WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 78-88. Research Article ISSN 2277– 7105

GAS CHROMATOGRAPHY-MASS SPECTROPHOTOMETRIC (GC-MS) STUDIES ON THERAPEUTIC POTENTIALS OF *COSTUS AFER KER GAWL* LEAVES

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Article Received on
13 March 2018,
Revised on 03 April 2018,
Accepted on 23 April 2018
DOI: 10.20959/wjpr20189-11232

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ABSTRACT

The ethanol leaf extract of *Costus afer* was subjected to qualitative phytochemical screening, column chromatography and GC-MS analysis to ascertain the compounds that exhibit anti-inflammatory and anti-oxidant properties as claimed by herbalists and traditionalists. The presence of Flavonoids, Saponins, Steroids, Terpenoids and Anthraquinones were indicated in the phytochemical screening. Eluent fractions from the moderately polar solvent of the column chromatography generated Gas Chromatogram that had 6 prominent peaks indicated that the prevailing compounds were oxirane hexadecyl (4.09%), n-Hexadecanoic acid(3.68%), 9-octadecenoic acid (z)- methyl ester(3.08%), 6-octadecenoic acid (z)- (13.49%) and two pentacyclic compounds identified as Cycloursan-3-ol, acetate (36.17%) and lupenone (39.50%). The two pentacyclic compounds confirmed the presence of steroids and validates the use of *C.afer* leaf for anti-inflammatory therapy and as an antidote for acute toxicity in traditional medicine.

KEYWORDS: *Anti-inflammatory, Anti-oxidant, Costus afer, GC-MS, lupenone.*

INTRODUCTION

Plants, microorganisms and many other forms of life are consistently evaluated for biologically active compounds and their therapeutic benefits. Most of the earliest pharmaceuticals were plant materials.[1] Medicinal plants contain bioactive compounds (phytochemical constituents) with reported therapeutic benefits.[2,3] Extracts from the roots, seeds, barks and fruits of such useful medicinal plants are important in the preparation of

syrups in traditional medicine as cough suppressants, insect repellants and in the treatment of various diseases.[4,5]

The search for new biochemical targets so as to obtain chemical structures for drug development is on the increase.[6,7] The plant *Costus afer ker gawl (costaceae)* is among 150 species of stout, perennial and rhizomatous herbs of the genus *costus*.[8] The plant is locally called kakil-zuwaa (Hausa), atare tete-egun (Yoruba), okpete (Igbo) and mbritem (Efik) all in Nigerian languages. It can be found in the forest belt of Senegal, Niger, South Africa, Guinea, Sierra Leone and Nigeria.[9] *Costus afer* leaf infusion is employed traditionally throughout tropical Africa to treat disorders such as fevers, diarrhea, vomiting, cough, rheumatism, hemorrhage and tachycardia (rapid heart rate). It is also used as an antipyretic, antidote for acute toxicity, anti-inflammatory and anti-diarrheal agent. Leaf sap of *Costus afer* is used as eye drops to treat eye itchiness and as nose drops to treat headache with vertigo; and in frictions to treat edema and fever.[10] A report on the use of stem-decoction of *Costus afer* for treatment of rheumatism has also been documented.[11] In Nigeria, the debarked stem is used to treat nausea and to quench thirst, stem sap is acidic and burns on open wounds, but it is also anodyne and is applied to different skin ailments as a suiting agent.[12] The present study has therefore been designed to identify the specific components of the *Costus afer* leaf that are responsible for some of the reported therapeutic properties exhibited by *C. afer*.

MATERIALS AND METHODS

Sample Collection and Authentication

Fresh leaves of *Costus afer ker gawl* plants were harvested from Umuelem, Ihiagwa, Owerri West Local Government Area of Imo state. The *C. afer* plant was identified by Prof. J. C Obiefuna in the School of Agriculture and Agricultural Technology (SAAT) Laboratory FUTO.

Sample preparation

The leaves of *C. afer* were washed under a running tap water to remove soil particles and adhered debris, finally washed with distilled water, allowed to dry under a shade at room temperature (25^{o}C) and pulverized with an electric blender. 30g of the powdered sample was transferred into the soxhlet apparatus for extraction. 400ml of 95% ethanol in soxhlet extractor was used for a period of three hours. The crude extract obtained was concentrated to dryness between 40^{0}C to 45^{0}C using water bath. A portion of the final residue was then

subjected to phytochemical screening and a second portion to Column chromatography. Eluents from the column chromatography were further subjected to GC-MS analysis.

Column chromatography

A thoroughly washed and dried glass column was plugged with a glass wool and the tap of the column was secured. A slurry of Silica gel (of 120 mesh size packing material which had earlier been activated in an oven) made with n-hexane was packed into the column. A homogenous packing of the silica gel was ensured by maintaining mild agitation while the slurry flowed through the column. Then, 0.70g of the extract was applied in the form of a concentrated solution on top of a cotton plug soaked with the solvent to protect the top of the column while the extract slowly drained and was covered with glass beads. The space around it was filled with solvent and the column was allowed to run with solvents of different polarities starting with n-hexane, chloroform and ethyl acetate to yield several fractions (85) which were collected in glass vials. The following fractions were clubbed together due to similarities in color and Thin Layer Chromatogram; 26-50 (40:60 n-hexane/methanol to 90:10 methanol/ethlyacetate), 51-53 (90:10 to 80:20 methanol/ethylacetate), 54-63(80:20 to 50:50 methanol/ethylacetate), 64-71(50:50 to 20:80 methanol/ethylacetate).

Phytochemical screening of test plant

The *Costus afer* leaf extracts and portions of eluents from different fractions were screened for Alkaloids (using Wagner reagent), Flavonoids, Tannins, Steroids, Saponins, Anthraquinones and Terpenoids by using documented standard methods.[13] The presence of Tannins, anthraquinones, flavonoids, alkaloids, terpenes and saponins were carried out by modifying (a pre-TLC treatment) the methods reported in previous studies.[14,15,16]

GC-MS Analysis

1µl of the ethanol extract of the *C. afer* leaf as well as eluted fractions from the column chromatography were respectively injected into the Gas chromatography unit Shimadzu GC-MS QP2010 instrument for analysis. The injector temperature was maintained at 250ºC. The detector used was flame ionization detector which was maintained at 280ºC. The pressure of the carrier gas, nitrogen was kept at 10 psi. The oven temperature was set at 60ºC to 280ºC with a gradual increment of 10ºC per minute with the DB-5 MS column of 30 m long and 0.25 mm inner diameter. The injected samples were separated into various constituents with different retention time which were eluted and detected by flame ionization detector. The GC

chromatogram (a plot of intensity against retention time) was recorded by the attached software.

Identification of Components

From the obtained GC chromatogram and the mass spectra, the compounds were identified by comparing the generated data with the existing software libraries- the database of National Institute Standard and Technology (NIST like NIST05 and NIST05s) having more than 62,000 patterns.

RESULTS AND DISCUSSION

3.1. The result of the qualitative phytochemical screening of the leaf extract is shown in table 1.

Table 1: Phytochemical Components of the ethanol leaf extract of *Costus afer ker.*

Phytochemical		Ethanol extract
1.	Alkaloids	-
2.	Flavonoids	+
3.	Saponins	+
4.	Tannins	-
5.	Steroids	+
6.	Terpenoids	++
7.	Anthraquinones	++

Where: ++ = Abundant, + = Trace and - = Absent.

Flavonoids, saponins and steroids were present in the ethanol extract of *Costus afer* leaf. The presence of terpenoids and anthraquinones were also indicated and more pronounced while the extract tested negative for tannins and alkaloids. Anthraquinones can induce laxative effect[17] and hence the use of *C.afer* as laxative and nervous system depressant may result from the presence of anthraquinones.[18] Steroids, flavonoids and saponins are potent water soluble antioxidants which prevent oxidizing cell damage suggesting anti-inflammatory properties.[19] [20]

Column chromatography

Table 2: Column chromatography Fractions of the leaf extract of *costus afer ker gawl*.

The fractions obtained, solvent (mobile phase) used and color of the various fractions is shown in table 2.

Fraction	Solvent (mobile phase)	Colour
1 - 25	100% n-hexane to 50:50 n-hexane/methanol	Colorless
26 - 50	40:60 n-hexane/methanol to 90:10 methanol/ethylacetate	Yellow
51 - 53	90:10 to 80:20 methanol/ethylacetate	Yellowish-brown
54 - 63	80:20 to 50:50 methanol/ethylacetate	Green
64 - 71	50:50 to 20:80 methanol/ethylacetate	Brown
72 - 85	20:80 to 100:0 methanol/ethyl acetate	Reddish - Brown

The observed yellow colour could be attributed the presence of anthraquinone, the green to saponin and the reddish color to steroids.

Gas chromatogram of eluent fraction from column chromatography 26-50 (40:60 n-hexane/ methanol to 90:10 methanol/ethylacetate) in Figure: 1 showed 6 prominent peaks.

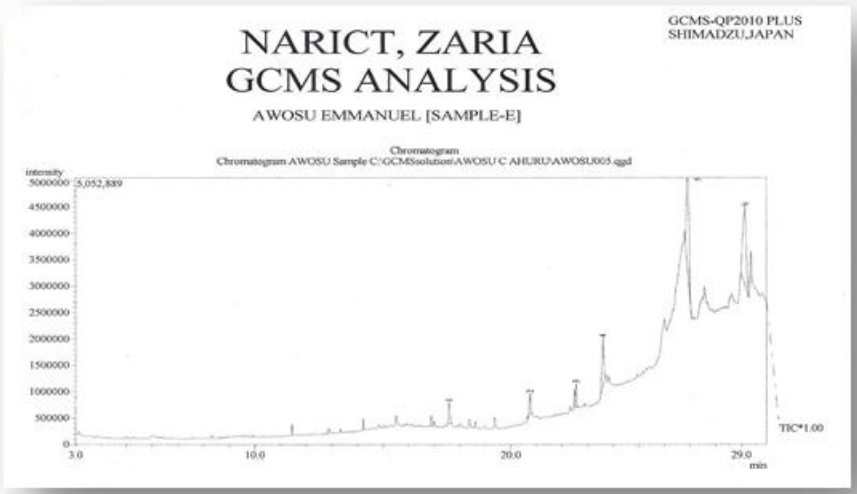
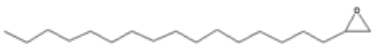
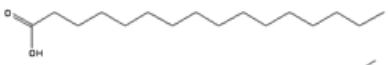
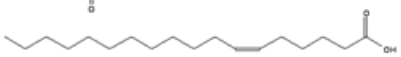
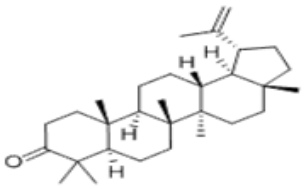


Figure 1: Gas Chromatogram of clubbed eluent fractions from column chromatography 26-50 (40:60 n-hexane/methanol to 90:10 methanol/ethlyacetate).

The details and characteristics of the 6 constituting peaks and the identified compounds with reference to the mass spectra in the NIST library are recorded in Table 3.

Table 3: Identified compounds in the clubbed eluent fractions from column chromatography 26-50 (40:60 n-hexane/methanol to 90:10 methanol/ethlyacetate) using GC-MS.

Identified Compound	Retention time(mins)	%tage conc.	Structures
1. Name: oxirane hexadecyl- **Formular:** $C_{18}H_{36}O$ **M.wt:** 268	17.59	4.09	
2. Name: n-Hexadecanoic acid **Formular:** $C_{16}H_{32}O_2$ **M.wt:** 256	20.75	3.68	
3. Name: 9-octadecenoic acid (z)- methyl ester **Formular:** $C_{19}H_{36}O_2$ **M.wt:** 296	22.55	3.08	
4. Name: 6-octadecenoic acid (z)- **Formular:** $C_{18}H_{34}O_2$ **M.wt:** 282	23.59	13.49	
5. Name: 13,27-Cycloursan-3-ol, acetate, (3.beta.,13.beta.,14.beta.)- **Formular:** $C_{32}H_{52}O_2$ **M.wt:** 468	26.89	36.17	
6. Name: lupenone **Formular:** $C_{30}H_{48}O$ **M.wt:** 424	29.14	39.50	

9-Octadecanoic acid is a common mono-unsaturated fat in human diet.[21] Mono-unsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol and possibly increased high-density lipoprotein (HDL) cholesterol. The GC-MS spectra of eluent fraction of the leaf extract revealed that previous reports on therapeutic benefits of *C.afer* could be attributed to its major constituents. Namely hexadecanoic acid which is a fatty acid may be an active antimicrobial and anti-diarrheal agent.[22] Acyclic diterpene alcohol and terpenoids can be used as a precursor for the manufacture of synthetic forms of vitamin E[23] and vitamin K.[24] Vitamin E is essential for the replenishment of worn-out cells and is also used as an antioxidant. Vitamin K is the antihemorrhagic factor related to blood clothing mechanism. Fresh leaf of *C. afer* is a good source of these two vitamins. This study is the first of its kind to evaluate the chemical constituents of leaf extract of *C.afer* using GC-MS analysis. The results of the GC-MS profile of the present study which showed

the existence of various compounds with different chemical structures of reported therapeutic benefits has therefore confirmed the application of *C.afer* for the alleviation of various ailments by traditional practitioners.

The prevailing compounds which were 13,27-Cycloursan-3-ol, acetate (36.17%) and lupenone (39.50%) had their retention time as 26.896 min (36.17%) and 29.143 min (39.50%) respectively. The identification of these two pentacyclic compounds could be used to explain the strong presence of terpenes indicated in the phytochemical screening of the *C.afer* leaf extract. Terpenes are made up of isoprene units which are the building blocks for steroids.

The individual fragmentation patterns of the two most abundant compounds are presented in figures 2_a and 2_b.

The mass spectrum of the compound with retention time of 26.896 min (36.17%) gave 18 major fragmentation patterns (plot of relative abundance against *m/z*) at 41, 43, 69, 81, 95, 109, 123, 138, 149, 161, 175, 189, 205, 218, 257, 393, 453 and 468 (Figure 2_a).

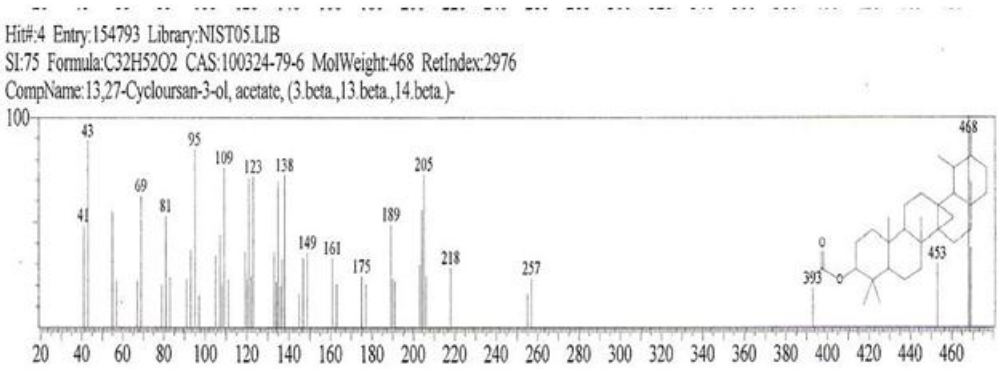


Figure 2_a: Mass spectrum of 13,27-Cycloursan-3-ol, acetate.

The base peak which is the fragment that appeared in greatest abundance was obtained at *m/z* 43 while the molecular ion, the peak with the highest *m/z* value in the spectrum *m/z* = 468 is the molecular ion. The height of the molecular ion peak also indicated the abundance of this ion, the stability of the compound and consequently its availability in the *C. afer* leaf as a therapeutic component.

The mass spectrum of the most abundant compound, Lupenone with retention time of 29.143 min (39.50%) gave 19 major fragmentation patterns (m/z) at 41, 55, 69, 81, 95, 109, 121, 135, 149, 161, 175, 189, 205, 218, 232, 245, 313, 409 and 424 (Figure. 2_b).

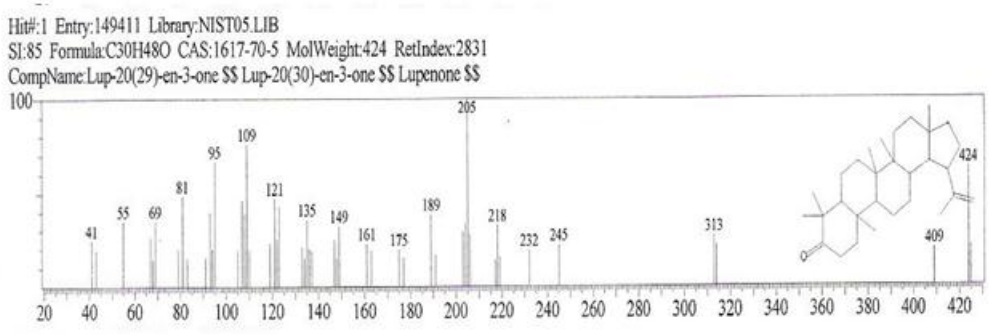


Figure 2_b: Mass Spectrum of Lupenone.

The base peak appeared at 205 *m/z* and the molecular ion at 424 *m/z*. The lupenone structure consists of the fundamental tetracyclic structures of steroids and specifically classified as triterpene with the base ion at 205 *m/z* molecular ion at 424*m/z*. The detection of lupenone in leaf extract of *Costus afer* has never been reported, although it has been identified in various therapeutic plants. Therefore, this is the first finding of these two triterpenoids in *Costus afer* leaf extract. The potential anti-cancer cell activities of Lupenone and its ability to promote the inhibition of protein tyrosine phosphatase and significantly decreased insulin activity in diabetes and treated obesity have been reported.[25] Known biological activities of the pentacyclic triterpenes include Analgesic, Anti inflammatory, Anti nociceptive, Gastroprotective, Hepatoprotective and Insectifuge.[26,27] Consequently a combination of all these attributes must have contributed to the successful application of *C. afer* leaf in traditional medicine.

CONCLUSION

GC-MS studies on the leaf extract of *Costus afer* showed the presence of relative amounts of one oxirane, three fatty acids and two triterpenoids identified as Cycloursan-3-ol, acetate (36.17%) and lupenone (39.50%). The two triterpenoid were noted for the first time in leaf extract of *Costus afer* and confirmed the presence of steroids and validate the use of *C. afer* leaf as an anti-inflammatory and antidote for acute toxicity in traditional medicine.

ACKNOWLEDGMENTS

The authors are grateful to the staff and management of the National Institute of Pharmaceutical Research and Development, Abuja and the staff and management of the National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria.

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