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Research Article

GC-MS Analysis and Antioxidant Activity of the Essential Oil Constituents from the Dried Leaves of *Gossypium arboreum* LINN

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About Article

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ABSTRACT

The name 'cotton plant' is actually used for four species in the genus *Gossypium*: *G. birsutum L. G. barbadense L. G. arboreum L. G. berbaceum L. Alongside* with their economic importance, the leaf, root, bark and seeds of Gossypium species have been widely explored for their medicinal values. *G. arboreum* possess wound healing and antioxidant properties. This study aims is to extract the essential oil of the dried leaves of *Gossypium arboreum*, determine the oil constituents, and assess the potential antioxidant activities of the oil. The essential oil from 150 g of air-dried leaves of *Gossypium arboreum* was obtained by hydrodistillation method in a Clevenger-type set up. The chemical constituents of the essential oil were analyzed by Gas chromatography coupled with mass spectrometry (GC-MS). The antioxidant activity of the essential oil was also evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) as the reagent and ascorbic acid as the standard. Results obtained was colourless and have a strong odour which was peculiar to the plant with a percentage yield of 0.05%. Twenty eight constituents representing 100% of the oil were identified from the GC-MC spectra. The major components of the essential oil were n-hexane (28.32%), 2-ethyloxetane (25.26%), pentane, 3-methyl (18.50%), and cyclopentane methyl (14.36%). Aliphatic hydrocarbon (66.25%), monoterpene hydrocarbons (3.21%), sesqueterpenoids (0.44%, oxygenated monoterpene (0.08%) and organosilicon compounds (0.38%) were the classes of compound found in the oil. Antioxidant activity at 517m of the essential oil of *G. arboreum* was investigated and the essential oil showed 71.70%, 81.53%, and 88.22% inhibition at concentrations 0.1, 0.2 and 0.3 mg/mL respectively. In conclusion, some of the oil constituents such as limonene, ɣ-terpinene, and caryophyllene that are present in *G. arboreum* has its medicinal uses.

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1. INTRODUCTION

G. arboreum L., also known as tree cotton, is indigenous to the Africa and other subcontinent. From *G. arboreum*, several races have evolved, including indicum, burmanicum, sinense, sudanense, cernuum, and bengalense. Despite accounting for only 2–3% of global cotton production, diploid species play a crucial role as they serve as a valuable source of essential genes for resistance against biotic and abiotic stresses (Parekh *et al.* 2018). Tree cotton, cultivated extensively, is well suited for rainfed conditions. With the rise of challenges like pink bollworm and the whitefly-Cotton Leaf Curl Virus complex, there is a renewed interest in tree cotton (Parekh *et al.* 2016).

These medicinal plant have displayed distinctive features in these modern era of herbal therapy in West Africa, mostly in Nigeria. It is noted to say that there are more than 1000 medicinal plants in Nigeria, and most of their bioactive compounds have not been investigated (Igoli *et al.*, 2005). Medicinal plants, which are known as traditional herbs, have been discovered and used in traditional medicine practices as drugs since inception time. Popularly known commercial medicinal plant species, secondary metabolites and biologically active phytochemicals, found in tropical and subtropical plants, spices, herb and root extracts, are used to prepare herbal concoctions (Ajani *et al.*, 2024; Umoren *et al.*, 2025) These plants synthesize a good number of bioactive compounds for functions that includes defense against insect bites, fungi diseases, and herbivorous mammals (Ahn, 2017). Lot of people depend on traditional herbs as their primary source of drug for use. Around 80% of these people in this world depend on kind of traditional drugs for health treatment. In most developing countries like Nigeria, the use of medicinal plants has been discovered as a basis for maintenance of good health (Tsai *et al.*, 2011) which is readily accessible for use, cheap, sustainable, and easily regenerated (Idowu *et al.*, 2025a).

The use of plants in the tropical and subtropical regions is diversified, and most of their uses are for drug use, source of food, clothing and shelter. However, these plants used for medicinal use are rapidly decreasing among the present generation of local people which is as a consequence of modernization and civilization (Cox, 2005). Major efforts have been contributed on researching and discovering of these great potentials of plant-extracted bioactive chemicals as well as their positive efficacy on human health (Chouhan *et al.*, 2017; Dar *et al.*, 2017). In this approach, useful biological and pharmacological activities have been attributed to essential oils extraction from plants bringing numerous medicinal values. (Angelini & Properzi, 2012).

In recent times, interest in essential oils from *Gossypium arboreum* as antimicrobial agents has grown because of the natural origins, wide range of activities and the "generally recognized as safe" (GRAS) status of essential oils (Pandey *et al*, 2017). Similarly, discoveries towards essential oil for antimicrobial applications has increased over the years as a result of consumers concerns regarding synthetic antimicrobial agents, preservatives and additives (Burt, 2004; Ozogul *et al.*, 2015). *Gossypium arboreum* as a medicinal plant have contributed immensely in the treatment of many diseases and ailments for years. More so, there is scarcity of information on essential oil of this specie of plant. Therefore, the research in the extraction of essential oil of *Gossypium arboreum* leaves will be carried out. For effective essential oil content, Clevenger - type apparatus will be used for hydro distillation method. The study aims to extract the essential oil of the dried leaves of *Gossypium arboreum*, determine the oil constituents, and also assess the potential antioxidant activities of the oil.

2. LITERATURE REVIEW

In Nigeria, broadly identified antidiabatic plant include *Magnifera Indica*, *Gossypium arboreum* and so on (Gbolade, 2009). *G. arboreum* which is known as "cotton plant" has been documented to be an antidiabetic medicinal plant found in different geo-political zones of Nigeria (Abo *et al.*, 2008; Etuk & Mohammed, 2009). *G. arboreum* leaves have been discovered to possess some medicinal properties of antibacterial and antifungal (Saidu & Abdullahi, 2011). The fresh leaf is used for the treatment of ulcers, while the macerated leaf is taken against vomiting, either as a covering (after heating) or as a powder dressing after heating with the fruit of *Piper guineense* Schumach and Thonn in Ghana. A cold inclusion of the leaf with lime juice is also used for the treatment of dysentery, while in Nigeria the flower is used for relieving dysentery. The plant root is considered an emmenagogue, to cause uterine contractions, and be taken as herb for an abortifacient. It was discovered that the juice of the plant root is used in the treatment of fever (Wendel *et al.*, 2010), these factors are associated with one another and change in one factor may lead to change in another which may lead to serious consequences (Idowu *et al.*, 2025b). In view of the presence of gossypol contained in the leaf extract of *G. arboreum*, has been used as oral contraceptive in humans (Coutinho, 2002). Annan and Houghton, (2008) reported that *G. arboreum* showed that it possesses wound healing and antioxidant properties.

The sesquiterpenes has been researched to occur in many cotton species and have been extracted from the leaves, flowers, seeds and boll of cotton plant (Essien *et al.*, 2011). Bell (1986) reported that the total concentration of some sesquiterpenes in essential oil extracted from whole cotton plants are up to 26.12% and 30.1% for *G. hirsutum* and *G. barbadense* respectively. Chemical composition was investigated on Nigerian-grown cotton leaf essential oil, *Gossypium barbadense L.,* (*Malvaceae*) analyzed by GC and GC/MS techniques which revealed the presence of nineteen components, accounting for 92.6% of the total oil fraction. The major constituents were the monoterpenes comprising of tricyclene (29.6%). bornyl acetate (18.6%), α-pinene (12.8%), α-terpinene (11.1%), isoledene (6.0%) and β-pinene (5.4%). There were conspicuous absences of oxygen-containing sesquiterpenoid compounds. It was seen that α- and β-bisabolol, bisabolene oxide, caryophyllene oxide and α-copaene. The specific markers of the essential oil of some Gossypium species, were not detected in the sample under study. The oil displayed moderate antimicrobial potentials to some tested organisms. The oil consists of 12 monoterpene hydrocarbons (66.1%), 1 oxygenated monoterpenoid (18.6%, 4 sesquiterpene hydrocarbons (7.4%) and 1 aliphatic aldehyde (0.5%). (Essien *et al.*, 2011). Also, previous investigation reveals fifteen constituents on this same essential oil of *G. barbadense* representing 75.11% of the oil. The main constituents of the oil were γ-terpinene (22.13%), Cyclohexene (13.28%),

(-)-β-pinene (11.93%) and β-bisabolol (11.15%). Monoterpenes (52.65%), Sesquiterpenes (9.76%) Sesquiterpenoids (11.62%) and diterpenoids (1.08%) were the classes of compounds identified in the oil. (Ogunmoye *et al.*, 2019).

3. METHODOLOGY

3.1. Materials used

n-hexane, 2, 2-diphenyl-1-picryhydrazyl (DPPH) reagent, ascorbic acid, methanol used for this investigation are in Analytical grades and were used without further purification. De-ionized water was used for the preparation of different concentration of samples (Peter *et al.*, 2025; Adisa *et al.*, 2025).

3.2. Collection of plant materials

Dried leaves of *Gossypium arboreum* were collected from Oke-Agbo, Ijebu-igbo Nigeria, and air dried in the laboratory for two weeks. The plant specie was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan.

3.2.1. Extraction of essential oil from plant

The dried leaves of *Gossypium arboreum* essential oil was extracted by hydrodistillation using Clevenger apparatus, using n-hexane as the solvent. The plant material was cut into smaller pieces and 150 g of plant materials were weighed with a weighing balance and was dispersed into a boiling flask of 5000 mL. 500 mL of water was added into the boiling flask which was then placed on the heating mantle. The set up for extraction consisted of a chiller which was filled occasionally with ice from the ice making machine. To prevent the escape of the liquefied oil, 4 mL of n-hexane was injected into the Liebig condenser and sealed with cotton wool to avoid evaporation of the oil. The temperature of the chiller did not exceed 20 °C. This process was experimented for 3 h before finally extracting the essential oil (British Pharmacopoeia, 1980). After the collection of the oil, it was preserved in the refrigerator to prevent it from escaping until the GC-MS analysis was carried out.

3.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the extracts of *G. arboreum* L was performed on an Agilent Technology HP 7890 plus gas chromatography fitted with HP-5 MS column (30 m x 0.320 mm, 0.25 um film thickness, Agilent Technology). The analytical conditions made were: carrier gas Helium (1 mL/min), injector temperature (PTV-programmed temperature 250 °C, column temperature programmed from 80 °C (2 min hold) to 240 °C (6 mins hold) at 12 °C/min. Samples were injected by mode of analysis split less. The volume injected was 1.0 pL. Inlet pressure was 6.1 kPa.

3.4. Antioxidant activity assay

The antioxidant activity of the essential oil of *Gossypium arboreum* was determined using DPPH free radical method and ascorbic acid was used as standard. 0.004 g of 2, 2-diphenyl-1-picryhydrazyl (DPPH) was weighed and dissolved on 100 mL of methanol then ascorbic acid was prepared by weighing 0.005 g of ascorbic acid and dissolving it in 5 ml of methanol. Nine test tubes was used for the analysis, three for each concentration (0.1, 0.2 and 0.3 mL). 2 mL of methanol was added to each test tube, followed by 1 mL of DPPH solution and the essential oil was added to each test tube depending on the concentration, the total solution was then made up to 4 mL by addition of methanol.

The samples were kept in a dark cupboard for 30 minutes after they had been thoroughly sealed with aluminum foil. After 30 minutes, the samples were taken to the UV-VIS spectrophotometer for analysis. Then, methanol was used to rinse the cuvette, after which the wavelength range was set to 517 mm and the absorbance of each sample was measured and recorded. The absorbance of the DPPH solution was also taken and recorded. The experiment was done in triplicate. This same process was repeated using ascorbic acid solution (reference sample), this was the blank experiment. The antioxidant activity of each solution was calculated as percent inhibition according to the following equation below:

$$\% \text{ Inhibition} = \frac{A_{blank} - A_{sample}}{A_{bank}} \times 100$$

Where,

A_{blank} = the absorbance of blank

A_{sample} = the absorbance of the test (sample).

4. RESULTS AND DISCUSSION

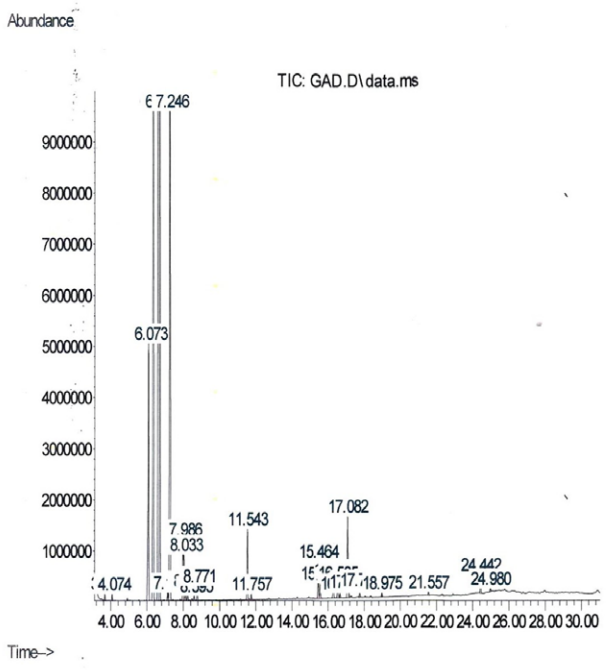

Figure 1. Chromatogram of essential essential oil from *Gossypium arboreum* leaves

Table 1. Chemical Composition of Essential Oil from *Gossypium arboreum*

Retention time	Compound Name	Kovat Index (Cal)	Kovat Index (Lit)	% Composition	M.F.
3.676	Propane	361	300	0.09	C_3H_6
4.074	1-propene,2-methyl	350	370	0.10	C_4H_8
6.073	Butane,2,3-dimethyl	577	573	5.00	C_6H_{14}
6.335	Pentane,3-methyl	600	595	18.50	C_6H_{14}
6.602	n-Hexane	524	N.A	28.32	C_6H_{14}
6.685	2-ethyloxetane	615	N.A	25.26	$C_5H_{10}O$
7.140	Butane,2,2,3-trimethyl-	600	626	0.13	C_7H_{16}
7.246	Cyclopentane,methyl-	687	675	14.36	C_6H_{12}
7.986	Cyclohexane	694	694	1.17	C_6H_{12}
8.033	Hexane,2-methyl-	634	650	0.75	C_7H_{16}
8.123	Pentane,2,3-dimethyl	700	684.1	0.71	C_7H_{16}
8.243	Hexane,3-methyl-	725	700	0.17	C_7H_{16}
8.595	Hexane,2,2-dimethyl	700	715	0.09	C_8H_{18}
8.771	Heptane	788	N.A	0.25	C_7H_{16}
11.543	Cyclotrisiloxane,hexamethyl	886	833	1.32	$C_6H_{18}O_3Si_3$
11.757	Heptane,2,4-dimethyl	622	797	0.11	C_9H_{20}
15.464	β-myrcene	803	967	0.69	$C_{10}H_{16}$
15.576	Cyclotetrasiloxane,octamethyl-	1000	1000	0.31	$C_8H_{24}O_4Si_4$
16.300	P-cymene	851	992	0.40	$C_{10}H14$
16.535	D-limonene	1000	N.A	0.32	$C_{10}H_{16}$
16.662	1,3,5-octatriene,3,7-dimethyl	1000	1035	0.09	$C_{10}H_{16}$
17.082	ɣ-terpinene	1170	1079	1.56	$C_{10}H_{16}$
17.157	Dodecane	1000	209	0.10	$C_{12}H_{26}$
17.754	Cyclohexane,1-methyl-4-(1-methylethylidene)	1134	1025.9	0.15	$C_{10}H_{18}$
18.975	Cyclopentasiloxane, decamethyl-	1064	1160	0.07	$C_{10}H_{30}O_5Si_5$
21.557	Bornyl acetate	1236	1247	0.08	$C_{12}H_{20}O_2$
24.442	Caryophyllene	1453	1449.01	0.36	$C_{15}H_{24}$
24.980	Humulene	1533	1491	0.08	$C_{15}H_{24}$

The essential oil obtained was colourless with a strong odour which was peculiar to the plant, also as a percentage yield of 0.05% (v/w). The compositions found in the oil are displayed above in table 1. The oil sample was analyzed by GC and GC-MS and the components were identified on the basis of their RI values, co-injection with the available authentic samples. The result obtained from the chromatographic analysis of *G. arboreum* L essential oil shown in figure 1. while the identities of the constituents as well as their percentage composition could be seen in table 4.0. Twenty eight constituents were identified in the oil of *G. arboreum* representing 100% of *G. arboreum*. The oil consists of 17 aliphatic hydrocarbon (95.89%, 6 monoterpene hydrocarbon (3.21%), 2 sesqueterpenoids (0.44%), 1 oxygenated monoterpene (0.08%) and 2 organosilicon compounds (0.38%). The major componenent of the essential oil are 2-ethyloxetane (25.26%), pentane, 3-methyl (18.50%), cyclopentane methyl (14.36%). Other significant constituents of the oil were butane, 2,3-dimethyl (5.00%), ɣ-terpinene (1.50%), cyclotrisiloxane, hexamethyl- (1.32%), cyclohexane (1.17%).

In comparison with the previous investigation of *Gossypium barbadense* by (Essien *et al.*, 2011) which has its major oil constituents as tricyclene (29.6%), bornyl acetate (18.6%), α-pinene (12.8%, and α-terpinene (11.1%) but this study shows that n-haxane (28.32%), 2-ethyloxetane (25.26%, pentane, 3-methyl (18.50%, and cyclopentane, methyl (14.36%) are the major components. Bornyl acetate which is the major

constituents in the previous study by Essien is a minor constituent in this study. Also, the previous investigation of *G. barbadense* by (Ogunmoye *et al.*, 2019) shows that r-terpinene (22.13%), cyclohexane (13.28%), (-)-β-pinene (11.93%), and β-bisabolol (11.15%) were the major constituents but in this study, ɣ-terpinene (1.56%) and cyclohexane (1.17%) occurs as the minor components.

Myrcene (0.6%), limonene (0.1%, β-caryophyllene (0.2%), α-humulene (0.1%) which were present in the previous study of *G. barbadense* by (Essien *et al.*, 2011) are absent in this study but p-cymene (0.40%) and ɣ-terpinene (1.56%) were detected in this study as minor constituent. Caryophyllene (4.50%) were also detected as minor constituents in the previous study by (Ogunmoye *et al.*, 2019).

4.2. Antioxidant activity result

Table 2. Percentage Inhibition of Dried *Gossypium arboreum*

Concentration (mg/mL)	Absorbance at 517 nm			Mean/SD	% Inhibition
0.1	0.323	0.328	0.567	0.406±0.114	71.70
0.2	0.312	0.260	0.224	0.265±0.036	81.53
0.3	0.179	0.167	0.160	0.169±0.005	88.22

A_{blank} = 1.435

Table 3. Percentage inhibition of Ascorbic Acid

Concentration (mg/mL)	Absorbance at 517 nm			Mean/SD	% Inhibition
0.1	0.021	0.024	0.022	0.022±0.001	98.47
0.2	0.020	0.021	0.021	0.021±0.001	98.53
0.3	0.020	0.019	0.021	0.020±0.001	98.54

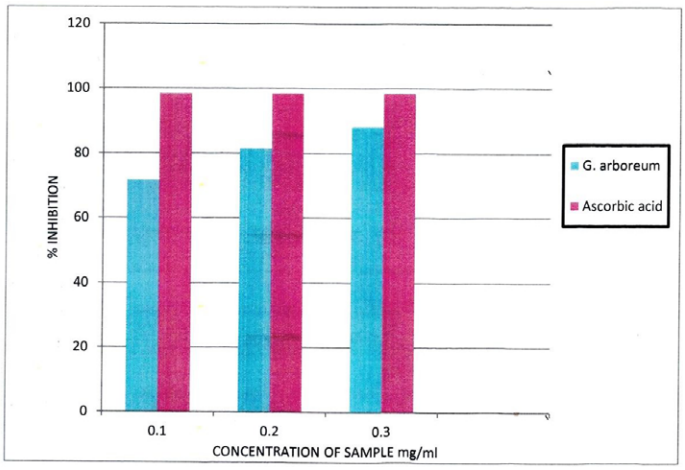

Figure 2. Bar chart of the antioxidant activity of *G. arboreum* and ascorbic acid.

The samples tested showed very good activity but lower than the standard at the same concentration (Table 1 and 2). Absorption at 517 nm was decreased as observed which indicates that the samples have the ability to donate hydrogen or can scavenge free radical. This was also shown in the calculated percentage inhibition. Figure 1 above also corroborated the *Gossypium arboreum* antioxidant activity. The concentration increases between 71.70 - 88.22% but still lower compared to the standard at the difference concentration.

5. CONCLUSION

In conclusion, the essential oil from the dried leaves of *Gossypium arboreum* grown in Nigeria has been documented as a medicinal plant for the treatment of bronchitis, cough and phlegm due to the presence of p-cymene, also used to prevent and treat cancer due to the presence of limonene. The leaves have been found to possess some anti-inflammatory, antimicrobial, antibacterial and antioxidant properties due to the presence of cryophyllene. Also, the oil sample of *G. arboreum* exhibit good antioxidant properties via the 2,2-diphenyl-1-picryhydrazyl (DPPH) method.

RECOMMENDATION

The plant can be used for several application in cosmetic, agricultural and pharmaceutical industries as its components can be used in the production of perfumes and personal care products, drugs, pesticides and insect repellents. Further work should be carried out to isolate and characterize the identified components from the oil.

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