ANTIMICROBIAL ACTIVITY AND CHEMICAL INFORMATION FROM GC-MS OF *Curculigo pilosa* RHIZOMES

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ABSTRACT

Chemical constituents of purified sample of Curculigo pilosa rhizomes were determined using GC-MS technique. The result revealed the presence of 3-eicosyne (8.98%), pentadecanoic acid (2.41%) hexadecanoic acid (31.18%), octadecanoic acid (1.52%) 9-octadecenoic acid (24.42%), linoleic acid ethyl ester (3.93%), androstan-3-one (5.90%), 1-phenanthrenenmethanol (5.78%), 1,2-benzenedicarboxylic acid (4.59%), hexanedioc acid (13.38%), 8,11-octadecadienoic acid (9.02%), nonadecane (3.52%), ethanol-2, 2-oxybis (20.75%), propane-1-(1-methylethoxy) (8.05%), and 2, 6, 10-dodecatriene-1-ol (5.14%). The antibacterial screening of the n-hexane, chloroform, ethyl acetate, n-butanol and methanol soluble fraction in comparison with commercial antibiotic were studied using the agar-well diffusion method against some pathogenic bacteria and yeast such as Escherichia coli, Staphylococcus aureus, Salmonella typhi, Staphylococcus feacali and Candida albicans. The results show that the methanolic fraction is more potent than other fractions.

Keywords: *Curculigo pilosa rhizomes, Chemical constituents, GC-MS, pathogenic bacteria, yeast*

INTRODUCTION

The use of plants as a complementary and alternative medicine has always maintained its popularity worldwide [1]. Understanding the overall composition and constituents of the medicinal plants is required to optimize its potential as a source of medicine. *Curculigo pilosa* belongs to Hypoxidaceae and is an herbaceous plant with stout, erect rhizomes bearing a cluster of grass-like leaves to 60 cm long and flower shoots to 20 cm at the end of the dry season [2]. It is popularly known as Golden eye grass and locally referred to as Echidungi (Nupe) and Epakun(Yoruba). The rhizome which is a tuberous root has been extensively used in indigenous systems of medicine in Nupeland, and Yoruba land. The active compounds that have been reported are flavones, tannin, glycosides, steroids, saponins, triterpenoids and other secondary metabolites [3]. It is therefore, very important to find out the compounds responsible for the activities of this plant as reported by many researchers.

MATERIALS AND METHODS

Collection of plant sample

Fresh rhizomes of *C. pilosa* were collected from Edozhigi forest along Bida-wuya road, Niger State, Nigeria. The sample was collected in month of June, 2012 and was identified by Plant Taxonomist, Dr Sherifat at NIPRD, Idu-Abuja.

Study Area

The present investigation was carried out in Nupeland of the Central Nigeria. The plant was collected in Edozhigi forest along Bida-wuya road, Niger State, Nigeria. Niger State is located at the North Central region of Nigeria between latitudes 8° 20'N and 11° 20'N and longitude 3° 30' E and 7° 20' E.

Preparation of samples

The preparation of samples collected from the farm was done according to method described [4]. The Fresh rhizomes of *C. pilosa* collected from the experimental sites were thoroughly washed with distilled water, air dried at room temperature. These were then pounded into uniform powder manually.

Extraction of the plant extract

100 g of the crushed C. *pilosa* was packed into a thimble before it was placed inside a soxhlet extractor and extracted with methanol for 48 h. Resulting solution was then concentrated using a rotary evaporator and evaporated to dryness on a water bath. Extract was weighed and labeled crude methanol extracts 'CMe'. The defatted residue material was air dried and then weighed to calculate percentage recovering.

Partition

The crude methanol extract was partition between two immiscible solvents in a separating funnels. The compounds have a "choice" of two solvents that they can dissolve in. Some compounds dissolve in one solvent and some compounds dissolve in the other solvent. The compounds in the mixture become separated into two groups depending on solubility of the compounds in the solvents used. The solvents used where n-hexane, chloroform, ethyl acetate, n-butanol and methanol which show two layers. The extracts were collected and evaporated on a water bath.

Antimicrobial susceptibility Testing

The antimicrobial activity of the extracts was carryout by using the agar well diffusion method as described by [5].

RESULT AND DISCUSSION

Table 1: Antibacterial activity against some selected pathogens

Extracts	*Escherichia coli*				*Staph. aureus*				*S. fecali*				*Salmonella typhi*				*Candida albicans*			
	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000
n-H_e	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C_e	–	–	4	4	–	–	–	4	–	–	–	–	–	–	–	4	–	–	–	3
ethyl	–	–	5	5	–	–	2	6	–	–	7	11	–	–	–	–	–	5	10	15
n-B_e	–	–	8	11	–	5	9	10	–	–	6	12	–	–	4	9	5	8	17	21
Me	–	3	10	13	–	8	10	13	–	–	5	12	–	–	6	11	7	11	19	25

Key: *n*-H_e= *n*-Hexane extract; C_e = Chloroform extract; ethyl = ethyl acetate extract; *n*-B_e= *n*-Butanol extract; Me =Methanol extract

Table 2: Analytical Parameters Deduced from GC-MS Spectrum first combined fractions (F_{10}-F_{32}) of *Curculigo pilosa*

Line no	IUPAC Name	Molecular Formula	Molar Mass	R.T	Area %	Fragmentation Peaks
1	3-Eicosyne	$C_{20}H_{38}$	278	17.467	8.98	(43), 95, 109, and 123
2	Pentadecanoic acid	$C_{17}H_{34}O_2$	270	19.207	2.41	57, (74), 87, and 101, 115, 129, and 143
3	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.640	31.18	(75), 115 129, 143, 157,171, 185, 213
5	Octadecanoic acid	$C_{19}H_{38}O_2$	298	22.760	1.52	43, 57, 74, (87), 115,129, 143, 157 171, 185, 199, 213 241, 255
6	9-Octadecenoic Acid	$C_{18}H_{34}O_2$	282	23.472	24.42	41, 55, (69), 83, 97
7	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	24.524	3.93	41, (67), 81, 95, 263
8	Androstan-3-one	$C_{19}H_{30}O_3$	306	25.854	5.90	41, 55, 69, (83), 97, 111, 125, 273, 288
9	1-Phenanthrenenmethanol	$C_{20}H_{30}O$	286	26.382	5.78	157, (253), 271
10	1, 2-Benzenedicarboxylic Acid	$C_{24}H_{38}O_4$	390	27.235	4.59	70, 112, (149), 261

Table 3: Analytical Parameters deduced from GC-MS Spectrum second combined fractions(F_{33}-F_{48}) of *Curculigo pilosa*

Line no	IUPAC Name	Molecular Formula	Molar Mass	R.T	% Area	Fragmentation Peaks
1	Nonadecane	$C_{19}H_{40}$	268	18.497	3.52	43, (57), 71, 85, 99
4.	8,11-Octadecadienoic acid	$C_{19}H_{34}O_2$	294	22.402	9.02	(67), 95, 81, 95, 109, 262
5.	Hexanedioc acid	$C_{22}H_{42}O_4$	370	26.069	13.38	57, (129), 241, 327, 341

Table 4: Analytical Parameters Deduced from GC-MS Spectrum first combined fractions (F_{49}-F_{64}) of *Curculigo pilosa*

Line no	IUPAC Name	Molecular Formula	Molar Mass	R.T	% Area	Fragmentation Peaks
1	Ethanol,2,2-oxybis	C_4H_{10}	106	5.265	20.75	(45), 75
2	Propane-1-(1-methylethoxy)	$C_6H_{14}O$	102	7.384	8.05	(43), 87
3	2, 6, 10-dodecatriene-1-ol	$C_{15}H_{26}O$	222	15.750	5.14	(69), 81

The present investigation proved that methanol soluble portion of the plant extract showed activity against all tested pathogens with maximum activity (25 mm) against *Candida albicans* as shown in Table 4. This finding also agrees with earlier report by Gbadamosi and Egunyomi [3] that the plant successfully inhibited some microbes; this result confirmed the use of *C. pilosa* in herbal medicine for disease prevention and treatment of infections. The chloroform soluble portion was not active against *Staphylococcus fecali* it inhibit the growth of *Escherichia coli, Staphylococcus aureus, Salmolella typhi, Candida albicans* and have least activity compare to other extracts that shows zone of inhibition. Generally, the activity of chloroform soluble portion extract was active against both gram negative and gram positive. The n-hexane soluble portion of plant did not show any activity against all the tested isolates. This result is different from the findings of analysis of same plant by Oliver [6] which reported that oil extracted from *C. pilosa* have slight antibiotic action and are used in the treatment of infections [6]. The ethyl acetate extract of *C. Pilosa* showed highest activity against *Candida albinca* (15 mm) followed by *Staphylococcus fecali* (11 mm), with *Staphylococcus aureus* (6 mm) and *E. coli* (5 mm). Generally the activity of the ethyl acetate soluble portion was not active at lower concentration of the extracts. The fraction does not show any activity against *Salmonella typhi.* The result from this investigation has showed that the methanol soluble portion extract was more active against the isolates compared to the other extracts. The control (clotrimazole) show maximum activity at 30 mm against *Candida albicans* . The activity of the methanol soluble portion extract compared favourably for *Candida albicans.* This indicates that the plant extract may be better at treating candidiasis. Ciprofloxacin control have 28mm maximum activity against the other pathogens it may also use to treat vomiting, diarrhea, throat, ostemyelitis, Meningitis, urinary track , hair follicle, wound infections and candidiasis caused by tested organism compared to other isolates. The extracts may be better than the control tablet since it is still in its crude form and due to the process of extraction the plant undergo and presence of other big compound which may not be active, will mask some of the active compounds in the plant [7] made a similar observation that the crude plant preparations have generally been reported to exhibit lower antimicrobial activity than pure antibiotic substance such as ciprofloxacin. The high activity of the methanol extract against *Candida albicans* is a proved that this pant can be use as anticandida to treat candidiasis which is common with females and uncircumcised male. The identified metabolites were believed to be responsible for the activity of the plant extract.

CONCLUSION

It can be concluded that antimicrobial screening showed that *C. pilosa* extracts have been found to be effective against gram negative and gram positive pathogenic micro-organisms involved in causing some infections. The methanol soluble fraction has more activity than others. The GC-MS of purified sample indicate that the plant contain some chemical constituents such as 3-eicosyne (8.98%), pentadecanoic acid (2.41%) hexadecanoic acid (31.18%), octadecanoic acid (1.52%) 9-octadecenoic acid (24.42%), linoleic acid ethyl ester (3.93%), androstan-3-one (5.90%), 1-phenanthrenenmethanol (5.78%), 1,2-benzenedicarboxylic acid (4.59%), hexanedioc acid (13.38%), 8,11-octadecadienoic acid (9.02%), nonadecane (3.52%), ethanol-2, 2-oxybis (20.75%), propane-1-(1-methylethoxy) (8.05%), and 2, 6, 10-dodecatriene-1-ol (5.14%) which were believed to be responsible for the activity of the plant extract. It is recommended that further work can be done on this plant so as to know actually the compound that inhibits the growth of the isolates as well as the dosage, toxicity and antioxidant.

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