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Phytochemicals, Proximate Analysis and Identification of Compounds in Cowpea (*Vigna unguiculata* (Walp) L.) Seeds using Gas Chromatography-Mass Spectrometry

***Emmanuel Eimiomodebheki Odion, Cyril Odianose Usifoh**

Department of Pharmaceutical Chemistry,
Faculty of Pharmacy,
University of Benin,
Benin City,
Edo State,
Nigeria.

Abstract

Vigna unguiculata seeds are used as food and contain different primary metabolites, however the identification of its secondary metabolites in the semi-polar solvent-dichloromethane could confirm some of its ethno-medicinal claims. The aim of this work was to determine the proximate parameters, phytochemical contents and identify the compounds in the dichloromethane extract of the seed of Vigna unguiculata. Proximate analysis and phytochemicals were determined using standard established methods while identification of the compounds in the dichloromethane extract was done by gas chromatography-mass spectrometric (GC-MS) analysis. Secondary metabolites include alkaloid, flavonoids, glycosides, phenols, saponins, sterols and triterpenoids. Primary metabolites such as carbohydrate content (48.32±0.34) %, moisture content (10.63±0.04) %, crude protein (25.78±0.04) %, fat content (0.65±0.05) %, crude fibre (6.48±0.05) % and ash content (2.78±0.07) % were reported. Twenty-one compounds were identified in the dichloromethane seed extract, they include fatty acids, esters, alcohols and ketones. Cowpeas contain both primary and secondary metabolites that have documented pharmacological applications.

Keywords: *Vigna unguiculata*, Identified compounds, Proximate parameter, Phytochemical screening.

INTRODUCTION

Cowpeas (*Vigna unguiculata* (Walp) L.) family Fabaceae is a yearly leguminous crop (Ibrahim *et al*., 2017; Singh *et al*., 2003) with Sub-Sahara origin, but widely grown in other parts of the world such as the Americas and Asia (Davis *et al*., 1991. The countries in the south of the Sahara remain the biggest cultivator and consumer of cowpea, in Nigeria the consumption rate is about 25 kg to 30 kg per annum (Falusi, 1997). The name "cowpea" may have been coined from the fact that the plant was used to feed livestock particularly cows in southern part of the United State (Timko *et al*., 2007). Its seeds are eaten as different delicacies by man, while its leaves are consumed as vegetable (Blade *et al*., 1997). The haulms are consumed by

Author for Correspondence

cows as an important source of protein (Kormawa *et al*., 2002). The grain is a worthy and inexpensive supplier of protein when compared to other protein sources (Aja *et al*., 2007). Cowpeas are also rich in carbohydrate (Bressani, 1985), fat, minerals and vitamins (Ehlers & Hall, 1997), these are considered to be primary metabolite. Secondary metabolites have been identified in the seeds, these include phenols, sterols and flavonoids (Alidu *et al*., 2020).

The presence of these secondary metabolites which have been termed phytochemicals have impacted on its use as herb (Londonkar & Awanti, 2014). Previous studies have shown its antioxidant (Marques *et al*., 2015), anticancer and antibacterial potentials of its peptides, phenols and sterols contents (Goncalves et al., 2015). These activities are due to plant chemicals in the leaves and seeds. A GC-MS analysis comparing the metabolomic profiles of seeds and pods from old Chinese landraces and the modern cultivars, yielded about 120 compounds (Perchuk *et al*., 2020). Previous study has used the GC-MS technique to evaluate ethanol extract of the leaves of cowpea and 50 compounds were identified (Aja *et al*., 2007). There is paucity of information on the GC-MS analysis of the dichloromethane extract of the seed extract of *Vigna unguiculata*. Thus, this study aim to evaluate the phytochemical constituents, proximate parameters and identify the compounds in the dichloromethane extract of the white cowpea seed by GC-MS analysis

MATERIALS AND METHODS

Sample collection, preparation and extraction

Cowpeas (*Vigna unguiculata*) were collected in the month of July from local market (Lagos street market) in Edo state, placed in zip-lock polyethylene bag and then stored at a temperature of 4^{o}C. They were identified by Dr Sunday Ogedegbe of the Department of Crop Science, Faculty of Agriculture. Fifty grams each of the grains collected from each market was carefully selected to exclude stones and unwanted materials, these were pulverised using hand milling machine (V-2, China) and extracted with 750 mL of dichloromethane by Soxhlet apparatus for 3 hrs at a temperature of 60^{o}C. The extracts were concentrated in vacuum using rotary evaporator at a temperature of 50^{o}C, then partially purified by adsorbing to silica (mesh size 160-200 µm) and eluting with 20 mL of dichloromethane in a column (15 cm x 9 mm I.D). The eluates obtained were concentrated, reduced to 2 mL and analyzed using GC-MS.

Gas Chromatography-Mass Spectrometric Analysis

Identification of the compounds in cowpeas was carried out using method described by Odion *et al*., (2020). It involves using a GC-MS-QP 2010 SE Shimadzu, (JAPAN). The column used was a Restek column with length, internal diameter and thickness (30m x 0.32mm x 0.5µm) with the following conditions: the GC was operated in the splitless injection mode with 1 ml/min flow rate for helium gas as carrier gas and make up gas. The injection temperature and volume are 250°C and 8 µl respectively. The column temperature program was as follows: 120 ^{o}C (0:00min), to 200 at 45 ^{o}C min^{-1} (0:00 min) to 230 ^{o}C at 12.5 ^{o}C min^{-1}, at 325^{o}C then it was held for 2 min, at 30 ^{o}C min-^{1}. Samples were injected automatically by split-less mode into the MS at an interface temperature of 250^{o}C with ion source at 230^{o}C, with ionization mode of electron impact ionization (EI) of 70 eV. Three ions specifically; the most abundant as quantification ion and two ions for confirmation.

Proximate analysis

The pulverised samples from Lagos street market were evaluated for the proximate parameters using the methods described by the Association of analytical Chemist (AOAC,

1990). Proximate parameters determined were moisture content, crude fibre, crude protein content, fat content and total ash value (AOAC, 1990).

Phytochemical content

Involves the screening for phenols, flavonoids, tannins, triterpenoids, cardiac glycosides, alkaloids, saponins and sterols. The procedures are as described by Sofowora (1993) and Trease and Evans (2002).

Presentation of results and statistical analysis

Results were presented as mean ± standard deviation (Mean ± SD), SPSS version 23 was used for the analysis and level of significance was set at $P \leq 0.05$.

RESULTS

Phytochemical screening revealed the presence of saponins, alkaloids, triterpenoids, sterols, phenols, flavonoids and glycosides (Table 1).

Table 1: Phytochemical analysis of the powdered seed of cowpeas

Phytochemicals	Inference
Saponins	+
Alkaloids	+
Triterpenoids	+
Sterols	+
Flavonoids	+
Phenols	+
Glycosides	+
Tannins	_

.key: +=Presence, -=Absence

From the proximate analysis result in table 2, it was revealed that the ash content (2.78 ± 0.07) %, carbohydrate (48.32±0.34) %, crude fibre (6.48±0.05) %, crude protein (25.78±0.04) %, fat content (0.65±0.05) % and moisture content (10.63±0.40) % were recorded.

Table 2: Proximate analysis of the powdered seeds of cowpea

Sample collected	Ash value (%)	Carbohydrate value (%)	Crude fibre (%)	Crude protein (%)	Fat content (%)	Moisture content (%)
Lagos street	2.78±0.07	48.32±0.34	6.48±0.05	25.78±0.04	0.65±0.05	10.63±0.40

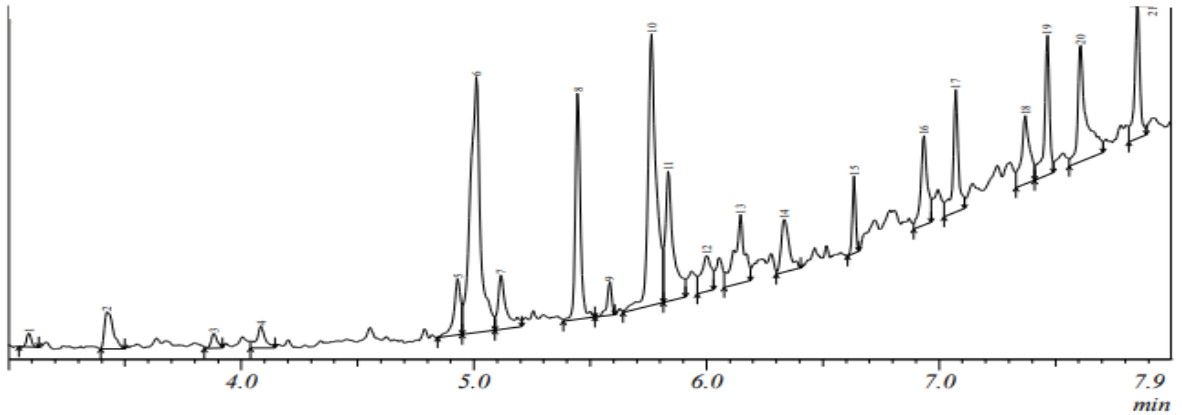

Figure 1: Representative Chromatogram of the dichloromethane extract of *Vigna unguiculata*

The GC-MS analysis identifies twenty-one (21) compounds which can be classified into branched alkane (2-methyloctacosane), aldehyde (2-ethyl-2-hexenal), alcohol (2-octyl-1-

decanol, 2-methylene cholestan-3-ol,(3β,5α), 2,2-dimethyl-6-methylene-1-[3,5-dihydroxy-1-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol, 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol), saturated fatty acid (2-octyldodecanoic acid, octadecanoic acid) and unsaturated fatty acids (palmitoleic acid), aromatic hydrocarbon (triphenylmethane), ester (isopropyl tricosanoate, vinyl 10-undecenoate, hexadecanoic acid, 1,1′-[1-(hydroxymethyl)-1,2-ethanediyl] ester), ketone (2-hydroxycyclopentadecanone, 2-methylcyclopentadecanone), steroid (trimethylsilyl ether-1-monolinoleoylglycerol), acid (2,6-bis[(trimethylsilyl)oxy]-benzoic acid)

Table 3: GC-MS analysis of the dichloromethane extract of cowpea seed

S/N	Name	RT	QI	MF	CI	MM
1	2-methyloctacosane	3.088	57	$C_{29}H_{60}$	43 71	408
2	2-ethyl-2-hexenal	3.422	55	$C_8H_{14}O$	97 111	126
3	2-octyl-1-decanol	3.881	57	$C_{18}H_{38}O$	43 71	270
4	2-octyldodecanoic acid	4.084	55	$C_{20}H_{40}O_2$	73 172	312
5	Palmitoleic acid	4.931	53	$C_{16}H_{30}O_2$	69 83	254
6	Butyl 4,8,12-trimethyl-tridecanoate	5.011	56	$C_{20}H_{40}O_2$	76 129	312
7	Triphenylmethane	5.116	167	$C_{19}H_{16}$	152 165	244
8	Chlorpyrifos	5.446	97	$C_8H_{11}Cl_3NO_3PS$	197 314	349
9	isopropyl tricosanoate	5.584	355	$C_{26}H_{52}O_2$	57 102	396
10	2-hydroxycyclopentadecanone	5.764	55	$C_{15}H_{28}O_2$	69 83	240
11	Octadecanoic acid	5.836	73	$C_{18}H_{36}O_2$	43 60	284
12	2-methylene cholestan-3-ol,(3β,5α)	6.000	69	$C_{27}H_{48}O$	81 95	400
13	2,2-dimethyl-6-methylene-1-[3,5-dihydroxy-1-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	6.145	69	$C_{14}H_{24}O_4$	55 81	256
14	hexadecanoic acid, 1,1′-[1-(hydroxymethyl)-1,2-ethanediyl] ester	6.334	43	$C_{35}H_{68}O_5$	57 98	568
15	trimethylsilyl ether-1-monolinoleoylglycerol	6.634	73	$C_{27}H_{54}O_4Si_2$	147 207	498
16	2-methylcyclopentadecanone	6.934	55	$C_{16}H_{30}O$	69 72	238
17	2,6-bis[(trimethylsilyl)oxy]-benzoic acid	7.070	73	$C_{16}H_{30}O_4Si_3$	267 355	370
18	Vinyl 10-undecenoate	7.369	55	$C_{13}H_{22}O_2$	69 149	210
19	N-Methyladrenaline, tri-TMS	7.464	73	$C_{19}H_{39}NO_3Si_3$	58 355	413
20	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)cyclohexanol	7.608	69	$C_{30}H_{52}O$	43 81	428
21	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl hexasiloxane	7.852	73	$C_{12}H_{38}O_5Si_6$	207 281	430

Key: RT= Retention Time, QI = Quantification ion, MF = Molecular formula, CI = Confirmation ion, MM = Molecular Mass

DISCUSSION

Most plants are considered as herbs with different ethno-medicinal uses. These claims are due to the presence of phytochemicals which are natural occurring plant synthesized compounds use for different purposes. Some of which could be beneficial to the plant or used by human or animal in the process of consumption. These beneficial effects are set as claims by herbalist and some of these claims have been validated through different pharmacological activities by researchers working in different fields (Derkyi *et al*., 2010). However the presence of these plant chemicals in this study are different from some earlier findings, this could be due to climatic variation (Madubuike *et al*., 2018). The presence of alkaloids, flavonoids, triterpenoids, saponins and glycosides in cowpeas may indicate a wide range of biological activities, because these chemicals have been shown to possess anti-inflammatory, hypocholesterolemic, immunomodulatory, hypoglycaemic, expectorant, vaso-protective and anti-tumour effect (Famata *et al*., 2013).

Proximate analysis provides important data and aid the evaluation of the feature of samples analyzed. It provide information on ash value, carbohydrate content, crude fibre and protein,

fat and moisture content [23]. Ash is the inorganic residue left following the removal of water and organic matter by heating. Additionally, it is a means of measuring the total amount of minerals in a sample [24]. In a study to determine the amount of minerals in different cultivar of cowpeas, Famata and co-workers in 2012, showed the ash content to be 3.00 %, while a study done in the same year by Alayande and co-workers(2012) indicated that the ash values obtained were below 3.00 % when they were compared. The ash content obtained in this study showed that Lagos street market I was below 3 %, while many other markets were above the 3 % mark (Aletan, 2018). These are indications that the minerals content in cowpeas can vary from place to place and could differ within extensive limits for cultivar of cowpeas because of flexible natural or physiological conditions. Its measurement is very vital, due to the fact that minerals may be responsible for the pharmacological effect and valuable in influencing the authenticity and purity of sample (Bhargava *et al*., 2013).

The crude fibre content obtained in this study do not agree with previous study carried out by Kirse & Karklina (2015), where the fiber content of cowpea ranged between 12.00 ± 0.15 % to 14.80 ± 0.20 %. Similarly, Eashwarage and co-workers (2017) similarly reported fiber content between 13.60 ± 0.15 % to 15.99 ± 0.49, these are higher than the values obtained in this study. The main contributors of the high fibre content of cowpeas are the seed coats and cell walls. This offers a lot of health gains to human, which include the regulation of blood cholesterol and glucose level, adds as roughage. Absorbs water in the gastrointestinal tract, thus guaranteeing easy evacuation of waste products (Eshwarage *et al*., 2017). Subsequently, occurrence of haemorrhoids and constipation are prevented (Jayathilake *et al*., 2018).

Moisture content of a food item influences its organoleptic properties and shelf life (Odedeji and Oyeleke, 2011). These properties may be affected adversely following a slight alternation from a set standard. Low moisture content prevent microbial attack of the seed or prematurely germination while storing (Ferreira *et al*., 2019). Moisture content recorded for cowpeas in this study were about 10.00 %, however seeds moisture content should ideally be between 3.00 % and 7.00 % for long-term storage (Gawrysiak-Witulska *et al*., 2012). In rainy season, moisture content may not be below 10 % due to high relative humidity levels especially in the tropic (Chappell *et al*., 2000).

The protein content of the sampled cowpeas showed no significant difference. The result from this study is in tandem with previously reported findings that protein content of both improve and local varieties ranged between 20 % and 27 %, this study showed values within these ranges. The high protein contents in these varieties could be used to address high levels of kwashiorkor (Tierney *et al*., 2010).

The fat content of the cowpeas sample in this study are below 0.7 %, while higher values such as 2.01 to 2.88 % have been recorded in brown seeds cowpeas (Tierney *et al*., 2010). However, previous report has shown that cowpeas are low in their fat or lipid content (NRCUSCDH, 1989). At $P > 0.05$, no significant difference in the level of the fat from cowpeas sampled in this study. Lipids provide high level of energy and could be responsible in the transportation of fat soluble vitamins such as vitamins A, D, E and K (Essien, 1987).

Carbohydrate content recorded in this study are in agreement with previous study carried out by Ashogbon & Akintayo (2013), that reported carbohydrate content of 45.64 to 57.12 %. Carbohydrate serve the purpose of providing energy and it storage in animal tissues could lead to it been converted to protein. Though cowpeas are labelled as proteineous foods, they also contain high level of carbohydrate.

The analytical methods used in this study included solvent extraction by dichloromethane and solid-phase extraction (SPE) for the purification of the extract and subsequent analysis using gas chromatography-mass spectrometry (GC–MS) for identification. This led to the identification of twenty-one which includes octadecanoic acid, hexadecanoic acid, 1,1′-[1-(hydroxymethyl)-1,2-ethanediyl] ester and 2-methylcyclopentadecanone which are fatty acids, ester and ketonic compounds. The fatty acids possess antimicrobial and anti-inflammatory properties (Pu *et al*., 2010; Choi *et al*., 2014; Apama *et al*., 2012). 2-methyloctacosane is the cuticular hydrocarbons of insects that also aids in chemical communication (Spikes *et al*., 2010). Palmitoleic acid exhibit antimicrobial activities specifically against skin-associated Gram-positive bacteria, which can explain their decrease. It was postulated to have anti-thrombotic effects which can aid stroke prevention (Abraham *et al*., 1970). It has also functions as an adipose tissue-derived lipid hormone that stimulates muscle insulin action and suppresses hepatosteatosis in mice deficient in fatty acid binding protein (Yang *et al*., 2011). Other positive effect reported include lower LDL cholesterol and higher high density lipoprotein cholesterol (HDL) (Mozaffarian *et al*., 2010). Beneficial effect have been reported for insulin sensitivity, cholesterol metabolism and haemostasis, with likely proposal of that it may prevent β-cell death induction of glucose or saturated fatty acids (Morgan & Dhayal, 2010). Trimethylsilyl ether-1-monolinoleoylglycerol have been reported to have antimicrobial activity (Meenaksh *et al*., 2009).

CONCLUSION

From the phytochemical and proximate parameters of the seeds of cowpea, it has shown to be a good source of healthy dietary and functional nutrition, this can be due to high and moderate levels of proximate parameters and presence of phytochemicals with wide range of documented pharmacological activities.

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