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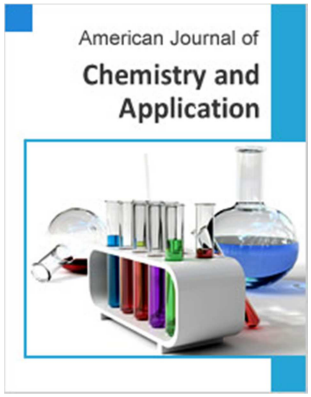

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Composition and Cytotoxicity of Volatile Metabolites of *Gossypium barbadense* Linn Fruits

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Abstract

Gossypium barbadense (cotton plant) is cultivated for economic and medicinal purposes. *G. barbadense* fruit volatiles were evaluated for the first time in order to ascertain the cytotoxic efficacy on cancerous cells. The essential oil was prepared by hydro-distillation and characterized by gas chromatography-mass spectrometry (GC-MS). The cytotoxic effects of *G. barbadense* fruit oil on human cervix adenocarcinoma (HeLa) and mouse fibroblast (3T3) cell lines were determined using the MTT-based cytotoxicity assay. Thirteen constituents were identified accounting for about 100% of the total oil composition. The oil was characterized by a significant amount of β-caryophyllene (40.97%), α-pinene (15.9%), α-caryophyllene (12.15%), and α-copaene (8.48%). This oil also exhibited notable cytotoxic activity against HeLa and 3T3 cell lines with the IC_{50} values of 47.0 µg/mL and 59.5 µg/mL respectively. *G. barbadense* fruit oil is a promising candidate for evaluation of various cell lines as a result of its rich β-caryophyllene content and peculiar compositional profile.

1. Introduction

Gossypium species, also known as cotton, are broadly classified into two groups-cultivated and wild. They belong to the family Malvaceae and consist of about fifty (50) species; *G. hirsutum, G. herbaceum, G. barbadense*, and *G. arboreum* are grown for both economic and medicinal purposes [1]. *Gossypium barbadense* Linn is a perennial under shrub, about 1-3 m in height, and indigenous to South America and now distributed from Senegal to Nigeria, and prevalently grown in tropics. An infusion of the leaf is effective 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 [2].

The cotton plant is vulnerable to insect, herbivore, and pathogen invasion. As a consequence of resistance to these predators, compounds are produced by the plant as a defense mechanism. Terpenes are an essential group of defense compounds synthesized in the cotton plant [3, 4]. Research has shown that these terpenes belong to two classes: constitutive compounds (occur in storage tissues and are liberated instantly after insect feeding or damage), and inducible compounds (which are produced de novo many hours

after exposure to pests and herbivores). The constitutive compounds comprise of α-pinene, β-pinene, limonene, caryophyllene, α-humulene, and myrcene, while the inducible compounds are β-ocimene, α-farnesene, β-farnesene, and linalool. A number of these terpenes exist in their enantiomeric forms in the plant with occurrence of the negative forms of α-farnesene, β-farnesene and β-ocimene [5-7]. Hedin *et al*. [8] and Essien *et al*. [9] reported the composition of the Egyptian and Nigerian cotton leaf oils respectively. The biological activity of *Gossypium* essential oils is limited in literature to antimicrobial efficacy and insect attractant [9, 10]. In this study, the cytotoxic potential of *Gossypium* essential oil and composition of fruit volatiles are reported for the first time.

2. Materials and Methods

2.1. Plant Sample

The fruits of *G. foetidum* were collected from mature plants cultivated in Uyo Local Government Area of Akwa Ibom State, Nigeria, in the month of November, 2015. The Sample was identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, where voucher specimen was deposited. The essential oil were obtained by hydrodistillation (4 h) of the fresh plant part using a Clevenger-type apparatus in accordance with the British Pharmacopoeia [11]. The oils were dried over sodium sulfate and stored in refrigeration (4°C) after estimation of percentage yield.

2.2. Gas Chromatography-Mass Spectrometry (GC–MS)

The volatile oil was subjected to GC-MS analysis on an Agilent system consisting of a model 7890 N gas chromatograph, a model mass detector Triple Quad 7000 A in EI mode at 70 eV (m/z range 40–600 amu) (Agilent Technologies, Santa Clara, CA, USA), and an Agilent ChemStation data system. The GC column was an HP-5 ms fused silica capillary with a (5% phenyl)-methyl polysiloxane stationary phase (30 m × 250 µm × 0.25 µm). The carrier gas was helium with a column head pressure of 9.7853 psi and flow rate of 1.2 mL/min. Inlet temperature and MSD detector temperature was 250°C. The GC oven temperature program was used as follows: 50°C initial temperature, held for 5 min; increased at 6°C/min to 190°C for 20 min; increased 7°C/min to 290°C for 15 min; increased 7°C/min to 300°C for 10 mins. The sample was dissolved in dichloromethane, and 2 µL was injected (split ratio 10:1; split flow 12 mL/min).

The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data [12]. The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5 ms column.

2.3. Cell Culture

HeLa (Cervical Cancer) cells were cultured in Minimum Essential Medium Eagle (MEME), supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with DMEM [13].

3T3 (mouse fibroblast) cells were cultured in Dulbecco's Modified Eagle Medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with medium [13].

2.4. Cytotoxicity Screening

Cytotoxic activity of the fruit oil was evaluated in 96-well flat bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay [13]. HeLa cells were plated into 96-well cell culture plates at 6 x 10^4 cells per well and 3T3 at 5 x 10^4 cells per well. The volume in each well was 100 µL for both cell types. After overnight incubation, supernatant fluid was removed by suction and 200 µL of fresh medium containing extract/compound was added in triplicate, giving a final concentration of 30 µg/mL and 100 µg/mL. Standard drug used in the MTT assay was doxorubicin. After the addition of the sample, plates were incubated for 48 h at 37°C; 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Formazan crystals, formed by reduction of MTT were dissolved in 100 µL DMSO and absorbance was taken at 570 nm using micro-plate reader (Spectra Max plus, Molecular Devices, CA, USA). The % inhibitions were processed by using Soft- Max Pro software (Molecular Device, USA). If extracts/compounds showed 50% or more percent inhibition, they were further processed for IC_{50} calculation. The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for both cell lines. The percent inhibition was calculated using the following formula:

$$\%\ \text{Inhibition} = 100 - \frac{\text{mean of O. D of test compound} - \text{mean of O. D of negative control}}{\text{mean of O. D of Positive control} - \text{mean of O. D of negative control}}\ \text{X}\ 100$$

3. Results and Discussion

The essential oil composition of *G. barbadense* fruit is presented in Table 1. A total of thirteen (13) constituents were identified accounting for 100% of the total oil composition and oil yield of 0.97%. The volatiles predominantly comprised of β-caryophyllene (40.97%), α-pinene (15.9%), α-caryophyllene (12.15%), and α-copaene (8.48%). The total monoterpenoid and sesquiterpenoid content of the oil were 33.57% and 66.45% respectively. The oil consisted of 6 monoterpene hydrocarbons (33.17%), 1 oxygenated monoterpene (0.4%), 5 sesquiterpene hydrocarbon (65.87%) and 1 oxygenated sesquiterpene (0.58%). Other significant components of the oil were γ-terpinene (6.07%) and β-pinene (5.86%). A comparison of *G. barbadense* leaf oil from Nigeria [9] and the fruit oil in this study reveal α-pinene, β-pinene, p-cymene, limonene, γ-terpinene, terpinolene, bornyl acetate and β-caryophyllene as common constituents. The leaf oil consist of high monoterpenoid content (84.7%) (tricyclene, bornyl acetate, α-pinene and α-terpinene as major compounds), whereas β-caryophyllene (40.97%), the main component of the fruit oil (Table 1) was identified in trace amount in the leaf volatile oil. Also, bornyl acetate (18.6%) in the leaf oil was detected as a trace constituent (0.4%) in the fruit oil. Similarly, most of the defense terpenes mentioned by Pare *et al*. [5] and Rose and Tumlinson [6] as constitutive compounds (α-pinene, β-pinene, limonene, and caryophyllene) were identified in the fruit oil in this study.

Table 1. *Composition of G. barbadense fruit essential oil.*

Compound	RI	Concentration (%)	QI (%)
α-Pinene	938	15.9	98
β-Pinene	983	5.86	96
p-cymene	1024	1.35	98
Limonene	1028	3.07	96
γ-Terpinene	1058	6.07	98
Terpinolene	1090	0.92	97
Borneol acetate	1287	0.4	98
α-Cubebene	1352	0.37	97
α-Copaene	1377	8.48	97
β-Caryophyllene	1419	40.97	99
δ-Cadinene	1524	3.90	96
α-Caryophyllene	1564	12.15	98
Caryophyllene oxide	1582	0.58	97

RI = Retention index in order of elution on HP-5ms column; QI, "quality index", indicates the fit comparison of experimental mass spectrum and NIST library spectrum

Evidence has also shown that the biological activities of essential oils are dependent on the oils composition which may be attributed to the presence of major constituents or a synergy between the major and minor components. Against this backdrop, the β-caryophyllene rich fruit oil of *G. barbadense*, in addition to its peculiar chemical profile, was exploited for possible cytotoxic effects against HeLa and 3T3 cell lines. It was observed that *G. barbadense* fruit oil demonstrated notable cytotoxic activity against HeLa and 3T3 cell lines *in vitro*. The cytotoxic effect of the fruit oil was more pronounced on HeLa cells compared to 3T3 cells base on percent inhibition and IC_{50} values, however the standard drug exhibited higher cytotoxic efficacy than the fruit oil at 30 µg/mL. It has been observed that the lower the IC_{50} value, the more potent the biological activity. It is worthy of note that the variation in the inhibitory action of the oil could be ascribed to difference in cell lines used in the assay. A number of β-caryophyllene rich essential oils and β-caryophyllene compound have exhibited several biological activities including anti-carcinogenic activity on some human cell lines [15-17].

Table 2. *Cytotoxicity of G. barbadense fruit oil.*

Percent Inhibition ± S. D		
Sample	**HeLa**	**3T3**
G. barbadense EO[a]	56.9 ± 0.43	32.7 ± 0.2
G. barbadense EO[b]	92.44 ± 0.7	76.25 ± 0.5
Doxorubicin[a]	95.90 ± 0.6	85.0 ± 0.15
IC_{50} Value ± S. D		
G. barbadense EO[b]	47.0 ± 0.05	59.5 ± 0.04
Doxorubicin[a]	20.4 ± 0.03	23.8 ± 0.02

[a] % Kill at 30 µg/mL; [b] % kill at 100 µg/mL; E. O = essential oil; values as mean ± standard deviation of triplicate determination.

4. Conclusion

G. barbadense fruits contain β-caryophyllene rich volatile oil. The notable cytotoxic activity of *G. barbadense* fruit volatile oil on HeLa and 3T3 cell lines is a function of its distinct compositional profile and a potential for further research on various cell lines.

References

[1] Gotmare, V., Singh, P., Tule, B. Wild and Cultivated Species of Cotton, Vol. 5. In: Technical Bulletin; Central Institute for Cotton Research: Nagpur, India; 2000.

[2] Busari, A. A. An annotated booklet of some medicinal plants ofSouth-west Nigeria. Armetu Publisher, Ibadan; 1984, p. 70.

[3] Opitz, S., Kunert, G., Gershenzon, J. Increased terpenoid accumulation in cotton (*Gossypium hirsutum*) foliageis a general wound response. *J. Chem. Ecol*. 2008, 34: 508–522.

[4] Degenhardt, J., Köllner, T. G., Gershenzon, J. Monoterpene and sesquiterpene synthases and the origin ofterpene skeletal diversity in plants. *Phytochem*. 2009, 70: 1621–1637.

[5] Pare, P. W., Tumlinson, J. H. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol*. 1997, 114: 1161–1167.

[6] Rose, U. S. R., Tumlinson, J. H. Systemic induction of volatile release in cotton: How specific is the signalto herbivory? *Planta Medica* 2005, 222: 327–335.

[7] Loughrin, J. H., Manukian, A., Heath, R. R., Turlings, T. C., Tumlinson, J. H. Diurnal cycle of emission of inducedvolatile terpenoids by herbivore-injured cotton plant. Proc. Natl. Acad. Sci. USA 1994, 91: 11836–11840.

[8] Hedin, P. A., Thompson, A. C., Gueldner, R. C., Rizk, A. M., Salama, H. S. Egyptian cotton leaf essential oil. *Phytochem*. 1972, 11: 2356-2357.

[9] Essien, E. E., Aboaba, S. O., Ogunwande, I. A. Constituents and antimicrobial properties of the essential oil of *Gossypium barbadense* (Linn.) leaf. *J. Med. Plants Res*. 2011, 5 (5): 702-705.

[10] Perveen, S. S., Qaisrani, T. M., Siddiqui, F., Perveen, R., Naqvi, S. H. M. Cotton plant volatiles and insect's behavior. *Pak. J. Biol. Sci*. 2001, 4 (5): 554-558.

[11] Medicines and Healthcare products Regulatory Agency (MHRA). British Pharmacopoeia; H. M. Stationery Office, Pharmaceutical Press: London, UK, 1980.

[12] Adams, R. P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed; Allured Publishing Corp: Carol Stream, IL, USA, 2007.

[13] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth*. 1983, 65: 55–63.

[14] Bell, A. A. Physiology of Secondary Products. In: Cotton Physiology; Mauney, J. R., Stewart, J. M. (Eds). The Cotton Foundation: Memphis, TN, USA; 1986, pp. 597–621.

[15] Langhasova, L., Hanusova, V., Rezek, J., Stohanslova, B., Ambroz, M., Kralova, V., *et al*. Essential oil from *Myrica rubra* leaves inhibits cancer cell proliferation and induces apoptosis in several human intestinal lines. *Ind. Crops Prod*. 2014, 59: 20–26.

[16] Fidyt, K., Fiedorowicz, A., Strządała, L., Szumny, A. β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Med*. 2016, 5 (10): 3007–3017.

[17] Gowda, P. J., Ramakrishnaiah, H., Krishna, V., Jagannath, N. Caryophyllene-rich essential oil of *Didymocarpus tomentosa*: chemical composition and cytotoxic activity. *Nat. Prod. Commun*. 2012, 7 (11): 1535-1538.