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CHEMICAL COMPOSITIONS AND ANTIMICROBIAL ACTIVITY OF THE LEAF ESSENTIAL OIL OF GOSSYPIUM HIRSUTUM

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ABSTRACT

The study was conducted to analyze the chemical constituent and to evaluate the antimicrobial properties of the leaf essential oil of Gossypium hirsutum. The oil was obtained by hydrodistillation and was analyzed by gas chromatography and gas chromatography –mass spectrometry (GC/GC-MS). This revealed the presence of fifty components accounting for - % of the total oil fraction. The leaf oil was dominated by patchoulane (14.70%), Ally 1-2, 6,6 – trimethyl bicyclo (3.1.1) heptane (5.95%). 1,7, 7 – trimethyl bicyclo (2.2.1) heptanes (5.95%) and 9- (1 – methylethylidene) bicyclo (6.1.0) nonane (5.95%). The oil displayed high antimicrobial potentials to some tested micro organisms.

KEYWORDS*: Gossypium hirsutum, patchoulane, aliphatic alcohol, antimicrobial potential.*

RESUMO

O estudo foi conduzido para analisar o constituinte químico e avaliar as propriedades antimicrobianas do óleo essencial de folha de Gossypium hirsutum. O óleo foi obtido por hidrodestilação e analisado por cromatografia gasosa e cromatografia gasosa-espectrometria de massa (GC / GC-MS). Isso revelou a presença de cinqüenta componentes representando -% da fração de óleo total. O óleo de folha foi dominado por patchoulane (14,70%), Ally 1-2, 6,6 - trimetilbiciclo (3.1.1) heptano (5,95%). 1,7, 7-trimetilbiciclo (2.2.1) heptanos (5,95%) e 9- (1-metiletilideno) biciclo (6.1.0) nonano (5,95%). O óleo apresentou potenciais antimicrobianos elevados para alguns microrganismos testados.

PALAVRAS CHAVE: *Gossypium hirsutum, patchoulene, álcool alifático, potencial antimicrobiano*

Introduction

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Plants are one of the five main groups of living things, these groups are called kingdoms. The plant kingdom includes such living things as grasses, trees, ferns, bushes and flowers. About 260,000 species of plant exist on the earth (14) plant contains complex and powerful substances known as essential oils. These are aromatic liquid derived from shrubs, flowers, trees, roots, bushes, herbs and seeds (15). Medicinal plants form an aspect of cultural heritage of the human race and their use is still expanding. Plants with useful medicinal properties have been obtained from folklore records of many indigenous communities. Egyptian and Chinese priests and physicians since time immemorial had been using extracted oils from plant for healing and this makes essential oil the earliest known medicine (15). An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. It is also known as volatile oils, ethereal oils or aethrolea or simply as the “oil of’ the plant from which they were extracted. An oil is “essential” in the sense that it carries a distinctive scent or essence of the plant (16).

Gossypium hirsutum L. (family malvaceae) is a cotton specie native to Central America and the Caribbean. After being domesticated in the United States, this cotton species now provides over 90% of commercial cotton worldwide (13). In Nigeria, cotton seed provides raw materials for local spinning and weaving industry (4). It is believed to be an indigenous effective medicinal plant. The dried root bark of cotton contains gossypol, this compound may cause abortion – inducing effect (3). Gossypol is a polyphenol that may be isolated more easily from the bark (5). There is on going research to use gossypol as an alternative to vasectomy (7), the plant is known to

posses anti-malaria properties and it has demonstrated in vitro effects on cell regulation and antitumor activity against mammary carcinoma a cell lines (9, 18, 6).

When the chemical constitution of the essential oil of six species within three genera of the family malvaceae were investigated, the essential oil of Gossypium tursutum L. was found to contain 67.0% hydrocarbon and beta-bisabolol was the major oxygenated component 13.7% (1),also (12) reported the presence of fifty nine constituents in the oil aroma of Gossypium barbadense in which patchoulane (7.94%), Bicyclo (4.1.0) heptanes -3-cylopropy 1-7-hydroxymethyl (trans) (7.86%), Z-Nonadecatetraene (6.19%), alpha-patchoulane (6.19%), caryophyllene oxide (5.75%) and Bicyclo (7.2.0) undec-4-ene, 11, 11-trimethy 1-8-methylene (IR*, 4z, 95*) (3.50%) as the major constituents. The oil also has antibiotic potential (12, 13).

Materials and Methods

Plant Material

Leaves of Gossypium hursutum were collected from Oyo, south West, Nigeria. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. Extraction of the hursutum was air dried in a well ventilated place till when the moisture content reduced to a minimum suitable for grinding. The plant material was pulverized and used immediately. The crushed plant material (400g) was subjected to hydrodistillation for 3 hours using a Clevenger – type apparatus (11). The oil collected was stored in a vial at low temperature until the time of the analysis.

Multidimensional GC X GC-MS Analysis

Analysis of leaf essential oil of Gossypium barbadense was performed using multidimensional chromatogram coupled with gas chromatography, mass spectrometer (Shimadzu, Japan,) equipped with double capillary columns (2.50m X 0.25^{N}m I.d, 0.25^{N}mdf) that have different characteristics (non polar and polar) High purity helium was used as the carrier gas at a constant flow rate of 0.99ml/min. A total of 1Nl sample was injected (Split ratio 100:1) into GC X GC MS using AOC 20i auton injector for analysis. The initial temperature was set at 60^{0}C treated at a rate of 30^{0}C minute to 28^{0}C and held isothermally for 6 minutes. Ion source temperature for this analysis was set at 200^{0}C while the interface temperature was set at 250^{0}C solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70eV as acquisition mass range from 40 – 700 a.m.N at 0.50cscan/S.

Antimicrobial Activities

The antibacterial activity of the leaf essential oil of Gossypium barbadense was measured against Grain-negative (Esherichia coli, klebsiellla pneumonae, salmonella typhimourion and proteins mirabilis) and Grain – positive (staphytlococcus aureus and streptococcus agalactiae) using a well diffusion method according to the National Committee for Clinical Laboratory Standard (13). Petri plates containing approximately 25-30ml of nutrient agar medium were swabbed using cotton applicator with 24 hours sub-cultured bacteria strains which were prepared in dilution to match the turbidity intensity of the MacFarland standard. Wells (6mm diameter) were punched in the agar

and filled 10^{N}L of the extract of different concentration (1000, 100 and 10Ngml^{-1}). The plates were incubated at 37^{0}C for 24 hours. The antibacterial activities were assessed by measuring the inhibition zone diameter (mm) around the well.

Results and Discussion

Exhaustive hydro distillation of the leaf of Gossypium hursutum gave a light yellow coloured oil with 0.4% V/W yield per 400g of dried leaf sample. The analysis of the leaf essential oil was carried out using GC X GC – MS and the percentage compositions are given in table 1. Fifty compounds were identified from the leaf essential oil amounting to –% of the oil. The leaf essential oil was dominated by patchoulane (14.70%) Allyl – 2, 6, 6 – trimethyl bicyclo (3.1.1) heptanes (5.95%), 1, 7, 7- Trimethyl bicyclo (2.2.1) heptanes (5.95%) and 9-(1-methyl-ethylidene) bicyclo (6.1.0) nonane (5.95%). The composition of the essential oil has similarity with the constituent of Gossypium barbadense reported by (12), patchovlane, been the compound with the highest percentage in Gossypium barbadense has the highest percentage in Gossypium hirsutum(14.70%). The aliphatic reported by (12) as part of the constituents of Gossypium barbadense are also in the present study even though it is quantitatively different (6 – Mehthyl-2-Vingl-5-hepten-01 (1.30%), 2- pentadecen-1-01 (1.30%) and Z – 10-pentadecen-1-01.

In addition, the oil displayed high antimicrobial activities to proteus micrabelis at 1,000, 100 and 10 Ngml^{-1}, Esherichia coli at 1,000, 100 and 10Ngml^{-1}, staphylococcus aureus at 1,000, 100 and 10Ngml^{-1} and klebsiell pneumonia at 1,000, 100 and 10Ngml^{-1} as shown in table 2. The oil also displayed low inhibitory activity against gram negative

salmonella typhimurium at all concentrations and this can antibiotics as shown in table 3.

Conclusion

The result obtained in this study showed that Gossypium hirustum possess essential oil and it has a great antibiotics potential and this confirm its use in traditional medicine as an antibiotics and anti-malaria.

Table I

Chemical Constituents of the essential oil of Gosspium Hirustum.

S/N	R.I	Compound	%Comp
1.	1061	2 (3H) – Furanone	2.00
2.	837	2 – Pentanethol	4.00
3.	935	Nitrohexane	2.00
4.	919	Pyruvic acid, Methylester oxime	2.00
5.	620	3 – Ethoxy-1-butene	0.58
6.	1273	3, 5 – Octanedione, 2, 2, 4, 7 – tetramethyl	0.58
7.	821	Pentanal, 2, 2 – dimethyl	0.58
8.	676	Tert – butylcarbinol	0.58
9.	729	Methyl -2-2methyoxyacrylate	0.58
10.	1515	Germacrene	0.2
11.	1221	Copane	0.4
12.	1440	Alpha – Amorphene	0.2

13.	1458	(Z, Z) alpha – Farnesene	0.2
14.		2, 6 – Bis (diazn) adamatane	0.32
15.	1050	2, 8 – Decadiyne	0.32
16.	1229	Ethanone-1-2-5 (methyl)-2 forylcycloprophyl	0.32
17.	2139	Benzene, 2-pheyl-2-propenyl) sulfonl oxacyclo tetradeca-4, 11-ddiyne	0.32
18.	1393	Patcheulane	14.70
19.	1148	1-Methyl-2-methylene-3, 5-divinyl cyclohexane	1.40
20.	1494	4,11, 11-Trimethyl-8-methylene bicyclohexane	1.40
21	1369	Cyclohexene, 4-180 propenyl-1-methoxymethyl	1.40
22.	1579	Alpha Humulene	0.63
23.	958	Ocimene	1.26
24.	976	Beta-ClS-Ocimene	1.26
25.		3-Methyl-4-(phenylthia)-2-prop-2-enyl-2, 5-dihydrothio phenel, 1-dioxide	0.57
26.		Lauryl pyridinium chloride	0.57
27.	1128	Pentanol-5- (methylene Cyclopropyl)	0.57
28.	1934	Z, Z, Z – 4, 6, 9 – Nonadecatriene	0.57
29.	1414	1, 2-Epoxy – 5, 9 – Cyclododecadiene	0.57
30	1187	Allyl – 2, 6, 6 – trimethyl bicoclo (3.1.1) heptanes	5.95
31.	1326	1, 7, 7 – trimethyl bicyclo(2.2.1) heptanes	5.95

32.	1242	9 - (1 – Methylethylidene) bicycle (6.1.0) nonane	5.95
33.	1066	1-(l-Methylene-2-propenyl) cyclopentanol	1.56
34.	1307	Bicyclo (4.1.0) heptanes-3-cyclopropy 1-7-hydroxymethyl trans	1.56
35.	1307	Bicuclo (4.1.0) heptanes-3-cycloprophy1-7-hydroxymethyl cis	1.56
36.	1078	1-(2 –Methylene cyclopropyl) cyclopentanol	1.56
37.	1143	Ethyhdene cyclo octane	1.56
38.	1196	Dihydrocarveol	1.30
39.	1169	6 – Methyl – 2-Vinyl-5-hepten-1-01	1.30
40.	1772	2 – Pentadecyn-1-01	1.30
41.	1292	Myrcenylacetate	1.30
42.	1079	Isothujol	1.30
43.	1968	Palmitic acid	3.27
44.	1272	Pelargic acid	1.09
45.	1769	Myristic acid	1.09
46.	2175	Cis-Oleic acid	4.00
47.	1763	Z-10-pentadecen-1-01	4.00
48.	2007	9 – Octadecenal	4.00
49.	1763	E-10-Pentadecen-1-01	4.00
50.	1293	10 – Undecenal	4.00
		Total	

Table 2

Zones of inhibition (mm) showing the antimicrobial activities of gram-negative and gram-positive bacteria.

Organism	**Escherichia Coil**	**Klebsiella Pneumonia**	**Salmonella Typhimouriu m**	**Proteus Mirabelis**	**Staphylococos**	**streptococcos**
Conc. ($Ngml^{-1}$)	1,000 100 10	1,000	1,000 100 10	1,000 100 10	1,000 100 10	1,000 100 10
Zone of inhibition	201710	151515	09 08 -	32 30 24	20 20 20	11 11 11

Keynote: - = no inhibition, 6-9mm = low inhibition, 10 – 15mm = moderate inhibition

And ≥ 15mm = high inhibition

+- = gram-negative bacteria

* = gram-positive bacteria

Table 3

Zones of inhibition (mm) showing the antimicrobial activities of antibiotics against gram positive and gram negative bacteria.

Organism/ antibiotics/ concentration	**Escherichia Coil**	**Klebsiella Pneumonia**	**Salmonella Typhimouriu m**	**Proteus Mirabelis**	**Staphylococus aureus**	**streptococcus agalactiae**
Nitrcoforantion (2Ng)	25	20	15	15	28	11
Ofloxacin (5Ng)	20	20	22	33	20	-
Gentamicin (10Ng)	24	15	18	16	-	-

Keynote: - Same as in table 2.

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