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RADICAL SCAVENGING ACTIVITY OF ESSENTIAL OIL FROM THE LEAVES OF GOSSYPIUM BARBADENSE (LINN)

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ABSTRACT

The essential oil constituents obtained by hydrodistillation in an all glass Clevenger apparatus of air-dried leaves of *Gossypium barbadense* growing in Nigeria was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). Fifteen constituents representing 75.11 % of the oil were identified from the GC-MC spectra. 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.

The examined *G. barbadense* essential oil for its antioxidant activity at 517 nm was able to reduce the stable, purple-coloured radical DPPH' into yellow-coloured DPPH-H. It can be seen that *G. barbadense* exhibited is lower than the DPPH % inhibition of the ascorbic acid at the same concentration.

Keywords: *Gossypium barbadense*, gamma terpinene, Cyclohexene and DPPH.

1. INTRODUCTION

The cotton genus *Gossypium* (Family Malvaceae) comprises of about 50 species and few new species continue to be discovered (Wendel *et al*., 2009). *Gossypium barbadense*, also known as extra-long staple (ELS) cotton, is a species of cotton plant that has been cultivated to have extra-long staple fibers - longer than 34 mm (1 3/8") that are associated with high quality products. *Gossypium barbadense* widely used in African folk medicine for the treatment of malaria and fever, stomach-ache, coughs, sexually transmitted diseases, tooth ache, breast cancer and constipation (Iwu, 2010). In South Eastern and part of the Midwestern Nigeria, the plant is used for the treatment of septic wounds and eye problems (Gill, 2012). In traditional medicine, *G. barbadense* leaves have been used as a treatment for swollen tissue around a wound and for nausea during pregnancy (Essien *et al.,* 2011). Phytochemical analysis of the plant revealed the presence of alkaloids and phenylpropanoids in the root, flavonoids and tannins in the leaf (Usman and Osuji, 2007). In continuation of our research into the essential oil components of poorly studied Nigeria plants (Ogunmoye *et al.,* 2015) we report in this paper the components and antioxidant activities in the essential oil of the leaves of *G. barbadense*.

2. MATERIALS AND METHODS

2.1 Plant materials

The plant materials were collected from their natural habitat on the ground of Oke pad, Ago-iwoye, Ogun State, Nigeria. Botanical identification and authentication was done at the Forest Research Institute of Nigeria (FRIN), Ibadan as ***Gossypium barbadense*** of the family Malvaceae with herbarium no. 110899. A voucher specimen was deposited at the herbarium.

2.2 Extraction of essential oil

The air-dried and pulverized leaves of *G. barbadense* (200 g) were subjected to hydrodistillation in a Clevenger-type glass apparatus for 3 h in accordance with established procedure (British Pharmacopoeia, 1980). The oil collected was preserved in a sample tube and stored under refrigeration until moment of analysis.

2.3 Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

GC-MS analysis of the oil was performed on an Agilent technology 7890A. Gas chromatograph equipped with a FID and fitted with a fused silica capillary HP-5 MS column (30 m x 0.32 mm id, film thickness 0.25 μm). The oven temperature was programmed from 80-240^{o}C at the rate of 8^{o}C/min. The ion source was set at 240^{o}C and electron ionization at 70eV. Helium was used as the carrier gas at a flow rate of 2 mL/ min. Scanning range was 35-425 amu. Diluted oil in n-hexane (1.0 μL) was injected into the GC/MS.

2.4 Identification of the Constituents

The identification of the constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of n-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST (Data base 69) and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values (Joulain and Koenig, 1998; Adams, 2007).

2.5 Antioxidant activity assay

The antioxidant activities or the capacity to scavenge the "stable" free radical DPPH was determined using the DPPH free radical scavenging method. Free radical scavenging activity of essential oil from *G. barbadense* plant was measured by 1, 1- diphenyl-2-picrylhydrazyl (DPPH). 9.0 mL of methanol was added to1.0 mL of the essential oil in the test tube, 5.0 mL, 7.5 mL and 8.75 mL of the mixture was put into a separate test tube, Then 1.0 mL each of DDPH methanol solution was added into the other three portion and allowed to stand for 30 minutes in the dark room temperature for any reaction to take place. Ultraviolent (UV) Absorbance of these solutions were recorded on a spectrometer at 517nm using a blank containing the same concentration of oils without DDPH. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

$$\text{AA} = \frac{A_C - A_S}{A_C}\ x\ 100$$

Where

A_c = the absorbance of control.

A_s = the absorbance of the test (sample).

AA = the antioxidant activity.

3.0 RESULTS AND DISCUSSION

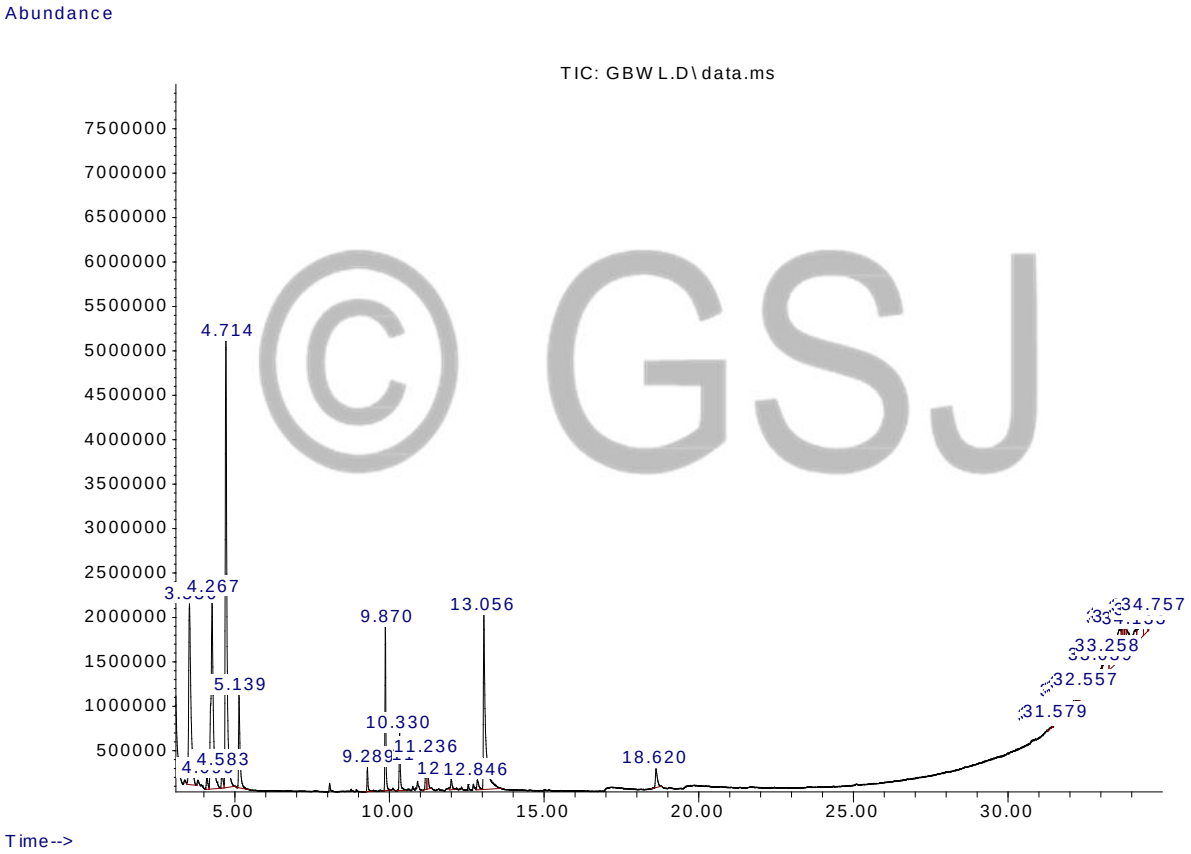

Figure.1.0 Chromatogram of *Gossypium barbadense* Essential oil Components

Table 1.0: Chemical Composition of Essential Oil from *Gossypium barbadense*

S/N	NAME	KOVAT INDEX$^{(cal)}$	KOVAT INDEX$^{(lit)}$	RETENTION TIME	% COMPOSITION
1	(-)-β-pinene	862	980	3.553	11.93
2	α-terpinene	1000	1000	4.100	0.53
3	Cyclohexene	1000	850	4.266	13.28
4	Ocimene	1000	1016	4.580	0.79
5	Γ-terpinene	1000	1022	4.712	22.13
6	α-terpinolene	1047	1050	5.141	3.99
7	Cubenene	1439	1432	9.290	0.70
8	Caryophyllene	1500	1504	9.867	4.50
9	β-Caryophyllene	1500	1467	10.331	2.02
10	Cis-γ-bisabolene	1500	1503	11.178	0.90
11	Naphthalene	1500	1508	11.235	1.08
12	β-Caryophyllene Oxide	1500	1542	12.002	0.47
13	Trans- β-farnescene	1500	1493	12.849	0.56
14	β-bisabolol	1521	1649	13.005	11.15
15	Hexadecyl pentyl ether	2140	-	18.622	1.08

The extraction brought about a cloudy light yellow with a strong odour. The essential oil obtained has a percentage yield of 3.275% (v/w). The result of the chromatographic analysis obtained for the essential oil shown in figure 1.0 while the identities of the constituents as well as their percentage composition could be seen in Table 1.0. Fifteen constituents were identified representing 75.11 % of *G. barbadense* with the following components; Γ-terpinene (22.13%), Cyclohexene (13.28%), (-)-β-pinene (11.93%) and β-bisabolol (11.15%) as the major

components. Other significant constituents of the oil were Caryophyllene (4.50%), α-terpinolene (3.99%), β-Caryophyllene (2.02%), Naphthalene (1.08%) and Hexadecyl pentyl ether (1.08%). Monoterpenes hydrocarbon (52.65%) was the maximum constituents in the extracts of the volatile composition while others are sesquiterpenes hydrocarbon (9.76%), sesquiterpenoids (11.62%) and diterpinoids (1.08%). It was observed that β-bisabolol and caryophyllene oxide which are among the specific markers of the essential oil of some *Gossypium* species, were detected in the sample under study.

A comparison of the present result with previously analysed samples of *G. barbadense* revealed some qualitative and quantitative variations. For example, 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%) were the main compound reported (Essien *et al.,* 2011) compared to what is obtained under the present study.

Similarly, the presence of (-)-β-pinene, α-terpinene, Ocimene, Γ-terpinene and β-Caryophyllene in this sample is in accordance with previously reported data, though non-quantitatively (Minyard *et al.,* 1965; Essien *et al.,* 2011). In addition, aliphatic alcohols such as cis-3-hexen-1-ol, trans-2-hexen-1-ol, 1-penten-3-ol and 6-octen-4-ol (Hedin *et al.,* 1971b), which are characteristics compounds of *G. hirsutum* var Deltaphine, were not detected in this investigation. Also, some of the components reported from the fruits oil (Essien *et al.,* 2017) are found in the leaves oil with some exception like p-cymene (1.35%), limonene (3.07%), borneol acetate (0.4%), α-copaene (8.48%) and δ-cadinene (3.90%) in varying quantity. The component with highest percent reported in the fruit oil is β-caryophyllene (40.97%) (Essien *et al.,* 2017), while that in leave oil is Γ-terpinene (22.13%). This suggested that there are qualitative and/or quantitative variations in the volatile compounds present in the different parts of *G. barbadense*. This may have been responsible for the observed compositional variations in the volatile oils of this plant from Nigeria.

Table 1.1: Percentage inhibition of ascorbic acid and GBWL

			Absorbance at 517 nm	
Ascorbic acid			GBWL	
Concentration (mg/ml)	Mean/SD	% inhibition	Mean/SD	% inhibition
0.1	0.130±0.006	68.67	0.165±0.000	60.24
0.2	0.123±0.014	70.36	0.180±0.002	56.62
0.3	0.131±0.002	68.43	0.170±0.001	59.04
0.4	0.126±0.004	69.64	0.187±0.002	54.94
0.5	0.123±0.006	70.36	0.167±0.002	59.76

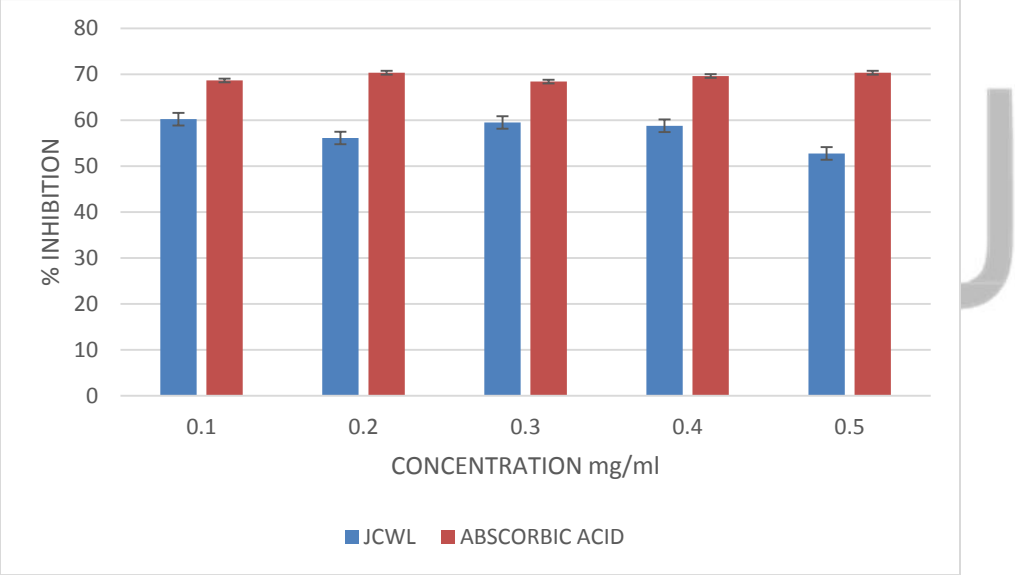

Fig. 1.1: The bar chart of scavenging activity of GBWL and ascorbic acid

The reduction in absorbance of DPPH at 517nm caused by the samples was measured in triplicate after 10 min. The tested samples showed very good activity but lower than the standard at the same concentration (Table 1.1). There was decrease in absorption at 517 nm indicating that the samples have hydrogen donating ability or can scavenge free radical. This was also shown in the calculated percentage inhibition. Figure 1.1 above also corroborated the *Gossypium barbadense* antioxidant activity. Generally the percentage inhibition fluctuated with increase in

concentration between 54.94 – 60.24% but still lower compared to the standard at the difference concentration.

CONCLUSION

In conclusion, it was observed that there were variation in some of the compositional pattern from previous studies on the essential oil from the plants from other part and this may be due to the ecological, age of the plant, period of collection, handling procedure and climatic condition. Also the oil sample of *G. barbadense* exhibit antioxidants properties via the DPPH method but not as effective as the standard used, therefore *G. barbadense* can be used to repair the damage tissue.

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