Innovations

Chemical Profiling and Antioxidant Activities of Leaf Extracts of *Gossypium Barbadense* Linn

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Abstract: *Across the world, Gossypiumbarbadense (G. barbadense) leaf herbal remedies are extensively used to treat microbiological infections and hypertension. Standard methods were used to identify the phytochemicals present in the crude extracts. The different chemicals contained in the extracts were evaluated using gas chromatography-mass spectrometry (GC-MS). Nitric oxide scavenging (NOS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis-3-ethylbenzothiozoline-6-sulphonic acid (ABTS) tests were used to assess the radical scavenging potentials of the crude extracts. Phenols, tannins, steroids, terpenoids, proanthocyanidins, and flavonoids were identified by phytochemical examination of the extracts. The presence of 20, 24, and 32 compounds which together account for 99.98%, 99.64%, and 99.49% of the extracts (methanol, ethyl acetate and hexane) was detected by GC/MS analysis. The principal constituents found in the methanol extracts arePropanal (45.78%), methylamine (N, N-dimethyl) (24.53%), and trimethylene oxide (19.47%). Conversely, the main constituents found in the leaf extracts made of ethyl acetate arecaryophyllene (31.79%), palmitic acid (15.80%), and α-linolenic acid (15.48%). The main constituents of the hexane leaf extracts includeβ-Bisabolol (21.85%), palmitic acid (20.99%), methyl-linolenate (12.00%), and γ-Curcumene (11.10%). The methanol extract of G. barbadense exhibited percentage DPPH, ABTS, and NOS radical scavenging activities of 63.49 $\pm$ 2.62, 53.29 $\pm$ 1.16, and 55.23 $\pm$ 0.96, respectively. The equivalent values for its ethyl acetate extract were 18.05 $\pm$ 1.08, 48.97 $\pm$ 0.45, and 40.02 $\pm$ 0.23. The leaf extracts showed antioxidant qualities that could be investigated for the production of novel drugs.*

Keywords: *Gossypiumbarbadense, Antioxidant, GC-MS*

Introduction

Man has used plants as food, shelter, and medicine since the beginning of time. According to Atanasov*et al*. (2015), this medication was in the form of unrefined pharmaceuticals such as powder, decoction, tincture, and tea. For their fundamental medical needs, over 80% of the world's population, according to the World Health Organization (WHO, 2002), uses traditional medicines, mostly herbal remedies. This is due to the fact that the plant kingdom contains a vast array of unexplored opportunities. Naturally occurring metabolites have been demonstrated to have more biochemical specificity than synthetic drugs, and the products of their transformations have extraordinarily advantageous therapeutic properties (Larayetan, 2017). Human body cells require oxygen to function; however, oxygen can harm cells oxidatively when it comes into contact with free radicals. Free radicals are generally produced in the body as a result of oxidation, a chemical reaction that results in the loss of electrons. These radicals can cause harm or even death to human cells. Free radical intermediates are eliminated by antioxidants, which also halt further oxidation events, breaking the chain reaction. Part of the chemoprevention of lipid peroxidation-induced sickness is the use of natural antioxidants derived from herbs and spices, which have been demonstrated to be beneficial against oxidative stress (Akbari*et al*., 2022). Commonly referred to as the "new antioxidants," plant-based chemicals like flavonoids and other polyphenols have positive effects on human health. The *Gossypium* genus in the *Malvaceae* family contains the cotton-producing species. Originally from the tropics and subtropics, the genus has roughly 400 species. Native to the West Indies, Gossypiumbarbadense Linn is a wood perennial that grows to a height of two meters. It is currently found all across Africa. *G. barbadense* is a popular herb in Nigeria for treating wounds, rheumatism, conjunctivitis, convulsions, jaundice, gastrointestinal issues, and STDs like gonorrhoea (Gbadamosi&Obago, 2013). According to Pieter's*et al*. (2004), there are other applications such as emmenagogue (menstruation stimulant), ergot substitute (fungus that produces particular alkaloids used in medicine), and haemorrhage control. According to Salako and Awodele (2012) and Ajayeoba*et al*. (2014), this medicinal plant also exhibits anti-malaria effectiveness when used in combination therapy. Triterpenoids, sesquiterpenoid, and polyphenols are a few of the active ingredients that have been identified in this therapeutic plant. The antifungal compounds 6-methoxy- and 6-dimethoxy-gossypol are found in the root. The plant also contains certain phytochemicals, including flavonoids, alkaloids, phlobatannins, tannins, and cardiac glycosides (Salako and Awodele, 2012). These phytochemicals are what give the plant its antibacterial and wound-healing characteristics (Ikobi*et al*., 2012). Additionally, the herb possesses anti-malarial effects (Gbadamosi*et al*., 2011; Salako

and Awodele, 2012). Antibacterial qualities (Essien*et al*., 2011, Ikobi*et al*., 2012) and a hypotensive impact (Pieter's*et al*., 2004). Plants are a good source of antioxidants as well, but they must be standardized by knowing their bioactivity and the bioactive components found in the various extracts of *G. barbadense*. For this reason, this study was carried out to extract, identify, and quantify the bioactive compounds in the *G. barbadense* leaf extracts that are responsible for some of their medicinal properties.

Materials and Methods

Collection of Plant Samples and Preparation of Extracts

At Agboye village, Oyo town, Oyo state, 7.874890 N, 3.92440 E, fresh leaves of *G. barbadense*were harvested. The samples were obtained by the researchers, who then had them identified and verified at the Forestry Research Institute of Nigeria (FRIN), located in Jericho, Ibadan. Voucher number FHI (Ogunmola) 113773 was then assigned to the sample. The leaf sample was ground into powder after being air-dried for seven days at room temperature via a mechanical grinder (MODEL LM-05). A hundred and fifty (150 g) of the grounded leaf sample was steeped for 48 h in hexane, ethyl acetate, and methanol in that order. It was then filtered through Whatman No. 1 filter paper and concentrated at 25 °C using a rotary evaporator. The extracts were refrigerated at 40 °C in tightly sealed vials until the analytical date.

Qualitative Phytochemical Screening of the Leaf Extracts

Test for saponins

Foam test: When precisely 5 mL of extract are added to 5 mL of water and heated, froth forms to indicate the presence of saponins.

Test for alkaloids

Hager's test: To 2 mL of extract, precisely 2 drops of Hager's reagent were applied. When there are yellow precipitates, alkaloids are present.

Test for Phenols

Precisely 4 mL of the extract was subjected to 1 mL of concentrated sulphuric acid and a few drops of sodium nitrate ($NaNO_3$). The mixtures received a precise addition of 2 mL of sodium hydroxide. The presence of phenol was revealed by the production of a blue precipitate. (Iwu*et al*., 2018)

Test for tannins

Precisely 2 mL of extract was combined with 2 mL of water, and two to three drops of 5% $FeCl_3$ were added. The green precipitate indicated the presence of tannin.

Test for Phytate

We used a Neogenphytotic acid test kit. If there is phytate present, a blue precipitate forms after precisely 0.5 mL of acetic acid and 2 drops of Neogen kit are applied to 1 ml of the extract.

Test for steroids.

2 mL of concentrated H_2SO_4 and 2 mL of $CHCl_3$ were combined with exactly 2 mL of extract. The presence of steroids is shown by the reddish-brown ring at the intersection.

Test for Terpenoids

In order to create a layer, precisely 5 mL of the extract were combined with 2 mL of chloroform and 3 mL of concentrated H_2SO_4. When the colour of the layer created turned a deep crimson, terpenoids were present.

Test for proanthocyanidins

By staining the extract with a recently made 1% vanillin-6-M HCl solution, proanthocyanidins become evident. If proanthocyanidin is present in the extract, a noticeable red coloration appears. (Engstrom*et al.*, 2014),

Test for flavonoids.

One mL of 10% $PbC_2H_3O_2$ was mixed with exactly 1 mL of the extract. If a yellow hue appears, flavonoids are present.

Quantitative Phytochemical Screening

Quantification of Total Phenolic Content

Using spectrophotometric analysis, the total phenolic content was determined (Kim *et al.*, 2003). Ten mL of distilled water were added after thoroughly combining 1 mL of the filtrate with 1 mL of the Folin-Ciocalteau reagent. After letting the mixture stand for seven minutes, 10 mL of a 7% Na_2CO_3 solution were added. For colour improvement, the resultant solution was vortexed for roughly 30 seconds and stored in a dark cabinet. The absorbance at 750 nm was measured. Using a standard calibration curve and a linear equation that is represented as y = 0.009x + 0.012 (R2 = 0.999), where x is the concentration and y is the gallic acid, the analysis was performed in triplicate, and the findings were expressed in mg per gallic acid equivalent (GAE/100 g).

Quantification of Total Flavonoid Content

The total flavonoid content of the extract was measured using the Ordonez *et al.* (2006) protocol. 2 mL of distilled water were added in addition to 1 mL of the extract.

Following vigorous vortexing for approximately 40 seconds, 0.4 mL of 5% aluminium chloride, 3 mL of sodium hydroxide, and 0.5 mL of 10% sodium nitrate were combined. The mixtures were then incubated for 50 minutes at room temperature in a dark area. Absorbance measurements were made at 420 nm. Using the linear equation y = 0.023x + 0.022 (R2 = 0.982), where x is defined as concentration dependent and y as the rutin equivalent scale, the total flavonoid contents of the two extracts were measured as mg RE/100 g of rutin.

Quantification of Total Tannin Content

Tannic acid was used as a standard, and tannin determination was assessed using the methodology outline by Kaur and Arora, 2009. In a conical flask, 250 mg of the extract was mixed with 50 mL of distilled water. A mechanical shaker was used to agitate the mixture for one hour. After filtering it into a 50-mL volumetric flask, distilled water was added to bring the final volume up to par. After treating an aliquot (1 mL) of the filtrate with 4 mL of distilled water and 0.1 M $FeCl_3$ diluted 10-fold in 0.1 M HCl and 0.008 M potassium ferrocyanide, 2 mL of the mixture was used. After carefully mixing the resulting solution and letting it stand for ten minutes, the absorbance was measured at 605 nm against the blank. The quantification process was conducted using the tannic acid (20, 40, 60, 80, 100, 140, and 200 mg/L) in distilled water standard calibration curve, which has seven points. Tannic acid equivalent, measured in mg per 100 g of dry material, was used to indicate the tannin content.

Quantification of Total Phytate

A modified version of Harland and Oberleas's (1977) method was used to extract phytate from the material. This method's basic workings depend on the conversion of free phytic acid and the colorimetric determination of the released organic phosphorus. The sample, weighing 2.0 g, was extracted using 40 mL of 2.4% HCl (68.6 mL of 35% hydrochloric acid in a total volume of 1 litre of D_2O) while being continuously shaken for three hours at room temperature (250 °C). After that, Whatman No. 1 filter paper was used to filter the extract. A spectrophotometric technique with an absorbance wavelength of 640 nm was used to quantify the amount of phytate (AOAC, 2005). A molecule of phytic acid, which contains six molecules of phosphorus (P), was assumed to be digested in order to compute the amount of phytic acid from the organic phosphorus (AOAC, 2005). The findings were expressed as the percentage of phytate in 100 g of the sample.

Quantification of Total Saponin Content

To analyze saponins, Brunner's (1984) spectrophotometric technique was employed. A 250-mL beaker containing 1 mL of extract was filled with 100 mL of isobutyl

alcohol. For five hours, the mixture was agitated on a shaker to guarantee even mixing. After that, 20 mL of a 40% saturated solution of magnesium carbonate was added to the mixture after it had been filtered through Whatman No. 1 filter paper into a 100-mL beaker. A clear, colorless solution was obtained by filtering the mixture made with saturated $MgCO_3$ through Whatman No. 1 filter paper once more. A 50-ml volumetric flask was filled with 1 mL of the colourless solution, 2 mL of 5% FeCl3 solution, and distilled water to bring the volume up to the mark. For thirty minutes, it was allowed to stand in order for the blood-red colour to emerge. Standard saponin solutions (Sigma 47036-50g-F) ranging from 0 to 10 ppm were made using the saponin stock solution. The 2 mL of 5% FeCl solution was applied to the standard solutions in the same manner as the 1 mL sample previously. A spectrophotonic 2lD spectrophotometer operating at 380 nm wavelength was used to measure the absorbance of both the sample and standard saponin solutions following colour development. The following formula was used to get the percentage of saponin:

$$\text{Saponin (mg/100 mL)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor} \times 100}{\text{Weight of sample x } 10{,}000}$$

Quantification of Phytosteroid Content

The procedure outlined by Trease and Evans (1989) was used to ascertain the plant sample's steroid concentration. A 2 mL fraction was extracted from a 2.5 g powdered plant material solution that was made in 50 mL of distilled water and vigorously shaken for a duration of one hour. After thoroughly cleaning the extract solution with 3 mL of 0.1 M NaOH (pH 9), 2 mL of chloroform and 3 mL of ice-cold acetic anhydride were combined, and two drops of concentrated H_2SO_4 were cautiously added. Spectrophotometric measurements were made at 420 nm to determine the absorbance of the sample and the blank.

Determination of Total Terpenoid Content

To each 2 mL micro-centrifuge tube containing 200µL of extract, precisely 1.5 mL of chloroform was added. To create the standard curve, mix 200 µl of a methanol-based linalool solution with 1.5 mL of chloroform. Then, do serial dilution at different levels (from 100 mg/200 µL to 1 mg/200 µL; 12.965 µM–1.296 µM) of the linalool concentrate. For the serial dilution, add 95% (v/v) methanol to make up the entire 200 µl of methanol. After carefully vortexing the sample mixture, it was left to stand for three minutes. A precise volume of 100 µL of concentrated sulfuric acid (H_2SO_4) was introduced into every 2 mL micro-centrifuge tube. The test tube was then left to incubate for 1.5–2 hours at room temperature in a dark environment. The standard

solution (linalool) needs to be incubated for no more than five minutes, and the micro-centrifuge tube cannot be disturbed during this period. Each test micro-centrifuge tube had a reddish-brown precipitation in it at the end of the incubation period. Without disrupting the precipitation, the reaction mixture containing the supernatant was gently and cautiously decanted. In the reaction mixture solution, the reddish-brown precipitate is partially soluble. After a gentle decantation, 1.5 mL of 95% (vol/vol) methanol was added, and the mixture was vigorously vortexed until all of the precipitate had completely dissolved in the methanol. The extract was moved to a colorimetric cuvette (95% (vol/vol)) from the test tube. The absorbance was measured at 538 nm, with methanol serving as the blank. To calculate total terpenoids, a standard curve was derived from the blank-corrected wave length of the Linalool standard at 538 nm. Using the regression equation of the Linalool standard curve, the total terpenoids concentration of the unidentified plant sample was also determined as Linalool equivalents.

Determination of Total Proanthocyanidin Content

Exactly 0.5 mL of 1 mg/mL aqueous extract was combined with 3 mL of vanillin-methanol (4% v/v) and 1.5 mL of hydrochloric acid and vortexed. After allowing the mixture to stand at room temperature for fifteen minutes, the absorbance at 500 nm was measured. The amount of proanthocyanidins in total was represented as mg/g of catechin equivalent.

GC-MS Determination of Bioactive Compounds

The Perkin Elmer Turbo mass spectrophotometer (Norwalk, CT06859, U.S.A.) with XLGC was the GC-MS instrument used. The column used was a Perkin Elmer Elite-5 capillary column, measuring 30m x 0.25mm with a film thickness of 0.25mm consisting of dimethyl polysiloxane. Helium was used as a carrier gas at a flow rate of 0.5 mL/min. The oven temperature was programmed to start at 110°C, then increase to 240°C over the course of four (4) minutes, and then rise to 280 °C over the course of five (5) minutes at a rate of 20 °C. Each sample took a total of ninety minutes to run.

***In vitro* antioxidant action**

DPPH Assay

The antioxidant and radical-scavenging properties of *G. barbadense* extracts were evaluated in relation to the free radical DPPH. The synthetic antioxidants vitamin C, gallic acid, and E were incubated with a DMSO solution of DPPH for approximately half an hour at room temperature and in the dark. The concentrations of the extracts ranged from 0.0025 to 0.8 mg/mL. Using a vortex machine, the solutions were meticulously mixed, and each sample's absorbance was measured at 517 nm. The

following equation was used to determine the extract's capacity to scavenge DPPH-free radicals in the solution.

$$\text{DPPH Scav activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance of DPPH + methanol;

Abs sample is the absorbance of DPPH radical + sample extract or standard. The results were expressed in percentages: inhibition = DPPH scavenging activity (%).

The dose-response curve was plotted, and the IC50value of the synthetic antioxidant and the extracts were calculated (Larayetan *etal.,* 2019).

ABTS Assay:

The extracts' ABTS activity was assessed using the modified Witayapen technique (Nantitanon et al., 2007). After oxidizing the ABTS stock solution (7 mM) and adding 2.4 mM of potassium persulphate in comparable proportions, the combination was allowed to react for 12 h at 25 °C to obtain the working solution. After 7 minutes, using a spectrophotometer, a fraction (1 mL) of the resulting solution was further diluted with 60 mL of methanol to get an absorbance of 0.706 ± 0.001 at 734 nm. In summary, each extract was combined with a methanolic ABTS solution at five distinct concentrations (0.025, 0.05, 0.1, 0.2, and 0.4 mg mL-1) and left for seven minutes at 25 °C in the dark. After that, the absorbance was measured spectrophotometrically at 734 nm, and the inhibition of the ABTS radical by the extracts and commercial antioxidants (Vitamin C and β-carotene) was computed using the DPPH assay equation.

Nitric oxide scavenging assay

The nitric oxide scavenging activity was performed using the method established by Rai *et al*. (2006). A mixture of 0.5 mL of 10 mM sodium nitroprusside in phosphate-buffered saline and varying amounts of the extracts (0.025, 0.05, 0.1, 0.2, and 0.4 mg mL^{-1}) was added. The mixture was then incubated for 150 minutes at room temperature in the dark. In this experiment, the sample for the control was precisely 1 mL of water. Following the incubation period, 1 mL of the sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) was mixed with 0.5 mL of the reaction mixture. After incubating for 5 minutes, add 1 mL of 0.1% naphthyl ethylenediamine dihydrochloride, stir, and incubate for 30 minutes at 25 °C. We measured the absorbance of the resultant chromophore at 540 nm. The nitric oxide scavenging capability of the extracts was tested using the Trolox standard curve; the

results were expressed as mole Trolox equivalent (TE) antioxidant capacity per 100 g sample. Three copies of each analysis were performed.

$$\% \text{ Inhibition } = \frac{\text{Abs control} - \text{ Abs sample}}{\text{Abs control}} \times 100$$

Results and Discussion

Percentage Yield of Crude Extracts

Exactly 150 g of the powdered leaves were used for the extraction, the various percentage yields are expressed using the equation below.

$$\% \textbf{ Yield} = \frac{\boldsymbol{Wt\ of\ extract}}{\boldsymbol{Wt\ of\ sample}} \times \mathbf{100}$$

The various percentage yields are given in Table 1;methanol leaf extract of *G. barbadense* has the highest yield (30.93%) while hexane leaf extract has the lowest yield (6.73 %) as shown in Table 1. This result is in agreement with the existing literature in which a higher yield was reported for aqueous and methanol extracts than hexane extract (Akobi *et al.,* 2012, Gbadamosi and Obago, 2013, Jokotagba *et al*., 2016, Olivier*et al.,* 2020).

Table 1: Percentage Yield of Crude Leaf Extracts of *G. barbadense*

Leaf Sample (Different Solvents Used forExtraction)	***G. barbadense*Leaves**	
	Yield (g)	Yield (%)
Methanol	46.40	30.93
Ethyl acetate	34.10	22.73
Hexane	10.10	6.73

Qualitative Phytochemical Screening of the Leaf Extracts of *G. barbadense* and *G.hirsutum*

The qualitative phytochemical screening of the aqueous, methanol, ethyl acetate and hexane extracts of *G. barbadense* revealed the presence of different bioactive components such as saponins, phenols, tannins, phytates, steroids, terpenoids and proanthocyanidins as shown in Table 2. Alkaloid was not found in all the four extracts and also phytate was conspicuously absent in the hexane leaf extract. The above results agree with the previous works of Akobi *et.al.,* 2014, Gbadamosi and Obago, 2013, Jokotagba, 2016 which reported the absence of alkaloid in the decocted aqueous extract of *G. barbadense* but is at variance with a study conducted in

Kaduna, Nigeria which reported the presence of alkaloid in a Nigerian sample of *G. barbadense* (Muhammad *et.al.*, 2014), the variation is likely to be due to environmental factors. Bioactive compounds found in *Malvaceae* family have been documented to have biological antibacterial activity that has no record of harmful effect on humans (Essien *et al.,* 2011, Oliver *et al.*, 2020).

Table 2: Qualitative Phytochemical Screening of Leaf Extracts of *G. barbadense*

Phytochemical Constituents	**Test**	**Methanol**	**Ethyl acetate**	**Hexane**
Saponins	Foam Test	+	+	+
Alkaloids	Mayer and Wager Test	-	-	-
Phenols & Tanins	Ferric Chloride Test	+	+	+
Phytates	AOAC (Method 2002)	+	-	-
Steroids and Terpenoids	Salkowski Test	+	+	+
Proanthocyanidins	Sun et al. (1998b)	+	+	+
Flavonoids	Lead Acetate Test	+	+	+

Key: + Presence - Absence

Quantitative Phytochemical Screening of the Leaf Extracts of *G. barbadense*

The quantitative phytochemical screening of the leaf extracts of G*. barbadense* in three different medium (methanol, ethyl acetate and hexane) showed that all the three extracts are rich in phenols, flavonoids, tannins, phytates, saponins, steroids, terpenoids and proanthocyanidins in different concentrations as shown in Table 3.

Total Saponin Content

The total saponin content in the extracts of *G.barbadense* ranged between 39.40 ± 1.47 mg/100g to 15.90 ± 1.92mg/100g. G. barbadense ethylacetate leaf extract (GB_{EA}) has the highest saponin content (39.40 ± 1.47mg/100g) while *G. barbadense* hexane leaf extract (GB_{hex}) has the lowest saponin content (15.90±1.92mg/100g) as shown in Table 3 and this is in the same range with what was reported by (Ade-Ademilua and Okpoma, 2018). Saponin are selectively referred to as triterpene glycosides, they are bitter tasting usually toxic plant derived organic chemical that have a foaming quality when agitated in water. They decrease blood lipids, lower cancer risk and lower blood glucose. A saponin diet can be used in the inhibition of dental caries and platelet aggregation and it is an antidote against acute lead poisoning.

Total Phenolic Content

The total phenolic content in *G. barbadense* leaf extract ranged between 941.32 ± 10.65 mg/100g to 119.55 ± 2.19 mg/100g. *G. barbadense* methanol leaf extract (GB_{aq}) has the highest phenolic content 941.32±10.65 while *G. barbadense* hexane leaf extract (GB_{hex}) has the lowest value 119.55 ± 2.19 mg/100g (Table 3). Ade-Ademilua and Okpoma, 2018, reported the absence of phenol in *G. barbadense* but reported its presence in *G. hirsutum*. The result obtained from this study is higher than that reported by Ade-Ademilua and Okpoma, 2018 and this is as a result of the variation in the time of harvest of the leaves and other environmental factors. Phenols are secondary metabolites synthesized through the Shikimic acid and Phenylpropanoid pathways. They posses numerous bioactive properties and although they are not nutrient but has antioxidant activity (Okudu *et al*., 1994, Ade-Ademilua and Okpoma, 2018).

Total Tannin Content

The total tannin content in the *G. barbadense* leaf extracts ranged from 665.45 mg/100g ± 10.29 to 189.77 ± 1.94 mg/100g. *G.barbadense* methanolic extract (GB_{me}) has the highest tannin content 665.45 ± 10.29 mg/100g while *G. barbadense* hexane leaf extract (GB_{hex}) has the lowest value 189.77 ± 1.94 mg/100g as shown in Table 3., this agrees with the works of Omojasola and Awe (2004) which reported the presence of tannin in *G. hirsutum* but Ade-Ademilua and Okpoma (2018) reported the absence of tannin in *G. hirsutum*. Tannin is a class of astringent polyphenolic biomolecules that bind to and precipitate proteins and various organic compounds including amino acids and alkaloids. Tannin has antioxidant, antibacterial and anti-inflammatory action in animals (Tong *et al*, 2022).

Total Phytate Content

The total phytate content in the leaf extracts of *G. barbadense* ranged from 195.67 ± 3.25 mg/100g to 181.22 ± 5.10 mg/100g. *G. barbadense* methanolic leaf extract (GB_{me}) has the highest phytate content 181.22 ± 5.10 mg/100g while *G. barbadense* ethyl acetate leaf extract (GB_{EA}) has the lowest value 181.22 ± 5.10 mg/100 g. It could be observed that there is no phytate in the hexane leaf extract andthis is the first time when phytate will be reported in significant amount and this is at variance with the works of Mohammad *et.al* (2014), Ade-Ademilua and Okpoma (2018). Phytate is a unique natural substance which could still be found in plant as phytic acid has received considerable attention due to its effects on mineral absorption. Phytic acid prevents the absorption of iron, zinc and calcium and may promote mineral deficiencies and that is why they are referred to as anti-nutrient, but they have some health benefits. Several factors have been documented to affect reduction in the levels of phytate which include sprouting, fermentation and boiling.

Total Steroid Content

The total steroid content in the leaf extracts of *G. barbadense* ranged from 188.26 ± 2.13 mg/100g to 109.70 ± 2.82 mg/100g. *G. barbadense* methanol leaf extract (GB_{met}) has the highest steroid content while hexane leaf extract (GB_{hex}) has the lowest 104.70 ± 2.82mg/100g as shown in Table 3. These values are higher than those reported for the same species in Nigeria (Ade-Ademilua and Okpoma, 2018). Steroids are biologically active organic compound with four rings arranged in a specific molecular configuration. They are important components of cell membrane which alter membrane fluidity. They act as anti-inflammatory agent.

Total Terpenoid Content

The total terpenoid content in the leaf extracts of *G. barbadense* ranged from 212.96 ± 1.49 mg/100g to 58.63 ± 0.92 mg/100g. Methanolic leaf extract (GB_{me}) has the highest value 212.96 ± 1.49 mg/100g while hexane leaf extract (GB_{hex}) has the lowest value 58.63 ± 0.92 mg/100g as show in Table 3 and this agrees with the works of Omojasola and Awe, (2004), Muhammad *et.al*, (2014). Terpenoids are also known as Isoprenoids, they are large and diverse class of naturally occurring organic chemicals, which have anticancer, antimicrobial, antifungal and antiviral properties (Franklin *et al*. 2001,Guimaraes, *et.al*, 2019).

Total proanthocyanidin content

The total proanthocyanidin in the leaf extract of *G. barbadense* ranged from 262.59 ± 4.11 mg/100g to 145.05 ± 4.45 mg/100g. Ethylacetate leaf extract (GB_{EA}) has the highest proanthocyanidin content 262.59 ± 4.11 mg/100g while methanolic leaf extract (GB_{me}) has the lowest value 145.05 ± 4.45mg/100g as shown in Table 3. From

the available literature, this is the first time when proanthocyanidins will be reported from the leaves of these two plants. This phytochemical is known to protect the heart and cardiovascular system and they work as antioxidants and block nitrosamines from forming. They work with vitamin C to lower the risk of breast cancer and prevent urinary tract infection.

Total Flavonoid Content

The total flavonoid content in the leaf extract of *G. barbadense* ranged from 638.88 ± 4.45 mg/100g to 150.37 ± 5.77 mg/100g. The methnol leaf extract (GB_{met}) has the highest flavonoid content 638.88 ± 4.45mg/100g while the hexane leaf extract (GB_{hex}) has the lowest 150.37 ± 5.77 mg/100g as shown in Table 3. Flavonoids are secondary metabolites polyphenol found in plants. They are utilized for the manufacture of pigments that attract insects for pollination in plants. They cannot be synthesized by animals and man (koes *et al.,* 2005), but are responsible for taste, colour, and impediment of fat oxidation and prevention of enzymes and vitamins degradation in food. In addition, they also exhibit significant anti-inflammatory, anti-allergic and anticancer activities. The result obtained in the leaf extract of *G. barbadense* in this study is higher than what was reported from Maiduguri, Borno State,Nigeria (Muhammed *et al.,* 2014) but the values are in the same range with another work done in southwest, Nigeria (Ade-Ademilua and Okpoma, 2018).

Table 3: Quantitative Phytochemical Screening of the Leaf Extracts of *G. barbadense*

Extracts	Phenols mg/100g	Flavonoids mg/100g	Tanins mg/100g	Phytates mg/100g	Saponins mg/100g	Steroids mg/100g	Terpinoids mg/100g	Proanthocyanidins mg/100g
GB_{met}	941.3 ± 10.65	638.88 ± 4.45	665.45 ± 10.29	181.22 ± 5.10	16.62 ± 0.72	188.26 ± 2.13	212.96 ± 1.49	145.05 ± 4.45
GB_{EA}	686.84 ± 7.55	178.47 ± 5.23	257.22 ± 25.06	195.67 ± 3.25	39.40 ± 1.47	137.37 ± 1.51	59.49 ± 1.75	262.59 ± 4.11
GBhex	119.55 ± 2.19	150.37 ± 5.77	189.77 ± 1.94	_	15.90 ± 1.92	104.70 ± 2.82	58.63 ± 0.92	147.71 ± 1.28

Key: GB_{met} = Methanolic extract of *G. barbadense*

GB_{EA} = Ethylacetate extract of *G. barbadense*

GB_{hex} = hexane extract of *G. barbadense*

GC/MS Analysis of the leaf Extracts

According to the results of the GC/MS analysis, there were twenty (20), twenty-four (24), and thirty-two (32) bioactive chemicals in the methanol, ethyl acetate, and hexane leaf extracts of *G. barbadense*. These compounds accounted for 99.98%, 99.64%, and 99.49% of the total compounds found in the extracts, respectively. A complex variety of substances belonging to many chemical classes, including fatty acids, steroids (sterols), diterpenes, triterpenoids, ethyl acetate, ethers, and monoterpenes, was revealed by the GC-MS components of the methanol, ethyl acetate, and hexane extracts of *G. barbadense*. Propanal (45.78%), methylamine (N, N-dimethyl) (24.53%), and trimethylene oxide (19.47%) are the main compounds found in the methanol extracts, while caryophyllene (31.79%), palmitic acid (15.80%), and α-linolenic acid (15.48%) are the key constituents found in the ethyl acetate leaf extracts. The leading components of the hexane leaf extracts include β-Bisabolol (21.85%), palmitic acid (20.99%), methyl-linolenate (12.00%), and γ-Curcumene (11.10%). Two bioactive compounds, vitamin E and palmitic acid, were found in all three extracts of *G. barbadense* in varied proportions, as seen in Tables 4, 5, and 6, while caryophyllene and humulene were seen in different proportions in both methanol and ethyl acetate extracts of the plant under investigation (Tables 4 and 5). A natural bicyclic sequiterpene, caryophyllene, also goes by the formal name β-caryophyllene. It is a component of numerous essential oils. Typically, it is found in mixtures with humulene and isocaryophylene. Having a trans-double bond in a 9-membered ring and a cyclobutane ring makes it noteworthy. In low ambient temperatures, caryophyllene enhances cold tolerance. When combined with paclitaxel, caryophyllene effectively combats cancer (Blowman, 2018). The fatty acid palmitic acid, also known as hexadecanoic acid, has a chain of 16 carbons. It is the most prevalent type of saturated fatty acid present in microorganisms, plants, and mammals. It is a significant part of the oil extracted from oil palm fruits. Based on evidence that it raises the risk of metabolic and cardiovascular disorders, this fatty acid's consumption ought to be managed (Laury, 2021). In most diets, the main saturated fatty acid is palmitic acid. It can be obtained through nutrition and makes up 20–30% of the total fatty acids in the human body. A study carried out in northern Italy revealed no link between the risk of breast cancer and palmitic acid and other saturated fatty acids (Pala *et al.*, 2001). However, there has been a documented association between blood fraction levels of palmitic acid and breast cancer risk.

Despite being present in a higher proportion, palmitic acid has also been discovered in the *G. herbaceum* hexane leaf extract (16.33%) (Larayetan *et al*., 2021). The values found in this investigation for *G. barbadense* methanol, ethyl acetate, and hexane leaf extracts are within the same range as those found by Larayetan *et al*. (2021) in *G. herbaceum*. A necessary polyunsaturated fatty acid (C-18, n-3) is α-linolenic acid. Either directly or via their oxylipin metabolites, this long-chain polyunsaturated fatty acid possesses anti-inflammatory properties. De Lorgeril *et al*. (2001) found that it also lowers the risk of cardiovascular disease. α-linolenic acid is frequently used to treat heart and blood vessel problems such as excessive blood pressure, heart disease, and artery hardening. Additionally, it is essential for the typical growth and development of humans (Web M-D, 2017). The white, waxy substance known as β-sitosterol is mostly produced by plants and shares structural similarities with cholesterol. According to Le Goff *et al*. (2019), clinical and prechemical research indicates that β-sitosterol lowers bad cholesterol (LDL), lowers the risk of coronary artery disease, atherosclerosis, and heart attacks, and prevents numerous cancer types. Vitamin E is a fat-soluble antioxidant that may aid in shielding cell membranes from reactive oxygen species(Lee*et al*., 2022).

Table 4: Chemical composition of methanol leaf extract of *Gossypium barbadense*

S/N	**R.T**	**Compound**	% **composition**
1.	1.379	Propanal	45.78
2.	1.522	Methylamine, N, N-dimethyl	24.53
3.	2.409	Oxirane, methyl-, (s) -	4.83
4.	2.460	Trimethylene oxide	19.47
5.	6.105	1,2-benzenedicarboxylic acid, bis (2,4,6-trimethylphenyl)ester	0.11
6.	6.282	Caryophyllene	1.10
7.	6.408	Humulene	0.27
8.	6.551	(Z)-1-methyl-4. (6-methylhept-5-en-2-ylidene) cyclohex-1-ene	0.24

9.	6.586	(Trimethylsilyl) methyltrifluoromethyldisulfide	0.09
10.	7.061	3-cyclohexen-1-o1, 1-(1,5-dimethyl-4-hexenyl)-4-methyl -	0.97
11.	7.461	5-Hexanal, 4-methylene	0.29
12.	7.524	3-cyclopropenoic acid, 1-butyl, methylester	0.11
13.	7.576	3,4-octadiene, 7-methyl	0.14
14.	7.702	Methyl palmitate	0.07
15.	7.827	Palmitic acid	0.77
16.	8.154	4-carbomoyl-2-phenyl-2-oxazoline	0.17
17.	8.274	linolenyl alcohol	0.54
18.	9.229	Bis (2-ethylhexyl) phthalate	0.13
19.	11.810	Vitamin E	0.15
20.	13.486	β-sitosterol	0.22
		Total	99.98

Table 5: Chemical composition of the ethyl acetate leaf extract of *G. barbadense*

S/N	**R.T**	**Compound**	% **composition**
1.	1.350	Propanoic acid, 2-chloro-ethylester	0.44
2.	1.442	Boron,[1-[(dimethylamino)methyl]-2-ethyl-1-methylbutyl] dimethylsinalonato (2-)] ethyl-1, (t-4)	0.74
3.	2.552	Tridecanone	3.20
4.	2.678	Serinol	1.84
5.	4.028	Butyl oxitol	2.72
6.	6.105	α -copaene	3.20

7.	6.282	Caryophyllene	31.79
8.	6.403	Humulene	4.43
9.	6.580	Cadina-1(6),4-diene	0.89
10.	6.998	p-Mentha-3,8-diene	0.48
11.	7.158	(Z,E)-Farnesol	0.52
12.	7.450	1-Ethynylcyclopentanol	0.99
13.	7.833	Palmitic acid	15.80
14.	8.171	Phytol	0.44
15.	8.285	α-Linolenic acid	15.48
16.	8.308	Stearic acid	2.11
17.	9.224	Bis(2-ethyhexyl) phthalate	1.03
18.	10.088	Squalene	1.23
19.	11.306	β-Tocopherol	0.44
20.	11.804	Vitamin E	2.56
21.	12.914	Stigmasterol	0.84
22.	13.486	γ-sitosterol	3.65
23.	13.996	β-Amyrin	3.65
24.	14.493	β-Elemene	1.17
		Total	99.64

Table 6: Chemical composition of hexane leaf extract of *G. barbadense*

S/N	R.T	Compound	% composition
1.	1.316	Propanoic acid, 2-chloro-methyl ester	0.51
2.	2.432	9-oximino-2,7-diethoxyfluorene	0.35
3.	4.652	Bicyclo [5.2.0] nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	3.17
4.	4.789	α-Bergamotene	2.39
5.	4.841	γ-Curcumene	11.10
6.	4.955	α-Longipinene	2.72
7.	5.007	1-chloro-2-methyl-1-(N-methyl-N-phenylamino)-1,3-butadiene	1.32
8.	5.127	1,E-8, Z-10-pentadecatriene	
9.	5.161	1H-3a,7-methano azulene, octahydrol,4,9,9-tetramethyl	1.86
10.	5.235	(+)-Spathulenol	3.35
11.	5.401	Trans-z-alpha, Bisabolene epoxide	1.80
12.	5.436	1-Hexen-3-yne,2-tert-butyl-	2.77
13.	5.49	β-Bisabolol	21.85
14.	5.916	Neophytadiene	2.85
15.	5.985	Preg-4-en-3-one, 17.alpha.-hydroxy-17 beta cyano-	1.82
16.	6.036	6-octadecyne	2.20
17.	6.162	Methyl palmitate	2.88
18.	6.197	clovane diol	0.53
19.	6.283	Palmitic acid	20.99
20.	6.500	(+/-)-Ipsdienol	0.32

21.	6.614	Methyl linolenate	12.0
22.	6.769	Stearic acid	1.26
23.	7.496	Benzonitrile, m-phenethyl -	0.49
24.	7.621	Bis (2-ethylhexyl) phthalate	0.37
25.	8.377	Supraene	0.34
26.	8.526	α-Tocospiro A.	1.92
27.	9.727	Stigmasta-3,5-diene	2.02
28.	9.830	Vitamin E	10.72
39.	11.226	beta-sitosterol	2.29
30.	11.650	β-Amyrin	1.09
31.	11.804	2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) -	1.75
32.	12.099	6-Aza-β-homo-5α-cholestano[6,7-d]tetrazole	2.02
		Total	99.49

Antioxidant Activities of the Leaf Extracts

Table 7 presents the antioxidant activity of the various crude extracts of *G. barbadense*. It is known that when testing for antioxidant activity, it is best to employ many methods. Since antioxidant qualities have been shown to be positively correlated with the amount of flavonoids and phenolics in a plant extract, the higher antioxidant activity of the methanol extract of *G. barbadense* is caused by higher quantities of phytochemicals found in the extract (Manach *et al*., 2005; Sharma *et al*., 2009; Zhang *et al*., 2014). Ade-Ademilua and Okpoma (2018) found in a prior study that *G. hirsutum*, a species of Gossypium, had greater levels of flavonoids and phenolic compounds, which translate into higher antioxidant levels.

Table 7: Antioxidant Activities of the leaf extracts of *G. barbadense*

Extracts	% Scavenging activity				EC_{50} @ 50%		
	% inhibition DPPH	**Trolox equivalent ABTS**	**Nitric oxide scavenging**		**DPPH mg/mL**	**ABTS mg/mL**	**NOS mg/ML**
GB_{meth}	63.49 ± 2.62	53.29 ± 1.16	55.23 ± 0.96		4.01	4.79	4.53
GB_{EA}	18.05 ± 1.08	48.97 ± 0.45	40.02 ± 0.23		13.13	5.08	6.20
GB_{hex}	12.63 ± 0.71	36.80 ± 1.94	36.85 ± 1.66		16.41	7.01	6.91
VIT C	69.85	-	90.26		0.007	-	0.005
Gallic acid	68.00	-	82.01		-	-	0.006
VIT E	16.92	-	7.40		-	-	0.067

Key: GB_{me} = Methanolic leaf extract of *G.barbadense*

GB_{EA} = Ethylacetate leaf extract of *G.barbadense*

GB_{hex} = hexane leaf extract of *G.barbadense*

Conclusion

Gossypium barbadense leaf extracts in methanol, ethyl acetate, and hexane were shown to have therapeutic qualities in this study. Active secondary metabolites like flavonoids and phenolic chemicals found in the plant sample's leaves may be responsible for the therapeutic effect. The research also identified *G. barbadense* leaves as a dietary source of antioxidants that may be useful in avoiding or delaying the onset of certain oxidative stress-related illnesses.

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