

Full Length Research Paper

Constituents and antimicrobial properties of the leaf essential oil of *Gossypium barbadense* (Linn.)

Emmanuel E. Essien¹, Sherifat O. Aboaba² and Isiaka A. Ogunwande^{3*}

¹Department of Chemistry, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

²Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

³Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway Ojo, P. M. B. 0001, Lasu Post Office, Ojo, Lagos, Nigeria.

Accepted 15 June, 2010

The chemical composition of the Nigerian-grown cotton leaf essential oil, *Gossypium barbadense* L., (Malvaceae) analyzed by GC and GC/MS techniques 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%), isodene (6.0%) and β -pinene (5.4%). There were conspicuous absences of oxygen-containing sesquiterpenoid compounds. It was observed 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.

Key words: *Gossypium barbadense*, essential oil composition, tricyclene, bornyl acetate, antimicrobial.

INTRODUCTION

Gossypium barbadense Linn (family Malvaceae) is a perennial under shrub 1 - 3 m high, native to South America and now distributed from Senegal to Nigeria and widely cultivated in tropics. In Nigeria, the cottonseed provides raw materials for local spinning and weaving industry. An infusion of the leaf is taken as an antedote for colds and bronchitis and the young shoots pulped for palpitations and as dressings for wounds and in the treatment of systematic diarrheas (Busari, 1998). A number of bioactive triterpenoid and sesquiterpenoid aldehydes compounds have been isolated and characterized from this and related species. They include gossypol (Zhou and Linn, 1988; Stipanovic et al., 2005), hemigossypol, 6-methoxyhemigossypol, 6-deoxyhemigossypol, 6-methoxyhemigossypol, 6, 6'-dimethoxy hemigossypol (Bell et al., 1975; Stipanovic et

al., 1975), helicoides H1 and H2 (Stipanovic et al., 1978) and gossyrubilone (Bell et al., 1978). Other non-aldehydic compounds that have been characterized as constituents of the plant are 3, 4-dihydroxy-5-isopropyl-7-methyl-2H - naphtho [1, 8-bc] furan, 3-hydroxy-5-isopropyl-4-methoxy-7-methyl-2H - naphtho [1, 8-bc] furan and desoxy-6-methoxyhemigossypol (Stipanovic et al., 1975). Flavonols (Waage and Hedin, 1984), tannins (Chan et al, 1978), cytotoxic sulfated compounds (Piccinelli et al., 2008); sesquiterpene glycosides (Stipanovic et al., 1980; Davila-Huerta et al., 1995) and sesterpenoid (Stipanovic et al., 1977) were also characterized from various species of *Gossypium*. Many of these compounds have exhibited several biological potentials such as antimicrobial, insecticidal and cytotoxic. Essential oils of the leaves, stems or flower buds of the cotton plant, as well as cottonseed oil are attractive to the boll weevil, *Anthonomus grandis* Boeham (Minyard et al., 1969) and the cotton leafworm, *Spodoptera littoralis* (Hedin et al., 1972). Earlier studies on the essential oils of *Gossypium* species have been mainly on *G. barbadense* L. var. Giza

*Corresponding author. isiaka.ogunwande@lasunigeria.org.
Tel: + 234 805 9929 304.

69 (Hedin et al., 1972a) and *Gossypium hirsutum* L. var. Deltapine Smoothleaf (Hedin et al., 1972b; Hedin et al., 1971a, b; Minyard et al., 1965; Minyard et al., 1966; Minyard et al., 1968).

As part of the systematic analysis of chemical constituents of the essential oil of relatively poorly studied species of Nigerian medicinal plants and herbs (Ogunwande et al., 2008), we report herein the chemical composition of the oil obtained from the leaves of *G. barbadense*. To the best of our knowledge, there has not been any literature report on the constituents of this plant species from Nigeria.

MATERIALS AND METHODS

Plant materials and isolation of the oils

The leaves of *G. barbadense* were harvested from cultivated plants within the vicinity of the Department of Chemistry, University of Ibadan, Ibadan, Nigeria, in May 2004. Mr. F. Usang of the Herbarium Headquarters, Forest Research Institute of Nigeria (FRIN), Ibadan, identified the plant species. Voucher specimen, FHI 107327 was preserved at the Herbarium of the Institute. The air-dried pulverized plant (300 g samples) was hydrodistilled (3 h) in an all glass Clevenger-type apparatus to obtain light yellow oil. The oil (yield 0.4% v/w) was stored under refrigeration until the moment of analysis.

Oil analysis

Gas chromatography (GC) analysis was performed on an Orion Micromat 412 double-focusing chromatography system, fitted with two capillary columns, coated with CP-Sil 5 and CP-Sil 19 (fused silica, 25 m x 0.25 mm, 0.15 mm film thickness) and flame ionization detector (FID). The volume injected was 0.2 mL and split ratio was 1:30. Oven temperature was programmed from 50 - 230°C at 3°C/min, using hydrogen as carrier gas. Injection and detector temperatures were maintained at 200 and 250°C, respectively. Quantitative data were obtained by electronic integration of FID area percentage without the use of correction factors.

For Gas chromatography-Mass spectrometry (GC-MS) analysis, a Hewlett-Packard HP5890A GC was interfaced with a VG Analytical 70-250s double-focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage, 70 eV; ion source temperature, 230°C. The GC was fitted with a 25 m x 0.25 mm fused capillary column coated with CP-Sil 5. The GC operating parameters were identical with those of the GC analysis. Retention indices for all the compounds were determined according to Kovats method, relative to the n-alkanes series.

Identification of compounds

The identification of the compounds was achieved by comparison of their retention indices and by matching their fragmentation patterns in MS with those of published MS data (Adams, 2001; Joulain and Koenig, 1998).

Antimicrobial activities

The antimicrobial activity of the oil was determined using agar well

diffusion techniques (Adeniyi, 1996; Cotter and Adley, 2001). Sensitivity test agar plates were each screened with 0.1 mL of an overnight cultured of each bacteria (equivalent to 108 CFU/mL) while the Sabourand dextrose agar plates were each similarly seeded with 0.1 mL of an overnight culture of each fungi (equivalent to 108 CFU/mL). Mueller Hinton agar was used to assay the *Niesseria gonorrhoea*, *Gardinella* spp and *Klebsiella aerogenes*. The seeded plates were allowed to set and then dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar into which was added 60 mL solution of the oil extract re-suspended in 1% DMSO. The plates were incubated at 37°C for 24 h and at 25°C for 72 h for bacteria and fungi respectively after which diameter of zones of inhibition were measured. 1% DMSO was included in each plated as negative control while gentamicin (10 mg/mL) and tioconazole at 2% were used as positive control for bacteria and fungi respectively. All assays were carried out in triplicates and diameter of zones of inhibition (mm) expressed as means and standard errors of means.

RESULTS AND DISCUSSION

The compositions of the oil are displayed 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 and by comparison of their mass spectra with those reported in the literature. Nineteen constituents were identified in the oil of *G. barbadense*, representing 92.6% of the total oil fraction. The oil consists of 12 monoterpene hydrocarbons (66.1%), 1 oxygenated monoterpene (18.6%), 4 sesquiterpene hydrocarbons (7.4%) and 1 aliphatic aldehyde (0.5%). There was a conspicuous absence of oxygen containing sesquiterpenoid compounds. The major constituents were tricyclene (29.6%), bornyl acetate (18.6%), α -pinene (12.8%), and α -terpinene (11.1%). Other significant constituents of the oil were isodene (6.0%), β -pinene (5.4%), p -cymene (2.1%) and terpinolene (2.0%).

By comparison of the present oil composition with the previous investigations into the volatile oils of *Gossypium* species, it could be seen that δ -cadinene, β -caryophyllene oxide and α -copaene (Hedin et al, 1972a), which are the major compounds of *G. barbadense* var. L. Giza, were not detected in the present study. Both α -humulene and β -caryophyllene, though could be identified, were present in insignificant quantity. 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), as well as the terpenoids α -terpineol, α - and β -bisabolol and bisabolene oxide (Hedin et al, 1972b; Hedin et al, 1971a, b; Minyard et al, 1966; Minyard et al, 1968), which are characteristics compounds of *G. hirsutum* var Deltaphine, were not detected in this investigation.

The Nigerian oil sample of *G. barbadense* was found to possess fewer number of aliphatic alcohols and sesquiterpene compounds than previously reported for other *Gossypium* species. A noteworthy observation was

Table 1. Chemical constituents of the essential oil of *G. barbadense*.

RI ^a	Compounds	Percentage composition (%)
	(<i>E</i>)-2-hexenal ^b	0.5
934	Tricyclene	29.6
935	α -Thujene	0.2
943	α -Pinene	12.8
956	Camphene	0.2
977	Sabinene	1.4
982	β -Pinene	5.4
991	Myrcene	0.6
1018	α -Terpinene	11.1
1022	p -Cymene	2.1
1033	Limonene	0.1
1045	(<i>E</i>)- β -Ocimene	0.3
1057	γ -Terpinene	0.3
1086	Terpinolene	2.0
1275	Bornyl acetate	18.6
1381	Isoledene	6.0
1421	β -Caryophyllene	0.2
1454	α -Humulene	0.1
1516	γ -Cadinene	1.1
Total		92.6

^a RI, relative retention indices on CP-Sil 5 capillary coated column; ^b identified only by MS.

Table 2. Antimicrobial profile of the essential oil of *G. barbadense*.

Organisms [#]	Zones of inhibition ^a
<i>Staphylococcus aureus</i> (NCTC 6571)	18 \pm 0.5
<i>Gardinerella spp</i> (UCHSTC 2182)	20 \pm 0.4
<i>Klebsiella aerogenes</i> (UCHSTC 2134)	-
<i>Neisseria gonorrhoea</i> (UCH 2303)	-
<i>Neisseria gonorrhoea</i> (ATCC 19424)	-
<i>Escherichia coli</i> (UCH 2306)	15 \pm 0.5
<i>Escherichia coli</i> (NCTC 9001)	18 \pm 0.3
<i>Pseudomonas aeruginosa</i> (UCH 2145)	-
<i>Candida albicans</i> (UCHSTC 2112) ^b	-

^a zones of inhibition as a mean of three replicates with standard deviation expressed as \pm , ^b tioconazole was used as positive control (zone of 25 \pm 0.3) [#] gentamicin was used as positive control for bacteria (zones of inhibition ca. 30 \pm 0.4), NCTC- National Collection Type Culture, ATCC- American Type Culture Collection, UCH/ UCHSTC- Clinical isolates, University of Ibadan Teaching Hospital Complex, College Hospital Collection.

the fact that tricyclene and bornyl acetate, the major compounds of the species under investigation, were not reported previously to be part of the constituents of the oil of *G. barbadense*. The presence of α -pinene, myrcene and β -ocimene in this sample is in accordance with previously reported data, though non-quantitatively,

(Minyard et al., 1965).

In conclusion, it may be mentioned that the oil of *Gossypium* species so far studied could be represented in three chemical forms-; (a) oils containing abundance of hydrocarbon and oxygenated monoterpenoids (this study), (b) oils containing an abundant of hydrocarbon and oxygenated sesquiterpenoids, and (c) oils rich in aliphatic alcohols, as previously described.

The quantitative composition and the relative proportions of the oil components are widely influenced by the genotype, ontogenic development, and the environmental and growing conditions, or on the plant species, the chemotypes and the climatic conditions. This might account for the reason for the observed compositional difference between the plant of study and results from previous investigations, considering the different ecological and climatic conditions between Nigeria and the rest of the world.

In addition, the oil displayed moderate antimicrobial activities to *S. aureus*, *Gardinerella spp*, *E. coli* and *C. albicans* when compared with the standard (Table 2). This observation may lend credence to the antimicrobial properties of the extracts of this species. The antimicrobial activities of an essential may be due to the major components or synergy between major and minor compounds. α -Pinene has been known to show efficient antimicrobial properties (Ganni et al., 2005). We have previously reported that the essential oil of *Eucalyptus torrelliana* (Jimoh et al., 2005) and *Taxodium distichum* (Ogunwande et al., 2007) rich in α -pinene exhibited antimicrobial and cytotoxic activities.

ACKNOWLEDGEMENTS

The authors are grateful to Late Mr. F. Usang of FRIN, Ibadan, Nigeria, for the identification of the plant sample. Dr. Adio formerly of the Institut fur Organische Chemie, Universitat Hamburg, Germany assisted in the GC and GC-MS analysis. Dr. (Mrs.) Adeniyi of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria, performed the antimicrobial assays.

REFERENCES

- Adams RP (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition, Allured Pub. Corp., Carol Stream, IL.
- Adeniyi BA (1996). Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae). Afr. J. Med Sci., 25: 221-224.
- Bell AA, Stipanovic RB, Howell CR, Fryxell PA (1975). Antimicrobial terpenoids of *Gossypium*: Hemigossypol, 6-methoxyhemigossypol and 6-deoxyhemigossypol. Phytochem., 14: 225-231.
- Bell AA, Stipanovic RD, O'Brien DH, Fryxell PA (1978). Sesquiterpenoid aldehyde quinines and derivatives in pigments glands of *Gossypium* Phytochem., 17: 1297-1305.
- Busari AA (1984). An annotated booklet of some medicinal plants of South-west Nigeria. Armetu Publisher, Ibadan, p. 70.
- Chan BG, Waiss Jr. AC, Lukefahr MJ (1978). Condensed tannin, an antibiotic chemical from *Gossypium hirsutum*. J. Insect Physiol., 24: 113-118.

- Cotter G, Adley CC (2001). Comparison and evaluation of antimicrobial susceptibility testing of Enterococci performed in accordance with Six National Committee Standardized Disk Diffusion Procedures. *J. Clin. Microbiol.*, 39: 3753-3756.
- Davila-Huerta G, Hamada H, Davis GD, Stipanovic RD, Adams CM, Essenberg (1995). Cadinane-type sesquiterpenes induced in *Gossypium cotyledons* by bacterial inoculation. *Phytochem.*, 39: 531-536.
- Ganni S, Silvia M, Mariavittoria M, Martina S, Safano M, Matteo R, Renato B (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem.*, 91: 621-632.
- Hedin PA, Thompson AC, Gueldner RC, Minyard JP (1971a). Isolation of α -bisabolol from the cotton bud. *Phytochem.*, 10: 1693-1694.
- Hedin PA, Thompson AC, Gueldner RC, Minyard JP (1971b). Constituents of the cotton bud. *Phytochem.*, 10: 3316-3318.
- Hedin PA, Thompson AC, Gueldner RC, Rizk AM, Salama HS (1972a). Egyptian cotton leaf essential oil. *Phytochem.*, 11: 2356-2357.
- Hedin PA, Thompson AC, Gueldner RC, Ruth JM (1972b). Isolation of bisabolene oxide from the cotton bud. *Phytochem.*, 11: 2118-2119.
- Jimoh ST, Ogunwande IA, Olawore NO, Walker TM, Schmidt JM, Setzer WN, Olaleye ON, Aboaba SA (2005). In vitro cytotoxicity activities of essential oils of *Eucalyptus torrelliana* F.v. Muell (leaves and fruits). *J. Essent., Oil-Bearing Pl.* 8: 110-119
- Joulain D, Koenig WA (1998). The atlas of spectral Data of sesquiterpene Hydrocarbons. E-B Verlag: Hamburg.
- Minyard JP, Tumlinson JH, Hedin, PA, Thompson, AC (1965). Isolation and Identification, Constituents of Cotton Bud. Terpene Hydrocarbons. *J. Agric. Food Chem.*, 13: 599-602.
- Minyard JP, Tumlinson JH, Thompson AC, Hedin PA (1966). Constituents of the Cotton Bud. Sesquiterpene Hydrocarbons. *J. Agric. Food Chem.*, 14: 332-336.
- Minyard JP, Thompson, AC, Hedin PA (1968). Constituents of the cotton bud. VIII. β -Bisabolol, a new sesquiterpene alcohol. *J. Org. Chem.*, 33: 909-911.
- Minyard JP, Hardee DD, Gueldner RC, Thompson AC, Wiygul G, Hedin PA (1969). Constituents of the cotton bud compounds attractive to the boll weevil. *J. Agric. Food Chem.*, 17: 1093-1097.
- Ogunwande IA, Olawore NO, Ogunmola OO, Walker TM, Schmidt JM, Setzer WN (2007). Cytotoxic effects of *Taxodium distichum* oils. *Pharm. Biol.*, 45: 106-110.
- Ogunwande IA, Essien EE, Ogunbinu AO, Karioti A, Saroglou V, Skaltsa E, Adebayo MA (2008). Essential oil constituents of *Klainedoxa gabonensis* Pierre Ex Engl (Irvingiaceae), *Brachystegia nigerica* Hoyle and A. Jones (Caesalpiniaceae) and *Acalypha segetalis* Muell Arg., (Euphorbiaceae). *J. Essent. Oil Res.*, 20: 211-215.
- Piccinelli AL, Lotti C, Severino L, Luongo D, Rastrelli L (2008). Unusual cytotoxic sulfated cadinene-type sesquiterpene glycosides from cottonseed (*Gossypium hirsutum*). *Tetrahed.*, 64: 5449-5453.
- Stipanovic RD, Bell AA, Mace ME, Howell CR (1975). Antimicrobial terpenoids of *Gossypium*: 6-methoxygossypol and 6,6'-dimethoxygossypol. *Phytochem.*, 14: 1077-1081.
- Stipanovic RD, Bell AA, Howell CR (1975). Naphthofuran precursors of sesquiterpenoid aldehydes in diseased *Gossypium*. *Phytochem.*, 14: 1809-1811.
- Stipanovic RD, Bell AA, O'Brien DH, Lukefahr MJ (1977). Heliocide H2: An insecticidal sesterpenoid from cotton. *Phytochem.*, 18: 567-570.
- Stipanovic RD, Bell AA, O'Brien DH, Lukefahr MJ (1978). Heliocide H3 an insecticidal terpenoid from *Gossypium hirsutum*. *Phytochem.*, 17: 151-152.
- Stipanovic RD, Bell AA, O'Brien DH (1980). Raimondal, a new sesquiterpenoid from pigment glands of *Gossypium raimondii*. *Phytochem.*, 19: 1735-1738.
- Stipanovic RD, Puckhaber LS, Bell AA, Percival AE, Jacobs J (2005). Occurrence of (+)- and (-)-Gossypol in Wild Species of Cotton and in *Gossypium hirsutum* Var. *marie-galante* (Watt) Hutchinson. *J. Agric. Food Chem.*, 53: 6266-6268.
- Waage SK, Hedin PA (1984). Biologically-active flavonoids from *Gossypium arboreum*. *Phytochem.*, 23: 2509-2511.
- Zhou RH, Lin XD (1988). Isolation of (-)-gossypol from natural plant. *Contracep.*, 37: 239-245.