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Phytochemical and Gc-Ms Profile of Fractions obtained from Methanolic Extract of *Acanthospermum Hispidum* Dc

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Abstract: The phytochemical screening of the combined fractions 87-93 and water fraction showed presence of flavonoids only while alkaloids, steroids, saponins, tannins were absent. The GC-MS analysis of the combined fractions 87-93 identified presence of seven compounds while that of the water fraction was eight. The identified compounds are polyfunctional alkene, esters, carboxylic acids and flavonoid.
Keywords: *A. hispidum*, methanol, extract, fractionation, GC-MS

Introduction

Acanthospermum hispidum dc is a member of the Asteraceae family[1]. It is an annual plant with a Y-shaped type of branching. The stems are densely covered with hairs which are stiff and bristly. The leaves are hairy, sessile, simple and opposite to each other. Some of the leaves can be as long as 11.5 cm. The margins of the leaves are irregular. The flower head has a 5-9 ray flowers. The ray flowers petals are yellow and about 1.5 mm long. The fruits are flat, hairy, triangular and about 5-6 mm long. The terminal spines are about 4 mm long. The main root is about 20 cm long. It can be found in East, West and Central Africa. Other places where found are Australia, North and South America[2,3]. Ethnomedicinally, *A. hispidum* dc is used in the treatment of yellow fever, malaria and stomach disorder[4-7]. Several compounds have been isolated and identified from the plant by different authors[8-10]. This research work is a continuation of ongoing work on the isolation and characterization of compounds present in the methanolic leaf extract of *A. hispidum* dc that grows in western Nigeria.

Materials and methods

Sample collection and preparation

The plant leaves were collected from a bush in Ogbomoso, Nigeria and taken to the Department of Pure and Applied Biology for identification. The leaves were dried under laboratory condition for three weeks, pulverized using a food blender and stored in a clean container.

Extraction

500 g of the pulverized sample was extracted in batches with n-hexane followed by methanol using a soxhlet extractor. The extracts for each solvent were pooled together and concentrated using a rotary evaporator and the weight determined.

Fractionation of crude methanolic leaf extract

The crude methanolic extract was partitioned between water and four organic solvents; n-hexane, chloroform, ethyl acetate and n-butanol successively. The organic phase of the different solvents were bulked,

concentrated to dryness and weighed. All the fractions were subjected to phytochemical screening[11].

Phytochemical screening of fractions

The fractions obtained from the fractionation of the crude methanolic extract and AGC were subjected to phytochemical screening[12]. These were analyzed for the presence of alkaloids, tannins, flavonoids, steroids, saponins, terpenoids and glycosides.

Accelerated gradient chromatography (AGC)

The ethyl acetate fraction obtained from the fractionation of the methanolic crude extract was dissolved in ethyl acetate and adsorbed on silica. The fraction-silica mixture was mixed thoroughly with pestle in a mortar and was allowed to air dry for 2 hours and later packed into a column layered with a separating portion. An eluting agent stronger than the one currently in use is added to the column in such a way that a continuous concentration gradient is established down the column; the rear part of each chromatographic band is then always in contact with a more strongly eluting solution than is the front, and each band is in a more powerful eluting medium than the band preceding. A total of 100 fractions were obtained.

Chemical identification of flavonoid type

1 ml of 50% NaOH and conc. H_2SO_4 respectively was added to 3 ml of the fraction. A yellow coloration observed in each fraction tested indicated the presence of flavone, isoflavones, flavanol or leucoanthocyanin in the fraction while an orange coloration indicates presence of flavanone.

GC-MS analysis

The water fraction and combined fractions 87-93 obtained from the accelerated gradient chromatography were subjected to GC-MS analysis. The analysis was carried out in GCMS-QP2010 PLUS Shamadzu. 2µl of the sample dissolved in ethyl acetate was used for the analysis which occurred within 32 minutes. The instrumentation method is as shown in table 1.

Table 1: GC-MS Instrumentation method

Gas chromatography programme	
Equipment	GC-MS-QP2010 PLUS Shamadzu
Column oven temperature	60^{o}C
Injection temperature	250^{o}C
Flow control mode	split
Pressure	100.2 kPa
Total flow	6.2 ml/min
Column flow	1.61 ml/min
Linear velocity	46.3 cm/sec
Purge flow	3.0 ml/min
Split ratio	1.0
IonSource temperature	200^{o}C
Interface temperature	250^{o}C
Solvent cut time	2.50 min
Detector gain mode	0.0 kV
Threshold	3000
Mass spectrometry	
Start time	3.00 min
End time	35.00 min
ACQ mode	Scan
Event time	0.50 sec
Scan speed	769
Start m/z	40.00
End m/z	400.00
Sample inlet unit	GC
Library used	NIST version- year 2005

Results

Phytochemical assay

The phytochemical screening of the extracts obtained from fractionation of the crude methanolic leaf extract of *A. hispidum* dc revealed the presence of flavonoids, steroids and terpenoids, and absence of alkaloids, saponins, tannins and glycosides in the chloroform, ethyl acetate and n-butanol extracts as presented in Table 2. Combined fractions 87-93 obtained from AGC of the ethyl acetate extract showed presence of flavonoids only. The water fraction also showed presence of flavonoids

Chemical identification of flavonoid type

The chemical test gave a yellow colouration which is an indication that the flavonoids present could be flavones, isoflavones, flavonol, flavonones or leucoanthocyanins.

Table 2: Phytochemical screening of extracts obtained from fractionation of the crude methanolic leaf extract

Extracts terpenoids	Steroids	Saponin	Alkaloids	Flavonoids	Tannins	Glycosides
n-hexane	-	-	-	-	-	-
Chloroform	+	-	-	+	-	-
Ethyl acetate	+	-	-	+	-	-
n-butanol	-	-	-	+	-	-
Water	-	-	-	-	-	-

+ , Present; -, Absent.

GC-MS analysis

For the combined fractions 87-93 a total of seven compounds were separated (fig. 1) and their mass to charge ratios obtained as shown in fig. 2. The molecular weights and molecular formulas of the compounds are given in table 3. Eight compounds were revealed from the water fraction as shown in fig. 3 and their mass to charge ratios are shown in fig. 4. Their molecular weights and molecular formulas are given in table 4.

Table 3: GC-MS analysis of combined fractions 87-93 obtained from AGC of the ethyl acetate extract

1	27.3	95638	74	$C_{17}H_{33}O_2$	269
2	28.5	125808	67	$C_{19}H_{32}O_2$	293
3	29.3	106429	93	$C_{15}H_{11}O_4$	254
4	29.7	52047	188	$C_{14}H_{10}O_6$	287
5	30.0	20024	41	$C_{17}H_{34}O_2$	270
6	31.1	333912	81	$C_{14}H_{19}O_2$	219
7	31.9	11617	55	$C_7H_5O_2$	121

AGC – Accelerated gradient chromatography

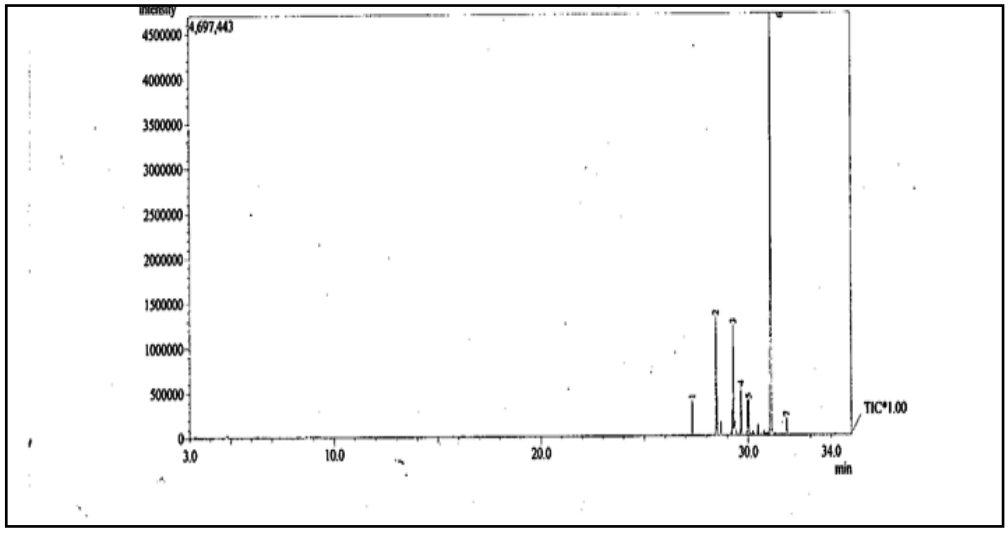


Fig.1 : GC analysis of combined fractions 87-93 obtained from AGC

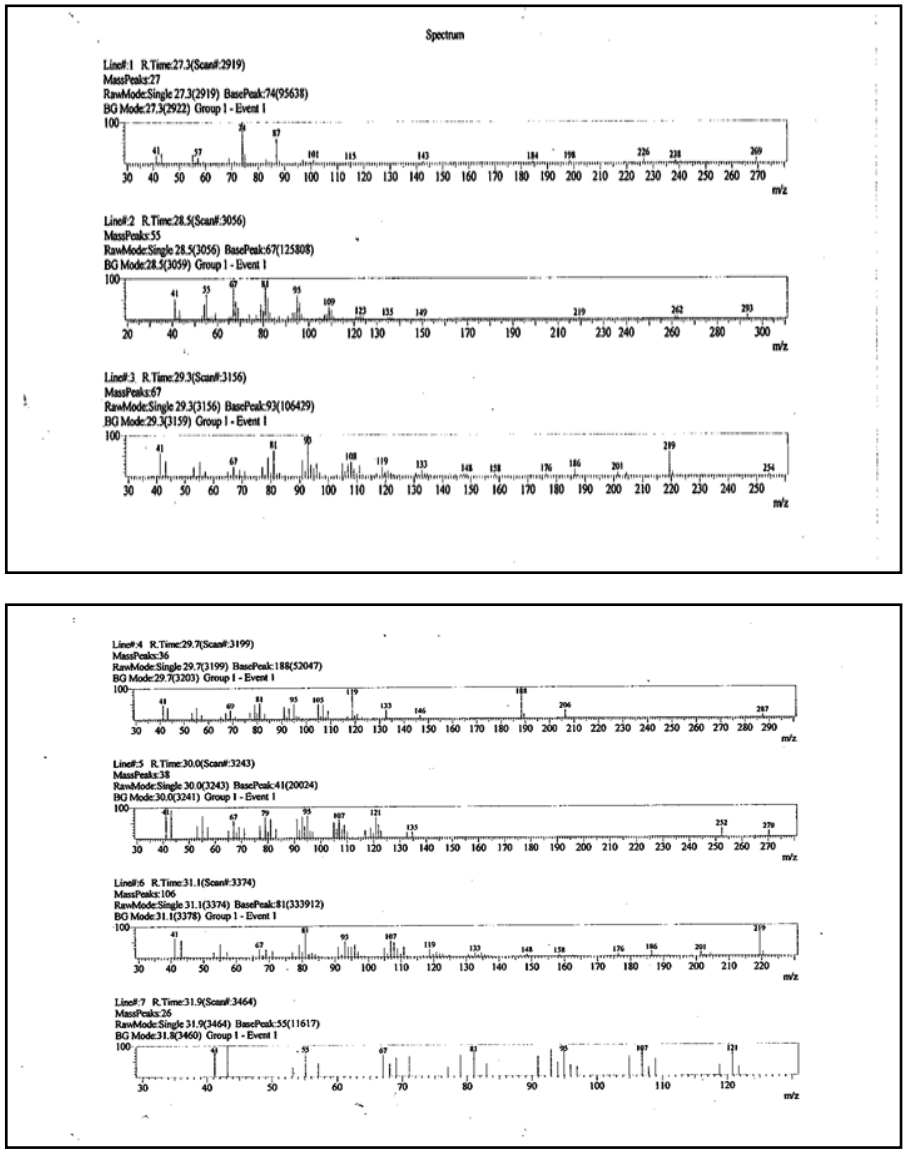


Fig. 2: MS analysis of fractions 87-93 obtained from AGC

Table 4: GC-MS analysis of the water fraction obtained from AGC of the ethyl acetate extract

1	24.9	3188	57	$C_5H_{11}O_2$	103
2	27.3	19612	74	$C_4H_7O_2$	87
3	27.9	6505	73	$C_5H_{11}O_2$	102
4	28.5	8738	55	$C_6H_6O_2$	110
5	29.3	12635	93	$C_6H_4O_2$	108
6	30.0	10351	219	$C_{14}H_{18}O_2$	219
7	31.1	11203	81		107

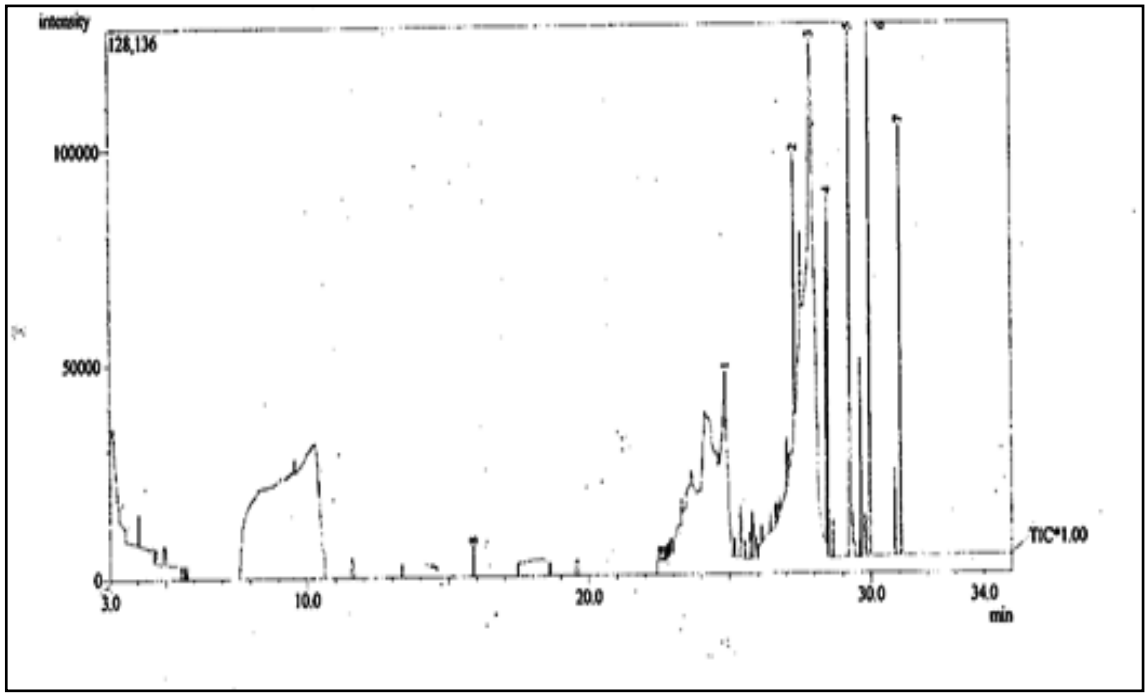
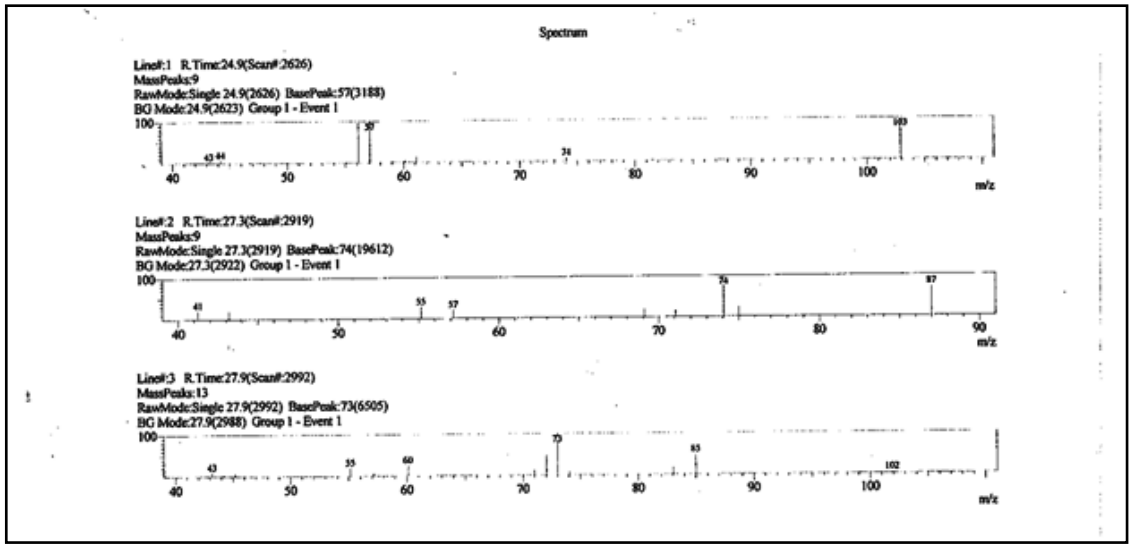


Fig. 3: GC analysis of the water fraction obtained from AGC of the ethyl acetate extract



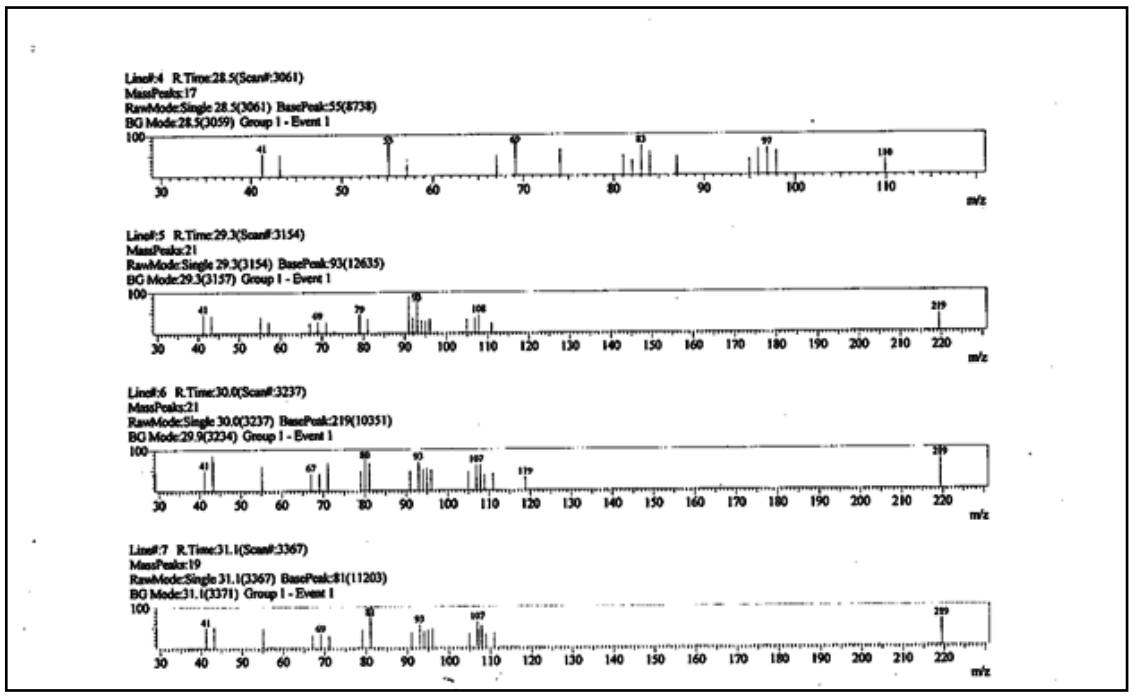


Fig. 4: MS analysis of the water fraction obtained from AGC of the ethyl acetate extract

Discussion

Wide range of chemical substances are synthesized by plants. Some of these phytochemicals have been found to be very important in the treatment of ailments. Several researchers have reported that these phytoconstituents exhibit biological activities such as antimicrobial, antifungal, antioxidant, anti inflammatory, antiallergy, etc. Some of the active phytochemicals that have been identified include flavonoids, terpenoids, saponins, steroids, etc[13]. Some of these compounds may also inhibit nitrosation or the formation of DNA adducts, stimulate the activity of protective enzymes such as phase II enzyme glutathione transferase or inhibit cyclooxygenase and lipooxygenase or possess antitumour activity[14-17]. The phytochemicals identified in the extracts could be responsible for the biological activity of the plant leaves. Due the very minute quantity of fractions 87-95 obtained from AGC, further purification using other separation techniques could not be done. GC-MS technique was used to effect complete separation and identification of the pure compounds in the combined fractions of 87-93 and the water fraction. The rule of 13 and Index of hydrogen deficiency coupled with the molecular weight obtained from the MS analysis aided in the elucidation of the molecular formula of each compound. The impossible Index of hydrogen deficiency (negative or fractions) was rejected and only the positive one was used in the chemical formula determination of the compounds. The presence or absence of functional groups in an organic molecule determines the manner in which that organic molecule will fragment. The mass to charge ratios of the fragment ions obtained from the MS analysis was used to determine the fragmentation pattern of the compounds, hence the molecular formula. The presence or absence of various mass peaks in each spectrum was used to deduce the structure of the compounds. The compounds identified are polyfunctional alkene, esters, carboxylic acids and flavonoids.

Conclusion

The compounds identified in the combined fractions 87-93 are 5-hydroxy-14-methoxyhexadec-2, 15-ene; 9, 12, 17-trienyloctadecanoate; flavone; 4, 5-dihydroxyflavone; hexadecanoic acid methyl ester and 3-enylhex-1, 6-heptadiyne while the water fraction contained pentanoic acid; butenoic acid; pentenoic acid; hex-2-yn-5-enoic acid; hex-2, 4-ynoic acid and 8-propenyl-2-yn-8-en nonyl methyl ester .

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