peroxidase, catalase, and superoxide dismutase, neutralize a variety of free radicals [2]. As a defensive strategy, antioxidants guard against numerous diseases in humans and are influenced by oxidative stress, either as a cause or an outcome. It has been established that the unchecked process of lipid peroxidation in cellular and subcellular membranes can induce or worsen pathogenic occurrences in degenerative illnesses such as osteoarthritis, cancer, atherosclerosis, and cataracts [3]. Research indicates that consuming a diet rich in antioxidants can have a positive impact on both the core intrinsic ageing process and numerous secondary age-related disease disorders [4]. According to [5], plants are the source of medication used for protective, therapeutic, preventive, or promotional purposes. A rich fruit and vegetable diet has been linked to a lower risk of degenerative diseases such as cancer and atherosclerosis. Additionally, dietary flavonoids have been shown to be able to repair a variety of oxidative radical damage that has been sustained by DNA [6]. For their basic needs such as the creation of food, shelter, clothes, transportation, fertilizers, flavors, scents, and medicines; humans have depended on nature throughout history [7]. Given that a variety of chemical components found in medicinal herbs are thought to interact synergistically, it is hypothesized that extracts from whole plants may be able to help alleviate some of the issues related to aging processes that are linked to damage caused by free radicals. Osteoarthritis and other chronic age-related illnesses have long been treated using herbal medicines. Interestingly, though, not much research has been done on how their antioxidant activity contributes to the treatment or prevention of these illnesses. The food industry has conducted the majority of the research on antioxidants related to dietary consumption. To reduce oxidative damage to cells, discovering antioxidants from natural sources is currently of great interest [8]. The widespread abuse of antibiotics has increased the prevalence of antibacterial resistance. Overuse of current medications has led to the development of drug resistance in pathogenic viruses, bacteria, fungi, and protozoa [9]. The need for novel antioxidant and antibacterial compounds never stops in order to combat the disadvantages because they have antioxidant qualities, medicinal plants' numerous active metabolites, such as tannins, flavonoids, and alkaloids which can be used to treat a variety of illnesses [10]. The study's objectives are to assess the phytochemical components, antioxidant activity, and GC-MS analysis of *Thespian garckeana's* (Malvaceae) methanol extract. We will learn more about the type and amount of phytochemicals found in *Thespian garckeana* fruits which might be extremely helpful in the development of novel medications that can effectively treat illnesses and enhance overall health.

Shoot Leaves and fruits Mature Fruit

Figure 1: Different parts of *Thespian garckeana.*

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Thespian garckeana fruit was collected in January 2023 from Tula in Kaltungo LGA, Gombe State and was identified and authenticated by Mr Felix Nwafor, a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The voucher number is UNH/05/0326A.

2.2 Chemicals and Reagents

Methanol, *n*-butanol, ethyl acetate, n-hexane, sulphuric acid, phosphate buffer (pH 7.4), chloroform, vanillin solution of 8 % (w/v), Na_2CO_3 7 %, gallic acid, distilled water, sodium nitrite (5 %) solution, aluminium chloride (10 %) solution, sodium hydroxide (1 M) solution, Folin's phenol reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, 10 M sodium nitroprusside, sulfanilic acid reagent (33% in 20 % glacial acetic acid), naphthylethylenediamine dihydrochloride (0.1% w/v), $K_3Fe(CN)_6$ (1% w/v), trichloro acetic acid (10 % w/v), $FeCl_3$ (0.1 %, w/v), 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate.

2.3 Equipment Used

Analytical weighing balance, beaker, test tubes, funnel, filter paper (whatman no.4), measuring cylinder, separating funnel, stopwatch, slides, crucible, oven, UV/Visible spectrophotometer Model 721G from Yoke Instruments Co., China, stirrer (glass rods), Soxhlet extractor and condenser, clamps and retort stand, round bottom flask, thimble, rotary evaporator, conical flask, test tube holders, measuring cylinder, cotton wool, centrifuge, spatula, electronic weighing balance, micropipette, bunsen burner, water bath, GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan).

2.4 Extraction of the Phytochemicals

Following thorough cleaning and air drying, the fruit was ground into a fine powder using a milling machine and placed in an airtight container for storage. The apparatus was assembled, and the thimble was filled with the powdered substance. The round-bottom flask that was connected to the condenser and Soxhlet extractor was filled with methanol. Subsequently, the solvent was heated to a boiling point of 64.7˚C using an isomantle. This allowed the solvent to evaporate as it passed through the apparatus and was collected by the condenser unit, which contained the evaporated methanol. One kilogram of the powdered material was utilized, and a Soxhlet extraction method was performed to obtain the plant material's methanol crude extract.

2.5 Qualitative phytochemical analysis of the methanol extract of Thespian garckeana

Qualitative phytochemical analysis of the methanol extract was done to determine the presence of phenols, tannins, terpenoids, alkaloids, steroids, saponins, flavonoids, glycosides and reducing sugar using standard methods described by [11] with slight modifications.

2.6 Quantitative Phytochemical Analysis of the methanol Extract Thespian garckeana

Using established procedures, a quantitative phytochemical analysis of the extract was conducted to determine the amount and distribution of the extract's total phenolics content (TPC), total flavonoids content (TFC), and total tannin content (TTC).

2.7 Determination of Total Phenolic Content (TPC)

To 1 ml of the liquid extract and 9 ml of distilled water, 1 ml of Folin-Ciocalteu reagent was added and shaken. The tube was incubated at room temperature for 5 minutes. To this was added 10 ml of 7 % Na2CO3 solution and the volume was made 25 ml with distilled water. Gallic acid standard solution was prepared in different concentrations

(20, 40, 60, 80, 100 and 200 ug/ml) and treated as the samples. These were incubated for 90 min at 30 ºC. The absorbance was read at 550 nm with UV-Vis spectrophotometer. The total phenolic concentration was read from the gallic acid standard curve and expressed as mg gallic acid equivalents (mgGAE)/g of extract.

2.8 Determination of Total Flavonoids Content (TFC)

The total flavonoids content was estimated using the aluminium chloride method. 1.0 ml of plant extract was diluted with 200 µl of distilled water followed by the addition of 150 µl of sodium nitrite (5 %) solution. This mixture was incubated for 5 min and then 150 µl of aluminium chloride (10 %) solution was added and allowed to stand for 6 min. Then 2 ml of sodium hydroxide (1 M) solution was added and made up to 5 ml with distilled water. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as quercetin equivalent (mg QE/g) of the extract from the standard curve.

2.9 Determination of Total Tannins Content (TTC)

Exactly 0.1 ml of the plant extract or standard was mixed with 0.5 ml Folin's phenol reagent and 7.5 ml of distilled water. Then 1 ml of 35 % sodium carbonate was added to the mixture and made up to 10 ml with distilled water. The mixture was shaken and allowed to stand for 30 minutes at 30 ºC. The blue colour produced was read at 640 nm using UV/visible spectrophotometer. The tannin content was calculated by calibration curve of tannic acid and the results were expressed as tannic acid equivalent (mg TAE/g) of the extract.

2.10 In vitro antioxidant assay of the methanol extract of Thespian garckeana

The use of a single method for determining in vitro antioxidant activity is not satisfactory because no one method can actually indicate with measurements the complexity of the system [10]. Therefore, no one approach is adequate and to account for the various ways that antioxidants work, multiple types of antioxidant capacity measurements must be carried out [12]. In this study, the approach was to apply a battery of tests - DPPH method, Phosphomolybdate method, Reducing Power, Nitric Oxide radical scavenging activity to compare the antioxidant capacity of the methanol extract of Thespian garckeana

2.11 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical Scavenging assay

The DPPH radical scavenging assay was performed using 1,1 diphenyl-2-picrylhydrazyl according to the method described by [13] with some modifications. Briefly, five different concentrations of the studied plant extract (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were prepared in methanol. The same concentrations were also prepared for ascorbic acid, which was used as a standard antioxidant. 1 ml of each studied extract was transferred into a clean test tube into which 0.5 ml of 0.3 mM DPPH in methanol was added. After giving the mixture a good shake, it was let lie at room temperature for fifteen minutes in the dark. As a baseline, blank solutions made up of 1 ml of methanol and 2.5 ml of the extract solution under study were utilized. Ascorbic acid was employed as the positive control at the same quantities as the extract under study, while 2.5 ml of DPPH solution and 1 ml of methanol made up the negative control. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. The experiments were performed in triplicate. The inhibition percentage (%) of radical scavenging activity was calculated according to the following in equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

Ascorbic acid was used as the standard and the curve of percent inhibition or scavenging effect against sample concentrations was plotted and the concentration of the sample required for 50 % inhibition (IC50) was determined.

2.12 Nitric Oxide Radical Scavenging Activity

Nitric oxide radical scavenging activity was determined by phosphomolybdate method using ascorbic acid as a standard as described by [14]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. 2 ml of 10 M sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of methanol extract at various concentrations 20, 40, 60, 80, 100 µg/ml and the mixture was incubated at 25 oC for 2hrs. From the incubated mixture 0.5 ml was taken out and added into 1ml sulfanilic acid reagent (33 % in 20 % glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1ml of naphthyl ethylenediamine dihydrochloride (0.1 % w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. Inhibition of nitrite formation by the plant extract and the standard antioxidant ascorbic acid were calculated relative to the control. The percentage inhibition and the IC50 of the extract required to reduce 50 % of the nitric oxide formation was determined. The nitric oxide radicals scavenging activity was calculated using the equation

% Inhibition of nitric oxide activity =

$$\frac{(A_0 - A_1)}{A_0} \text{ X } 100$$

where A_0 is the absorbance value of the blank sample or control reaction and A_1 is the absorbance value of the test sample. Ascorbic acid was used as standard.

2.13 The Total Antioxidant Capacity

The total antioxidant capacity of the methanol extract was determined by phosphomolybdate method using ascorbic acid as a standard as described by [14]. The stock solution (1 mg/ml) of plant extract was diluted to lower concentrations 20, 40, 60, 80, 100 µg/ml. An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). After being sealed, sample tubes were incubated for 90 minutes at 95 °C in a water bath. The absorbance of the mixture was measured at 695 nm against a blank on a UV-visible spectrophotometer once the sample had cooled to room temperature. A standard blank was incubated under the same conditions and contained 1 ml of the reagent solution and the appropriate volume of solvent. The antioxidant capacity of plant extract solution was estimated using the equation below:

$$\text{Total Antioxidant Capacity (\%)} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \text{ x } \frac{100}{1}$$

2.14 Gas Chromatography-Mass Spectrometry Analysis

The Gas chromatography-Mass spectrometry (GC-MS) analysis of methanol extracts of Thespian garckeana was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The column oven temperature was programmed from 50 °C to 300 °C for 2 °C min-1. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The temperature of the injector was fixed to 240 °C and one of the detectors to 200 °C. Helium (99.9995 % purity) was the carrier gas fixed with a flow rate of 1.5 mL min-1. At a rate of 3.0 scans/second, the mass range of 40-1000 m/z was examined. Using a Hamilton syringe, 1.0 µL of Thespian garckeana

methanol extract was manually injected into the GC-MS for total ion chromatographic analysis using split injection technique. The GC-MS runs for thirty-five minutes in total. The percentage with peak area normalization was used to express the relative percentage of each extract ingredient

2.15 Identification of Compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Research Institute of Chemical Technology (NARICT) having more patterns. By comparing the average peak area of each component to the total areas, the relative percentage amount was determined. The known component's spectra, which was kept in the NARICT data library, was compared to the spectrum of the unknown component. The test material's component names, structures, molecular weights, and molecular formulas were ascertained.

3. RESULTS AND DISCUSSION

3.1 Percentage yield

The percentage yield of the methanol extract of *T. garckeana* was expressed as percentage of the starting material (powdered crude drug). The yield (%) of methanol extract was calculated to be 40.09 % using the relation:

Yield (%) = (Weight of extract (g) ÷ Weight of powdered crude drug (g)) ×100

= (400.85 ÷ 1000) × 100

= 40.09 %

3.2 Phytochemical Analysis

3.2.1 Qualitative Phytochemical Analysis of the Methanol extract of Thespian garckeana

The results of the qualitative phytochemical analysis of *Thespian garckeana* as shown in (Table 1) shows the presence or absence of the secondary metabolites and its corresponding relative abundance.

Table 1: Qualitative Phytochemical analysis of the extract of *Thespian garckeana* fruit

Phytochemical Tests	Results
Alkaloid	Nd
Phenols	+
Tannin	+
Glycosides	+
Saponins	+
Terpenoids	+
Steroids	+
Flavonoids	+
Reducing Sugar	+

KEY: + = Present, Nd = Not detected

3.2.2 Quantitative analysis for Total Phenolics Content (TPC), Total Flavonoids Content (TFC) and Total Tannin Content (TTC) in crude of *Thespian garckeana* fruit extract.

Table 2: Quantitative Phytochemical analysis of the extract of *Thespian garckeana* fruit

Phytochemicals	Methanol extract (mgGAE/g)
TPC	54.00 ± 0.02
TFC	11.99 ± 0.01
TTC	24.67 ± 0.01

The total phenolics, total flavonoids and total tannin contents shown in Table 2, were determined using Folin-Ciocalteu reagent expressed in milligram Gallic acid equivalent with a standard curve ($Y = 0.002x + 0.031$; $R^2 = 0.973$). Aluminum chloride colorimetric assay expressed in milligram quercetin acid equivalent with a standard curve ($Y = 0.003x + 0.016$; $R^2 = 0.998$). Folins's Phenol reagent expressed in milligram Tannic acid equivalent with a standard curve ($Y = 0.0037x + 0.0464$; $R^2 = 0.982$). The quantitative analysis of *Thespian garckeana* showed a Total Phenolic Content of 54.00 mgGAE/g, Total Tannin Content of 24.67 mgTAE/g and Total Flavonoid Content of 1.19 mgQE/g.

3.3 *In Vitro Antioxidant Activity*

In vitro antioxidant activity of *Thespian garckeana* fruit extract were determined using

Reducing power assay, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, Total antioxidant capacity and Nitric oxide (NO) scavenging activity and the results were obtained and showed in the figures below.

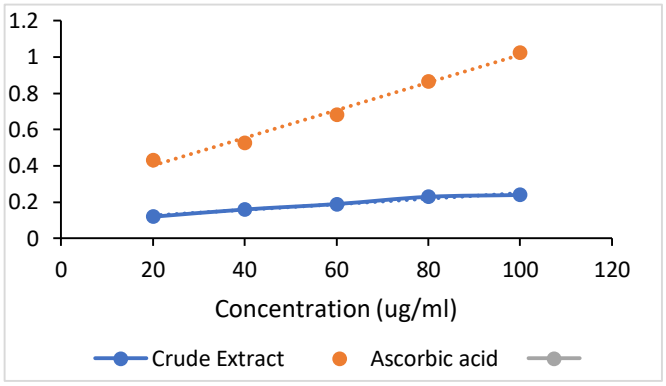

Figure 2: Reducing power activity of *Thespian garckeana* fruit extract plotted against ascorbic acid (standard).

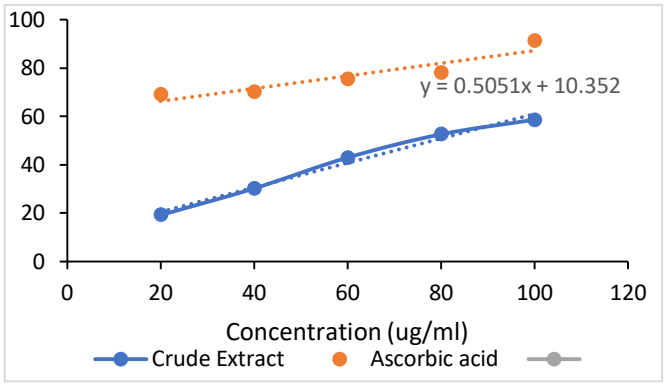

Figure 3: DPPH scavenging activity of *Thespian garckeana* fruit extract against ascorbic acid

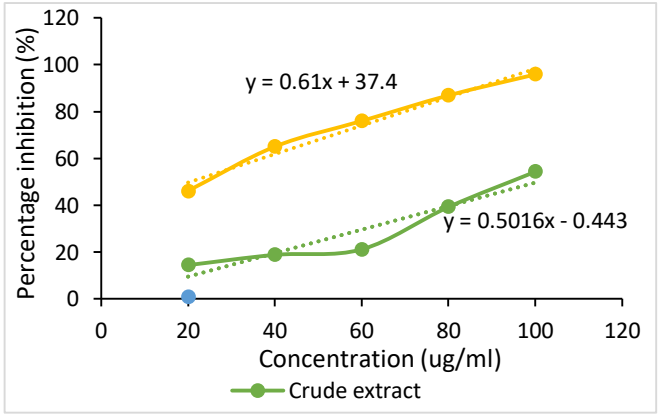

Figure 4: Total Antioxidant Capacity of *Thespian garckeana* fruit extract against ascorbic acid

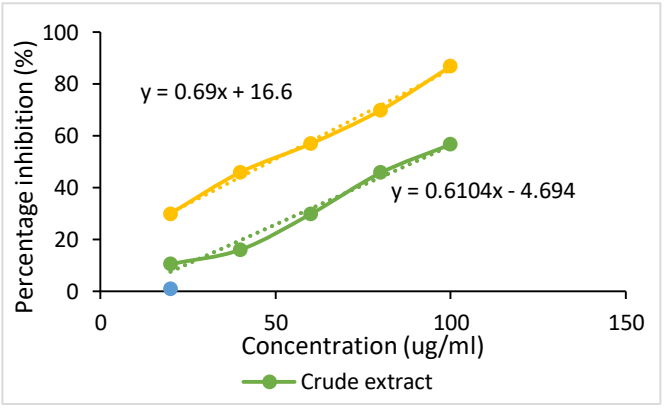

Figure 5: Nitric oxide scavenging activity of *Thespian garckeana* fruit extract against ascorbic acid

Table 3: IC50 Values for methanol crude extract of the fruit extract of Thespian garckeana and the standard (Ascorbic acid) for Antioxidant Models.

Antioxidant Models	IC50 (µg/ml)	
	Methanol extract	Ascorbic acid
DPPH	78.50	19.63
Total antioxidant capacity	100.56	20.66
Nitric oxide scavenging assay	82.68	48.41

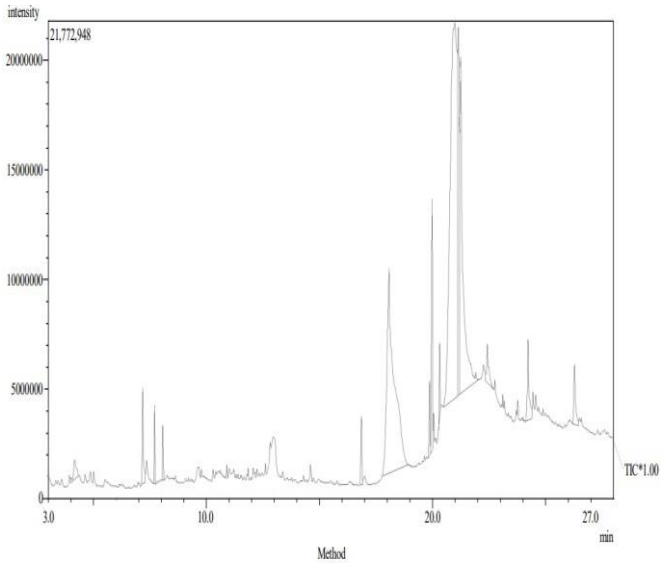

Figure 6: GC-MS spectrum of methanolic fruit extract of Thespian garckea

Table 4: GC-MS Identified Components of the methanol extract of *Thespian garckeana*

Peak	RT (min)	Area (%)	Name of Compound	Class of Compound	Reported Activity
1	4.136	0.83	Propanoic acid, 2-methyl-, butyl ester	Esters	Activities not tested yet
2	7.157	1.48	Ethanol, 2-(2-methoxyethoxy)	Ethers	Anti-icing agent in military jet fuel [15].
3	7.684	0.74	Butane, 1,1-dibutoxy	Ethers	Antibacterial properties and have potent antagonist activity against *Vibro anguillarum* [16].
4	8.055	0.59	Ethanol, 2-[2-(2-methoxyethoxy) ethoxy]	Polyethers	Activities not tested yet
5	16.859	1.10	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid methyl esters	Antifungal, Antimicrobial [17].
6	18.083	21.85	*n*-Hexadecanoic acid	Carboxylic acids	Antioxidant, antiinflammatory properties, antitumor [17].
7	19.882	0.94	9,12-Octadecanoic acid, methyl ester (Z,Z)	Fatty acid methyl esters	Anticancer [18].
8	19.992	3.60	11-Octadecanoic acid, methyl ester	Fatty acid methyl esters	Antibacterial, antifungal and antioxidant [19].
9	20.33	0.89	Octadecanoic acid, methyl ester	Fatty acid methyl esters	White crystal semisolid ester, flavour component in food, lubricant, used in the manufacture of pharmaceuticals, cosmetics, soaps, surfactant and softening agents [20].
10	21.00	40.3	Cyclopropaneoctanal, 2- octyl	Aldehydes	Activity not tested yet
11	21.16	7.92	12-Octadecadienoic acid-(Z,Z)	Fatty acids	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge [21].
12	21.26	15.12	Oleic acid	Fatty acids	Antitumor, Antiinflammatory [22].
13	22.444	1.08	Decane, 1-fluoro	Haloalkanes	Activities not tested yet
14	24.25	1.97	9-Tetradecanal, (Z)	Aldehyde	Activities not tested yet
15	26.31	1.53	9-Octadecanoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Fatty acid ethanolamides	Inhibit the proliferative effect in keloid fibroblasts [23].

3.5 DISCUSSIONS

The antioxidant potential of medicinal plants has been attributed to the redox effect of phenolic compounds which scavenge single oxygen, donate proton and act as reducing agents [24]. According to [25], Thespian garckeana offer promise for use in the creation of novel culinary and beverage products. It has also been reportedly used in

traditional medicine for treatments of management of more than 20 human diseases and ailments. The plant is used as herbal remedy for diseases like cough, chest pains, infertility, menstruation abnormalities, sexually transmitted infections and hepatic impairments [26,27]. A qualitative phytochemical analysis was conducted using a UV-visible spectrophotometer to evaluate the medicinal potential of the methanol extracts of Thespian garckeana fruit. Flavonoids, glycosides, saponins, tannins, terpenoids, reducing sugar, phenols, and steroids were found in Thespian garckeana, according to the results of the phytochemical screening; alkaloids were not found, as indicated in (Table 1). Plant secondary metabolites are valuable as therapeutic plants since they are known to have a variety of bioactivities. However, flavonoids are recognized for their anti-inflammatory, hypolipidemic, antiviral, antibacterial, tumor-inhibiting, vasoprotective, anti-thrombotic, and antioxidant properties [28]. According to [29], plants that contain saponin may have benefits for the immune system, inflammation, cholesterol reduction, cancer prevention, and diarrhea prevention. Among other things, tannins have anti-microbial, anti-inflammatory, and anti-diarrheal qualities [30]. The broad range of applications for these medicinal herbs may be attributed to the previously noted characteristics of the secondary metabolites present in the Thespian garckeana extract. The methanol extract was found to have a total phenolic content of [54.00 ± 0.02 (mgGAE/g)], a total tannin content of [24.67 ± 0.01 (mgTAE/g)], and a total flavonoid content of [11.99 ± 0.01 (mgQE/g)] as indicated in (Table 2). The antioxidant activity of T. garckeana fruit methanol extract was ascertained using three models; the DPPH, Total Antioxidant Capacity, and Nitric Oxide scavenging activity. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay is predicated on the capacity of this stable free radical to decolorize when antioxidants are present. The process is predicated on the reduction of methanolic DPPH-solution in the presence of an antioxidant that donates hydrogen, as a result of the reaction that forms non-radical form DPPH-H [31]. The extract was able to absorb the diphenyl picryl hydrazyl, which has a yellow color, and the DPPH radical, which is evident as a deep purple color. According to [32], the reaction between the antioxidant molecules and the radical causes the radical to be scavenged by hydrogen donation, which reduces the absorption of DPPH. Consequently, it was believed that the plant extract's capacity to donate hydrogen was the reason behind Thespian garckeana extract's action on DPPH scavenging. According to [29], a higher absorbance corresponds to a stronger capacity for decreasing power. Based on the findings, this higher absorbance corresponds to reducing power. The amount that the antioxidant reduces Mo (VI) to Mo (V) is measured in the Total Antioxidant Capacity model. It is believed that an extract's antioxidant activity increases with a lower IC_{50} value, and that an antioxidant's potency increases with its anti-radical strength [9]. The fruit had a good inhibitory action with the antioxidant models, as demonstrated by the IC_{50} values of 78.50 ug/ml, 100.56 ug/ml, and 82.68 ug/ml for DPPH, Total antioxidant activity, and Nitric Oxide scavenging activity, respectively.

The result of the Gas chromatography and Mass Spectroscopy analysis of the methanol crude extract of Thespian garckeana fruit revealed several compounds with varying biological activities. The analysis indicated the presence of 15 compounds from the methanolic fruit extract of Thespian garckeana. The GC-MS chromatogram of the fifteen peaks of the compounds detected is shown in Fig 6. The identification of phytochemical compounds based on the peak area, retention time, chemical structure, molecular formula and reported activities were shown in (Table 4 and 5). In the identification study of the metabolites of the Thespian garckeana methanol fruit extract, it was found that the compounds obtained were Propanoic acid, 2-methyl-, butyl ester, Ethanol, 2-(2-

methoxyethoxy), Butane, 1,1-dibutoxy, Ethanol, 2-[2-(2-methoxyethoxy)ethoxy], Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid, 9,12-Octadecanoic acid, methyl ester (Z,Z), 11-Octadecanoic acid, methyl ester, Octadecanoic acid, methyl ester, Cyclopropaneoctanal, 2- octyl, 9,12Octadecadienoic acid-(Z,Z), Oleic acid, Decane, 1-fluoro, 9-Tetradecanal, (Z) and 9-Octadecanoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester. The result showed maximum percentage of Cyclopropaneoctanal, 2-octyl (40.36 %), followed by n-Hexadecanoic acid also known as palmitic acid (21.85 %), Oleic acid (15.12 %), 9,12-Octadecadienoic acid-(Z,Z) (7.92 %), 11-Octadecanoic acid, methyl ester (3.60 %), 9-Tetradecanal, (Z) (1.97 %), 9-Octadecanoic acid (Z)-, 2-hydroxy-1(hydroxymethyl) ethyl ester (1.53 %), Ethanol, 2-(2-methoxyethoxy) (1.48 %), Pentadecanoic acid, 14-methyl-, methyl ester (1.10 %), Decane, 1-fluoro (1.08 %), 9,12-Octadecanoic acid, methyl ester (Z,Z) (0.94 %), Octadecanoic acid, methyl ester (0.89 %), Propanoic acid, 2-methyl-, butyl ester (0.83 %), Butane, 1,1-dibutoxy (0.74 %) and Ethanol, 2-[2-(2-methoxyethoxy)ethoxy] (0.59 %). Most of these compounds had been reported to have pharmacological, antimicrobial, antioxidant and anticancer activities [33, 34, 35]. Also, Butane, 1,1-Dibutoxy has been reported to have potent antagonist activity against Vibrio anguillarum [17]. Ethanol, 2-(2-methoxyethoxy) is used as an Anti-icing agent in military jet fuel [16]. It is well known that 9, 12-Octadecadienoic acid (Z, Z) has anti-inflammatory, anti-cancer, hypocholesterolemic, hepatoprotective, and antihistaminic qualities [18].

Anticancer compounds are recognized to aid in the prevention of cancer since a large body of research in the laboratory has demonstrated that they may delay or even stop the growth of cancer in chemical, cell culture, and animal investigations. Moreover, 9-Octadecenoic acid methyl ester, 11-Octadecenoic acid methyl ester, and 9,12-Octadecanoic acid, methyl ester (Z,Z) were discovered to possess antioxidant qualities and to have an impact on cancer. Hexadecanoic acid has been demonstrated to possess not only antioxidant and anticancer qualities, but also anti-inflammatory, anti-androgenic, and 5-alpha reductase (5AR) inhibitory activity. By inhibiting the enzyme 5-alpha reductase (5AR), the chemical *n*-Hexadecanoic acid, which is made up of free and esterified fatty acids, may have an anti-androgenic action [36, 37]. The enzyme 5AR catalyzes the conversion of androstenedione to androsterone, cortisol to allotetrahydrocortisol, and testosterone to the far more potent 5-alpha Dihydrotestosterone (5a-DHT), which has been related to enlarged prostates and prostate cancer, among other things. In both men and women, increased 5AR activity is linked to insulin resistance and obesity [38]. According to [39], increased 5AR activity in women is also linked to hirsutism, a condition in which there is excessive growth of dark or coarse hair in a male-like pattern on the face, chest, and back, often caused by excess androgens. Elevated testosterone levels are linked to a hormonal disease called polycystic ovarian syndrome, or PCOS [39, 5]. Therefore, in situations where 5AR activity is observed to be increased, such as those mentioned above, 5AR suppression may be desired. A frequent non-cancerous swelling of the prostate gland that can cause difficulties urinating and premature baldness in males is known as benign prostatic hypertrophy (BPH) and is linked to elevated 5AR activity [39, 5].

As demonstrated with 5AR inhibiting medications, natural 5AR inhibitors such as n-Hexadecanoic acid can offer efficient and mild inhibition without completely blocking 5AR. Pentadecanoic acid, 14-methyl-, methyl ester which belongs to the class of fatty acid methyl esters has been reported to show antifungal and antimicrobial activities [17]. 11-Octadecanoic acid, methyl ester which is also in the class of fatty acid methyl esters has also been reported to have antibacterial, antifungal and antioxidant properties [19].

Octadecanoic acid, methyl ester is a white crystal semi-solid ester, flavour component in food, lubricant, used in the manufacture of pharmaceuticals, cosmetics, soaps, surfactant and softening agents [20]. Reports on oleic acid shows antitumor and anti-inflammatory activities [22]. 9-Octadecanoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester inhibit the proliferative effect in keloid fibroblasts as reported by [23]. These results could validate and encourage the use of Thespian garckeana fruit in traditional medicine.

4. CONCLUSION

Due to their strong antioxidant qualities and economic viability, plants' therapeutic qualities have drawn the attention of scientists in recent global scientific advancements. The study's findings demonstrated that Thespian garckeana's methanol extract exhibited remarkably strong antioxidant properties. Its antioxidant properties are supported by the phytochemical investigation, which revealed a considerable presence of total flavonoids, total phenolics, and total tannin content. Thespian garckeana fruit methanol extracts were subjected to GC-MS analysis, which revealed the presence of many bioactive chemicals. These compounds have been shown to have health-promoting properties and may be utilized in biotechnology. We can conclude that these natural and promising chemicals have the potential to be employed as affordable feed and food additives that promote the health of both people and animals.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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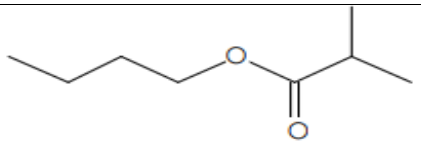
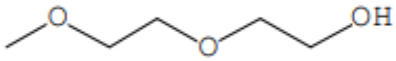
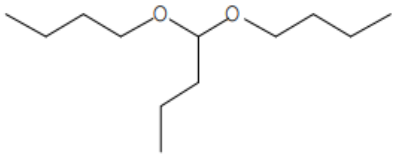
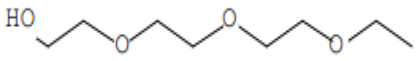
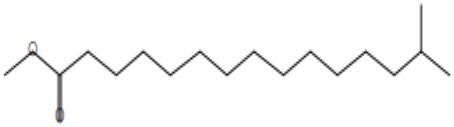
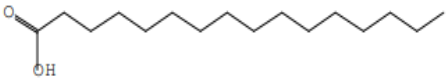
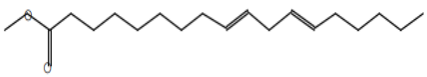
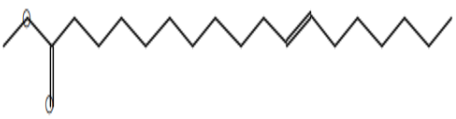
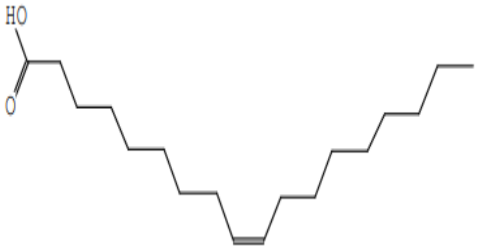
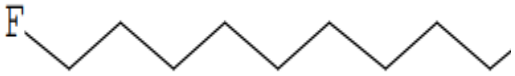
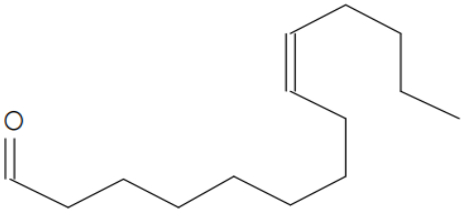
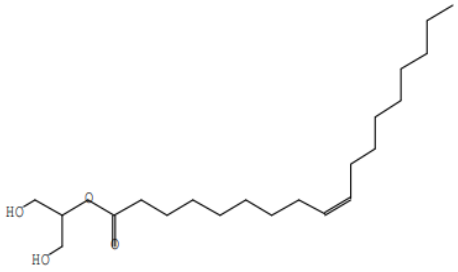
Table 5: Structures of GC-MS Identified Components of the Methanol extract of *Thespian garckeana*

S/N	Formula	***Structure***	Molecular Weight (g/mol)
1	$C_8H_{16}O_2$		144
2	$C_5H_{12}O_3$		120
3	$C_{12}H_{26}O_2$		202
4	$C_8H_{18}O_4$		178
5	$C_{17}H_{34}O_2$		270
6	$C_{16}H_{32}O_2$		256
7	$C_{19}H_{34}O_2$		294
8	$C_{19}H_{36}O_2$		296
9	$C_{19}H_{38}O_2$		298

10	$C_{19}H_{36}O$		280
11	$C_{18}H_{32}O_2$		280
12	$C_{18}H_{34}O_2$		282
13	$C_{10}H_{21}F$		160
14	$C_{14}H_{26}O$		210
15	$C_{21}H_{40}O_4$		356