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Original Article

Phytochemical profiling of the bioactive principles of *Alysicarpus glumaceus* (Vahl) DC. aerial parts

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ABSTRACT

Background and Aims: *Alysicarpus glumaceus* is a plant used in Africa, Asia and some parts of the Arabian Peninsula with folkloric claims of having anti-tussive, anti-asthmatic, anti-diarrheal, abortifacient, anti-psychotic, anti-inflammatory, diuretic and stimulatory activities. The aim of this study was to determine the phytoconstituents present in the methanol extract (ME) of *Alysicarpus glumaceus* and its fractions.

Methods: Standard qualitative phytochemical screening methods such as thin layer chromatography (TLC), gas chromatography mass spectrometry (GC-MS) and fourier-transform infrared (FT-IR) spectroscopy were employed for the profiling of the plant and identification of the phytoconstituents.

Results: The phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenes. GC-MS chromatogram showed a total of 57 peaks with 38 different compounds identified, out of which 15 of the compounds were fatty acids mainly 3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester; cis-Vaccenic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- hexadecamethyl-;1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one; 2-Pentanone, 4-hydroxy-4-methyl-; Hexadecanoic acid; Palmitoleic acid; 9,12-Octadecadienoic acid and 9,17-Octadecadienal. While FT-IR spectras indicated the presence of carbonyl, alcohol, carboxylic acid, and aliphatic functional groups. Additionally, ethyl acetate fraction showed the peak characteristics of the aromatic (=C-H)/olefinic (=C-H) functional group.

Conclusion: The study showed that fatty acids were the major constituents of *Alysicarpus glumaceus*.

Keywords: *Alysicarpus glumaceus*, phytoconstituents, gas chromatography-mass spectrometry (GC-MS)

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INTRODUCTION

Medicinal plants have existed since the creation of man (Saric-Kundalic Dobe, Klatte-Asselmeyer & Saukel 2010). An estimated 14-28% of higher plant species are used for their therapeutic purposes and 74% of therapeutic agents in use were discovered from folkloric use of some of these plants (Ncube, Afolayan & Okoh , 2008). The search for chemical compounds in medicinal plants is of great importance because it gives information about the quality of the plant. The fundamental reason for quality control of herbal medicines is based on the concept of phytoequivalence of herbs, and then to use this concept to identify the real herbal medicine from the false one and further to doquality control (Ingole, 2016). Traditionally, medicinal plants possess different secondary metabolites with an array of pharmacological or biological activities such as antimicrobial, antioxidant, antifungal, antibacterial and antiviral agents.

Phytochemicals are natural bioactive compounds found in plants to protect against diseases and parasites along with the defense system of the body (Krishnaiah, Devi, Bono & Sarbatly, 2009). It is therefore important to know the phytochemical constituents of plants as that can lead to a search for the development of new and improved therapeutic agents (Phani, Anilakumar, & Naveen., 2015). Identification of chemical compounds in plants adds more knowledge to the medicinal value of plants as reported by Wintola & Afolayan (2011).

Alysicarpus glumaceus (*A. glumaceus*) is commonly known as alyce clover, alysicarpus, buffalo clover. It belongs to the family of Leguminonae. *It* is very similar to its other species *Alysicarpus rugosus* and *Alysicarpus ovalifolius* during the vegetative stages, thus difficult to identify (CIRAD, 2010). It is a shrubby, loosely branched, creeping and ascending non-climbing annual to 1m high in the grassy savanna mostly found in Northern Nigeria, where it is locally known as "*gadagi*" in hausa and "*bundiya*" in Fulani (Burkill, 1985).

The leaf is generally used for healing by application on old wounds, burns and leprosy; respiratory diseases including nasopharyngeal infections, cough and asthma; stomach aches and protection against the evil eye. The leaf and root are used as anti-motility agents. The root is used for its anti-inflammatory (gout and edema), aphrodisiac, abortifacient and is taken orally for snake bites (Haerdi, 1964; Burkill, 1985; Bekalo, Woodmatas & Woldemariam, 2009; Pandya, 2009; Umberto, 2016). The aerial parts are used for neuropsychiatric diseases especially depression and a poly herbal preparation having *Alysicarpus ovalifolius* as the main ingredient along with other substances of abuse is commonly available as "gadagi tea" in Kano State-Nigeria for over fifty years, believed to be medicinal. It is taken for extra energy and also increases alertness (Aminu, 2017), thus a stimulant.

The aim of this work was to identify the active principles in both the methanol extract of *A. glumaceus* and its fractions using phytochemical screening methods, thin layer chromatography (TLC), fourier transform infrared (FT-IR) spectroscopy and gas chromatography and mass spectrometry (GC-MS).

MATERIALS AND METHODS

Collection and identification of the plant

The whole plant of *A. glumaceus* was collected from Turunku of Igabi Local Government Area in Kaduna State, Nigeria in the month of September 2017 and was authenticated at the Department of Biological Sciences, Herbarium Section, Ahmadu Bello University Zaria, Nigeria by comparison with an existing specimen number, 446.

Plant extraction and fractionation

Two kg of shade dried coarse aerial parts of *A. glumaceus* were obtained after being pulverized in the pestle and mortar was macerated in methanol 70% for 10 days with occasional shaking. The menstrum was filtered after collection and left in an evaporating dish at room temperature for the filtrate to concentrate to a consistent weight. The concentrated filtrate was referred to as methanol extract (ME). The ME was successively partitioned using *n*-hexane, ethyl acetate, chloroform and *n*-butanol. These were subsequently concentrated as *n*-hexane (HEX), ethyl acetate (EAF), chloroform (CCF), *n*-butanol (BUT) and residual aqueous (RA) fractions respectively. The ME and its fractions were stored in a desiccator until use.

Qualitative phytochemical screening of the methanol extract of *Alysicarpus glumaceus* and its fractions

Phytochemical screening of the methanol extract of *A. glumaceus* and its fractions was carried out in accordance with standard protocols as described by Sofowora (1993) and Trease & Evans (2004).

Thin layer chromatography (TLC)

TLC ascending technique was employed and the stationary phase was pre-coated silica gel 60 PF_{254} (0.2 mm thick) TLC plate. Spots were applied manually using capillary tubes and the plates developed at room temperature in an air tight chromatographic tank containing various developing solvent systems. The developed chromatograms were air dried and visualized under ultraviolet light (254 and 366 nm), sprayed in the fume cupboard with 10% concentrated sulphuric acid followed by heating as a detecting agent. The positions of the various phytoconstituents were marked and their retention factor (R_f) was calculated (Harbone, 1973).

Identification of phytochemicals using gas chromatography and mass spectrometer (GC-MS)

The Phytochemical constituents from ME and fractions (HEX, EAF, CFF, N-But and RAF) of *A. glumaceus* were identified using gas chromatography-GC (model: Agilent, 7890A) interfaced with mass spectrometer-MS (Model: Agilent, 5977A) which was equipped with Agilent J & W GC capillary column with HP-5MS Ultra Inert (30 m x 250 µm x 0.25 µm) and composed of (5%-phenyl)-methylpolysiloxane. An electron ionization system with ionization energy of 70 eV with Emission current 0–315 µA was used. GC-grade helium gas (99.999%) was used as the carrier gas at a flow rate of 3.6839 mL/min and an injection volume of 3 µL (split ratio of 5:1). With the injector temperature at 250°C and ion-source temperature at 280°C, the oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min, to 200°C,

then 5°C/min to 280°C (isothermal for 9 min). Mass spectra were taken at 70 eV (a scan interval of 0.5 seconds and fragments from 40 to 550 Da). The total GC running time was 650 mins. The eluted constituents were detected by a flame ionization detector and the Gas chromatogram of each plant extract was recorded.

Phytochemicals present in the 6 samples were determined based on molecular weight and molecular structure. The relative percentage of each component was calculated by comparing its average peak area to the total area. The software used to handle the mass spectra and chromatograms was MassHunter and classic MSD ChemStation. The mass spectra of the compounds present in each of the samples were matched with National Institute of Standards and Technology (NIST) library 2014.

Identification of the functional groups using Fourier-transform infrared (FT-IR) Spectroscopy

FT-IR spectrum of methanol extract of *A. glumaceus* and its fractions was recorded using an Agilent Cary 630 FT-IR spectrometer with a resolution of 8.0 cm^{-1} in the transmission mode. The prepared sample was compressed into a self-supporting pellet and introduced as a spot on a thin film slide into an IR cell in the region 4000–650 cm^{-1}. The exposure time was about 15s and 16 scans were taken, IR spectrums were automatically generated after the analysis. The functional groups present in the 6 extracts were identified based on their wavelengths.

RESULTS

Yield of the methanol extract of *Alysicarpus glumaceus* and its fractions

2 kg of the powdered aerial parts of *A. glumaceus* gave a yield of 15.78% of the methanol extract. Partitioning of 250 g of the methanol extract with solvents of varying polarity resulted into *n*-hexane (45.53 g -Dark green), chloroform (10.06 g - light green), ethyl acetate (5.29 - light brown), *n*-butanol (57.17 g - Brownish red) and residual aqueous (126.59 g - coffee brown) fractions as shown in Table 1.

Table 1. Yields of the methanol extract of *Alysicarpus glumaceus* and its fractions.

Extract	Yield (g)	Yield (%)
ME	315.63	15.78
Fractions		
HEF	45.53	18.21
EAF	10.06	4.02
CCF	5.29	2.12
NBF	57.17	22.87
RAF	126.59	50.64

ME- Methanol Extract, HEF- Hexane Fraction, EAF- Ethyl Acetate Fraction, CCF-Chloroform Fraction, NBF- *n*-butanol Fraction, RAF- Residual Aqueous Fraction

Qualitative phytochemical screening of the methanol extract of *Alysicarpus glumaceus* and its fractions

The result of the phytochemical analysis revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenes but antraquinones were absent in the methanol extract of *A. glumaceus* and all its fractions. The hexane fraction was devoid of carbohydrates, flavonoids, saponins and tannins while the ethyl acetate and *n*-butanol fraction showed an absence of steroids and terpenes. Besides, he residual aqueous fraction lacked saponins and steroids (Table 2).

Thin layer chromatography

The analysis from thin layer chromatography profile of the methanol extract, *n*-hexane fraction, chloroform fraction, ethyl acetate, *n*-butanol and residual aqueous fractions revealed the presence of various phytochemicals when developed in different solvent systems of varying polarity. The R_f values of the various spots and the number of spots obtained after the development in various solvent systems are presented in Table 3.

Compounds identified in the methanol extract of *Alysicarpus glumaceus* and its fractions following GC-MS analysis

A total of 57 peaks were identified through the NIST (2014) library search of GC-MS, in methanol extract and the fractions of the plant, however some were overlapped. Thirty-eight compounds in all the extracts were identified and are listed in Tables 4-9.

Pharmacological activities of the identified compounds by GC-MS

Some of the identified compounds by the GC-MS that gave the large peak area and their pharmacological activity or activities that have been reported in the literature are all summarized in Table 10.

Functional chemical groups identified by Fourier-transform-infrared (FT-IR) spectroscopy analysis in the methanol extract of *Alysicarpus glumaceus* and its fractions

The study revealed most of the functional groups identified were in the frequency range of 3306 – 1025 cm^{-1}. These groups were almost similar for the methanol extract and its fractions. They included the saturated carbons, hydroxyl and carboxylic acid as presented in Table 11. However, the ethyl acetate fraction also had unsaturated carbons and esters identified in addition to the others.

DISCUSSION

The aerial parts of *A. glumaceus* were extracted with 70% methanol (70% methanol:30% water) yielded 15.78%, a similar yield (13.86%) of the methanol extract of *A. glumaceus* was reported by Bawa (2012). The high yield obtained in the *n*-butanol fraction and residual aqueous fraction suggests that the phytoconstituents present in the aerial parts may be more polar thus more soluble in polar solvents.

The results obtained from the qualitative phytochemical screening indicated a similarity in the profile of phytochemicals obtained from the methanol extract of *A. glumaceus* and

Table 2. Phytochemical constituents of the methanol extract of *Alysicarpus glumaceus* and its fractions.

Chemical constituents	Test	ME	HEF	EAF	CCF	NBF	RAF
Carbohydrates	Benedicts	+	-	+	+	+	+
	Fehlings	+	-	+	+	+	+
	Molisch	+	-	+	+	+	+
Alkaloids	Dragendroffs'	+	+	+	+	+	+
	Mayers	+	+	+	+	+	+
Flavonoids	Sodium hydroxide	+	-	+	+	+	+
Anthraquinones	Free anthraquinone	-	-	-	-	-	-
	Combined anthraquinone	-	-	-	-	-	-
Saponins	Frothing	+	-	+	+	+	-
Tannins	Ferric chloride	+	-	+	+	+	+
Steroids		+	+	-	+	-	-
Terpenes		+	+	-	+	-	+
Cardiac glycosides		+	+	+	+	+	+

ME- Methanol Extract, HEF- Hexane Fraction, EAF- Ethyl Acetate Fraction, CCF-Chloroform Fraction, NBF- *n*-butanol Fraction, RAF- Residual Aqueous Fraction. += Presence of constituents, - = absence of constituents

its fractions. They included cardiac glycosides, steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and devoid of both free and combined anthraquinones. Although, the hexane fraction was devoid of flavonoids, saponins and tannins, the result of this study correlates to a study done by Bawa (2012) on the same species. Similar classes of phytochemicals have also been reported to be present in another member of the genus, *Alysicarpus ovalifolius* and other members of the genus (Bashir, Uzair, & Bashir., 2018). Furthermore, alkaloids, saponins and tripenes have been previously isolated from the genus, Alysicarpus (Allen & Allen, 1981).

Phytochemicals have been reported to have many nutritive, biological and therapeutic properties (Benedec *et al*., 2013). They serve as useful taxonomic markers in identifying particular species as well as to distinguish it from related species, hence are helpful in the delimitation of taxa (Jonathan & Tom, 2008; Du *et al*., 2014). Phytochemicals are sources of energy required for a number of other physiological processes in humans (Hoffman, Friedmann, Saltman & Polich., 1999; Dingman, 2002). Flavonoids, saponins and tannins have been found to possess appreciable anti-inflammatory and central nervous system (CNS) action (Jäger & Saaby, 2011; Kauri & Arora, 2015). Flavonoids and neuroactive steroids were found to be ligands for gamma-aminobutyric acid (GABA) receptors in the CNS (SajidBijan, Zamiul, Mominul & Ekramul, ., 2013). Tannins have been used to tan animal hides and some isolated from certain plants have been shown to possess antidepressant and antihemorrhagic activities (Pemminati *et al*., 2010). Flavonoids, terpeniods and tannins possess antioxidant activity (Dutta, 2013; Phani *et al*., 2015). Triterpeniods have been reported to possess a wide range of neuropharmacological activities like anxiolytic, sedative, hypnotic, antidepressant and antinoceptive (Scott, Wright & Angus 2004; Morris,Dawson, Reynolds, Atack & Stephens ., 2006; Parmar *et al*., 2013). Alkaloids, flavonoids, terpenoids, tannins and saponins also give neuroprotection (Phani *et al*., 2015). Alkaloids and flavonoids have immunomodulatory activity (Middleton, Kandaswami & Theoharides 2000; Horrigan,Kelly & Connor 2006; Lantz, Chen, Sarihan, Sólyom, Jolad & Timmermann., 2007; Kure,Timmer & Stough ., 2017).

TLC profiling of the methanol extract of *A. glumaceus* and its fractions showed that there were a number of spots implying the presence of secondary metabolites. Various R_f values of the compounds gives an idea about their polarity that may also help in selecting a particular solvent system for further isolation of any compound from the plant extracts using chromatographic and spectroscopic techniques (Biradar & Rachetti, 2013). Compounds with high R_f value in less polar solvent system have low polarity while those with a low R_f value have high polarity (Talukdar, Choudhury, Chakraborty & Dutta (2010). In relation to other chromatographic methods, TLC offers the simplest and cheapest means of detecting natural product constituents.

GC-MS technique is used for the identification and quantification of compounds (Aneesh, Thomas, Thomas & Anandan., 2013; Senthil, Rameashkannan & Mani ., 2016). The 3, 7, 28, 6, 6 and 7 peaks were seen in the GC-MS chromatograms of methanol, hexane fraction, ethyl acetate fraction, chloroform fraction, *n*-butanol fraction and residual aqueous fraction, respectively. Fifteen out of the 38 different compounds that were identified in all the 6 extracts of *A. glumaceus* were fatty acids, thus indicating that they are the major constituents determined from the GC-MS analysis. Hexane and chloroform fractions had most fatty acids and occupied (match >90%) most of the total peak area while other extracts had less than 50%. These included both unsaturated (essential fatty acid 9, 12-octadecadienoic acid, methyl ester; [1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester; 9, 12, 15-octadecadienoic acid, methyl ester (Z,Z,Z)-; etc) and saturated fatty acids (hexadecanoic acid; pentanoic acid, heptyl ester; methyl stearate; pentadecanoic acid, 2,6,10,14-tetramethyl- methyl ester).

In addition, other classes of compounds like lactones, ethyl ester alkaloids, amide, ketones and unsaturated alcohols were also identified by the GC-MS.

Fatty acids are known to be responsible for many important physiological processes. They provide energy to the cell and act as substrates in the synthesis of fats, lipoproteins, liposac-

Table 3. Thin layer chromatography of the methanol extract of *Alysicarpus glumaceus* and its fractions.

Sample	Solvent system	No of Spots	R_f
Plate I & III			
ME	CF: EA		
	1:2	0	–
	2:1	3	0.89, 0.684, 0.937
CCF	CF: EA		
	1:2	7	0.073, 0.293, 0.489, 0.013, 0.756, 0.793, 0.866.
	2:1	7	0.165, 0.316, 0.379, 0.506, 0.684, 0.873, 0.949
EAF	CF: EA	2	0.013, 0.073
	1:2	2	0.063, 0.189
Plate II			
EAF			
	2:1	6	0.069, 0.139, 0.205, 0.625, 0.819, 0.889
	EA: CF: M: W		
	15:8:4:1		
RAF	EA: CF: M: W		
	15:8:4:1	0	–
Plate IV			
ME	Hexane : EA		
	3:2	3	0.571, 0.596, 0.974
HEX	Hexane : EA	6	0.532, 0.714, 0.819, 0.870, 0.909,
	3:2		0.974
CCF	Hexane : EA		
	3:2	7	0.104, 0.338, 0.530, 0.714, 0.819, 0.780, 0.909
Plate V	CF: Toluene		
HEX	90:10	5	0.063, 0.203, 0.281,0.438, 0.75
CCF	90:10	3	0.063, 0.438, 0.75
EAF	90:10	4	0.063, 0.203, 0.75
NBF	90:10	3	0.063, 0.203, 0.75
RAF	90:10	1	0.0469
Plate VI	CF: AA: M: W		
sNBF	64:32:12:8	4	0.246, 0.344, 0.557, 0.623
RAF	64:32:12:8	6	0.0492, 0.082, 0.328, 0.459, 0.557, 0.623

AA-Acetic Acid, CCF-Chloroform Fraction, CF-Chloroform, EA- Ethyl Acetate, EAF- Ethyl Acetate Fraction, HEF- Hexane Fraction, M-Methanol, ME- Methanol Extract, NBF- *n*-butanol Fraction, RAF- Residual Aqueous Fraction, R_f- Retention Factor, W-Water.

Table 4. Compounds identified in the methanol extract of *Alysicarpus glumaceus* following GC-MS analysis.

PK	RT	Peak Area	Library/ID/Class of the compound
1	5.3809	1.0341	2-Pentanone, 4-hydroxy-4-methyl- (KETONE)
2	55.9738	0.102	11,14,17-Eicosatrienoic acid, methyl ester (ESTER)
3	95.4662	98.8639	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl- (LACTONE)

PK-Peak, RT-Retention Time, ID-Identity

Table 5. Compounds identified in the hexane fraction of *Alysicarpus glumaceus* following GC-MS analysis.

PK	RT	Peak Area	Library/ID/Class of the compound
1	6.7732	0.0977	Propanedinitrile, methylene- (NITRILE)
2	47.2548	0.1058	1,4-Cyclohexanediol, trans- (ALICYCLIC ALCOHOL)
3	50.149	1.3733	Hexadecanoic acid, methyl ester (FATTY ACID ESTERS)
4	55.7908	0.958	9,12-Octadecadienoic acid, methyl ester (FATTY ACID ESTERS)
5	56.0106	2.7726	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (FATTY ACID ESTERS)
6	56.8898	0.5085	Methyl stearate (FATTY ACID ESTERS)
7	98.5071	94.1841	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester (FATTY ACID ESTERS)

PK-Peak, RT-Retention Time, ID-Identity

Table 6. Compounds identified in the ethyl acetate fraction of *Alysicarpus glumaceus* following GC-MS analysis.

PK	RT	Peak Area	Library/ID/Class of the compound
1	5.3809	5.0383	2-Pentanone, 4-hydroxy-4-methyl- (KETONE)
2	10.5098	0.451	Methanamine, N-methoxy- (AMINE)
3	15.712	0.4783	Cyclopropane, 1,1-dimethyl- (ALICYCLIC HC)
4	35.0552	0.0632	2-(1,2,3,4-Tetrazol-1-yl)acetonitrile (NITRILE)
5	37.3998	0.0896	3-Methyl-4-amino-5-methylamino-1,2,4-triazole(AROMATIC HETEROCYCLE)
6	41.1732	0.3714	(SR)- or (RS)-4-methyl-2,3-pentanediol (ALCOHOL)
7	42.8218	0.1124	Propanoic acid (ORGANIC ACID)
8	44.8734	0.2417	4-Methyl-1,4-heptadiene (ALKENE)
9	45.8991	0.1319	1,2:4,5:9,10-Triepoxydecane (EPOXIDE)
10	46.5586	0.1532	1-Propanol, 2-methyl- (ALCOHOL)
11	47.0348	0.609	1-Methoxy-3-(2-hydroxyethyl) nonane (ALKANE)
12	47.2546	0.8256	Oxirane, [(dodecyloxy)methyl]- (OXIRANE)
13	47.9141	0.0973	2-Nonen-1-ol (UNSATURATED ALCOHOL)
14	48.0973	0.2592	Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester (ESTER)
15	48.5369	0.2575	1-Octadecyne (ALKYNE)
16	48.83	0.1028	Propanoic acid (ORGANIC ACID)
17	49.3428	0.2902	1,6-Octadiene, 5,7-dimethyl-, (R)- (ALKENE)
18	49.7458	0.1352	2-Octylcyclopropene-1-heptanol (ALCOHOL)
19	50.1488	8.2493	Hexadecanoic acid, methyl ester (FATTY ACID ESTER)
20	50.9548	0.0757	2-Pentyn-1-ol (UNSATURATED ALCOHOL)
21	51.431	3.4969	Dibutyl phthalate (ESTER)
22	52.4202	0.5035	2-Dodecanol (ALCOHOL)
23	53.5559	0.3231	13-Tetradecynoic acid, methyl ester (FATTY ACID ESTER)
24	55.7906	5.4353	9,12-Octadecadienoic acid, methyl ester (FATTY ACID ESTER)
25	56.047	15.079	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (FATTY ACID ESTER)
26	56.4867	0.4996	Undec-10-ynoic acid, undecyl ester (FATTY ACID ESTER)
27	58.9412	53.9042	cis-Vaccenic acid (ORGANIC ACID)
28	67.4039	2.7255	9,17-Octadecadienal, (Z)- (ALDEHYDE)

PK-Peak, RT-Retention Time, ID-Identity

Table 7. Compounds identified in the chloroform fraction of *Alysicarpus glumaceus* following GC-MS analysis.

PK	RT	Peak Area	Library/ID /Class of the compound
1	6.7731	0.1402	Pentanoic acid, heptyl ester (FATTY ACID ESTERS)
2	50.1488	1.0016	Hexadecanoic acid, methyl ester (FATTY ACID ESTERS)
3	55.7906	0.453	9,12-Octadecadienoic acid, methyl ester (FATTY ACID ESTERS)
4	56.0104	1.4074	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (FATTY ACID ESTERS)
5	56.853	0.3809	Methyl stearate (FATTY ACID ESTERS)
6	97.0415	96.6169	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester (FATTY ACID ESTERS)

PK-Peak, RT-Retention Time, ID-Identity

Table 8. Compounds identified in the *n*-butanol fraction of *Alysicarpus glumaceus* following GC-MS analysis.

PK	RT	Peak Area	Library/ID/Class of the compound
1	5.3811	1.3888	2-Pentanone, 4-hydroxy-4-methyl- (KETONE)
2	50.149	0.134	Hexadecanoic acid, methyl ester (FATTY ACID ESTER)
3	56.0106	0.3103	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (FATTY ACID ESTER)
4	98.8368	98.1669	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (ETHER)

PK-Peak, RT-Retention Time, ID-Identity

Table 9. Compounds identified in the residual aqueous fraction of *Alysicarpus glumaceus* following GC-MS analysis.

PK	RT	Area Pct	Library/ID/ Class of the compound
1	11.2425	0.1503	Methanamine, N-methoxy- (AMINE)
2	42.4921	0.2933	Propanamide, 2-methyl- (AMIDE)
3	52.4935	0.1174	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester (ESTER)
4	60.773	4.409	Palmitoleic acid (FATTY ACID)
5	87.9194	95.03	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one (KETONE)

PK-Peak, RT-Retention Time, ID-Identity

Table 10. Some of the chemical compounds identified from the methanol extract of *Alysicarpus glumaceus* and its fractions by GC-MS and their reported pharmacological activity.

S/No	Compound	Derivatives	Pharmacological activity
1.	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	Fatty acid esters	Anti-inflammatory, analgesic, precursors for other drug molecules with activities like anticancer, anxiolytic, antioxidant, anti-Parkisons', antibacterial agents and antidepressants. (Rajeev, Prabodh & Mohammed. 2011; Aly, Hassan, Makhlouf, & Bräse, 2020),
2.	Hexadecanoic acid	methyl ester Fatty acid	Anti-inflammatory, antioxidant, inhibit production of uric acid activity, urine acidifier, mainly used to produce soaps, cosmetics, release agents, antifibrinolytic, heamolytic, lubricant, nematicide, antialopecic and antidepressant. (Rahman, Ahmad, Mohamed, & Ab Rahman, 2014)
3.	9,12-Octadecadienoic acid	methyl ester Fatty acid	Hepatoprotective,antihistaminic, hypocholesterolemic, antieczemic, antioxidant and anticancer properties (Yu., *et al,* 2005; Akpuaka, Ekwenchi, Dashak, & Dildar, 2013)
4.	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy	ethyl ester Alkaloid	Anti-inflammatory, inhibit production of uric acid activity, urine acidifier, increae aromatic amino acid decarboxylase acitivity, 17-beta-hydroxysteriod dehydrogenase inhibitor, testosterone-hydoxylase inducer,
5.	Cis-Vaccenic Acid	Fatty Acid	Anticancer and antimicrobial properties (Lim, *et al.,* 2013; Hamazakic *et al.,* 2016)
6.	Dibutyl phthalate	Fatty acid	Antimicrobial, antifouling, solvent for perfumes, nitrocellulose and cellulose acetate, alcohol denaturant (toilet preparations), nail polish, as a fixative in perfumes and in fingernail elongators as a plasticizer, ectoparasitic agent. (Senthil, Rameashkannan & Mani., 2016; Ingole, 2016)

Table 10. Continue.

S/No	Compound	Derivatives	Pharmacological activity
7.	Palmitoleic acid	Fatty acid	anti-thrombotic effects, lowers LDL cholesterol and higher high-density lipoprotein cholesterol, has beneficial effects on insulin sensitivity, cholesterol metabolism, and hemostasis. (Morgan & Dhayal, 2010)
8.	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	Fatty acid ester	saturated fatty acid, flavoring agent (Smedman, Gustafsson, Berglund, & Vessby 1999; Jost, 2002; Aneesh, Thomas, Thomas, & Anandan, 2013)
9.	Propranoic Acid	Organic Acid	Anti-inflammator(Prostaglandin Inhibitor (present in NSAIDS-Ibuprofen), anti-asthmatic drugs, preservative and as an anti-microbial agent in foods produced for human and livestock consumption. (Dracheva *et al.,* 2009; Flavouring agent, antifungal agent (Lim, Wong, Rosli, Ng, Seow, & Chong, 2009)
10.	2-Dodecanol	Alcohol	Antimicrobial (Falowo, Muchenje, Hugo, Aiyegoro. & Fayemi., 2017)
11.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl-	Lactone	

Table 11. Functional groups identified by Fourier-Transform-Infrared FT-IR spectroscopy analysis of the methanol extract of *Alysicarpus glumaceus* and its fractions.

Sample/Fraction	I.R Frequency(cm^{-1})	Assignment	Inference
Methanol	3306	O – H Str	- Saturated carbons
	2922	-C – H Str (asym)	- Alcohol
	2855	-C – H Str (sym)	- Carboxylic acids
	1718	-C = O	
	1036	-C – O	
n-Hexane	3354	O – H Str	- Saturated carbons
	2922	-C – H Str (asym)	- Alcohol
	2855	-C – H Str (sym)	- Carboxylic acids
	1710	-C = O	
	1039	-C – O	
Chloroform	3339	O – H Str	- Saturated carbons
	2922	-C – H Str (asym)	- Alcohol
	2855	-C – H Str (sym)	- Carboxylic acids
	1718	-C = O Str	
	1036	-C – O Str	
Ethyl acetate	3343	O – H Str	-Saturated and Unsaturated carbons
	3011	=C – H Str	
	2922	-C – H Str (asym)	-Carboxylic acid
	2855	-C – H Str (sym)	-Alcohols
	1710	-C = O Str	-Esters
	1073 – 1170	O - C – O Str	
Residual aqueous	3261	O – H Str	- Saturated carbons
	2926	-C – H Str	- Alcohol
	1580	-C = O Str	- Carboxylic acids
	1036	-C – O Str	
Butanol	3272	O – H Str	- Saturated carbons
	2929	-C – H Str	- Alcohol
	1707	-C = O Str	- Carboxylic acids
	1025	-C – O Str	

IR- Infar Red, Str-Stretching, sym-Symertic, asym- Asymertic

charides and eicosanoids (Ansorena, Gimeno, Astiasarán, & Bello., 2001). They aid in the of modulation of enzymes activities (Zamaria, 2004), receptor site (Facchini, 2001), as well as neurotransmission, cell survival and signaling pathways, which ultimately affects mood and cognition (Parekh, Smeeth, Milner & Thure2017; Fernandes,Mutch, & Leri ., 2017). Other fatty acids found in *A. glumaceus* with their reported pharmacological activities includes the following: essential fatty acid 9, 12-Octadecadienoic acid, methyl ester (Saradha & Paulsamy, 2013; Aneesh,Thomas, Thomas, & Anandan, 2013; Venkatesh, Vidya,& Kalaivani ,2014); dibutyl phthalate, hexadecanoic acid (Easwaran & Ramani, 2014; Senthil, Rameashkannan & Mani., 2016; Abbasi-Maleki &Mousavi, 2017) Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-; (Kumaradevan, Damodaran, Mani, Dineshkumar & Jayaseelan.,2015), Pentadecanoic acid (Smedman, Gustafsson, Berglund, & Vessby ., 1999; Jost, 2002, Aneesh, Thomas, Thomas, & Anandan, 2013)), Palmitoleic acid (Abraham, Riemersma, Wood, Elton, & Oliver, , 1989; Morgan & Dhayal, 2010; Mozaffarian *et al*., 2010) and [1,2,4] Triazolo[1,5-a] pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester (Rajeev, Prabodh & Mohammed., 2011).

The FT-IR data obtained for all the extracts indicated the presence of the carbonyl (C=O), alcohol and carboxylic acid (O-H), and aliphatic (-C-H) functional groups, as evident by the appearance of prominent bands observed at 1707-1718 cm^{-1} (C=O stretching vibrations), 1025-1039 cm^{-1} (C-O stretching vibrations), 3261–3354 cm^{-1} (O – H stretching vibrations) and 2990 cm^{-1} (Aliphatic Csp3-H stretching vibrations) for both the methanol and its fractions. Additionally, the ethyl acetate fractions showed peak characteristics of aromatic (=C-H)/olefinic (=C-H) functional group at 3011 cm^{-1} (aromatic Csp2-H stretching vibrations), although the presence of aromatic function in the other fractions could not be ruled out due to the broad O-H stretching peak which might be overlapping with it and also the presence of a peak around 1500 cm^{-1} (C=C stretching vibrations) (Crews, Rodriquez, Jaspars & Crews 1998; Pavia, Lampman, Kriz & Engel ., 2011) for all the extracts. These functional groups or pharmacophores are usually the ones responsible for the therapeutic action and similar pharmacophores found in the extracts have been reported in tricyclic antidepressants (Sagdinc, Azkeskin, & Eşme., 2018). The fact that there was no absorbance observed in the region 2220-2260 cm^{-1} indicated that there was no cyanide group which is a toxicophore and suggested that the plant can be considered as safe (Ragavendran, Sophia, Raj & Gopalakrishnan., 2011; Abdoun, Hassan, Gaber, & El- Sharekh., 2019).

These spectral data obtained from the extracts conformed with the qualitative phytochemical screening revealed the presence of flavonoids, saponins, tannins, steroids and cardiac glycosides in all the fractions except for hexane fraction which was devoid of flavonoids and saponins. The functional groups identified (alcohols, acids and ketones) are characteristics of these phytochemicals (Pavia, Lampman, Kriz & Engel., 2011). These identified functional groups further confirm the presence of the secondary metabolites in the methanol extract of *A. glumaceus* and its fractions.

In addition, the result from FT-IR spectrum correlate to the chromatograms obtained from GC-MS analysis of the methanol extract of *A. glumaceus* and its fractions where the most significant fragments (compounds) identified were found to be fatty acids, alcohols, ketones, and esters.

Further study is needed to isolate and characterize the specific active principle(s) responsible for the biological activity of the plant. Additional investigations are ongoing to evaluate its pharmacological activities and to elucidate the probable mechanism(s) of some of the activities including antidepressant, anxiolytic, analgesic and anti-inflammatory.

CONCLUSION

A. glumaceus contains several classes of phytoconstituents such as alkaloids, flavonoids, tannins, saponins, terpenes, steroids and cardiac glycosides. It also contains many compounds whose biological activities have been previously reported and this may provide a scientific rationale for various folkloric claims of the plant.

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