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Chemical Constituents and Nutrient Composition of *Carica papaya* and *Vernonia amygdalina* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author OO designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AR and SMR managed the literature searches, analyses of the study and performed the spectroscopy analysis. Author UAV managed the experimental process. Authors OJ and EC reviewed the draft for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

The present study evaluates the chemical constituents and nutrient composition of leaf extracts of *Carica papaya* and *Vernonia amygdalina*. The chemical constituents were analysed using gas chromatography mass spectrometry (GC-MS). The constituents of *C. papaya* leaf extract showed twenty constituents, dominated by oleic acid (28.98%) with molecular weight of 282, with the least compound Trans-Geranylacetone (0.17%), with molecular weight of 194. However, *V. amygdalina* leaf extracts contained sixteen constituents, dominated by 9-octadecenoic acid (35.18%), with molecular weight of 282, and Methyl tridecanoate (0.45%) as the least compound. The proximate analysis showed significantly (*P<0.05*) higher quantities of ash, crude protein and crude fibre

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content in *C. papaya,* when compared with *V. amygdalina*. In the anti-nutrient analyses, *C. papaya* had significantly (*P<0.05*) higher amounts of oxalate, phytate and flavonoid as compared with *V. amygdalina*. Evaluation of mineral composition showed higher amounts of magnesium and zinc in *V. amygdalina* except in copper content, compared with *C. papaya*. This study revealed the potential compounds present in the plant samples for their acclaimed biological activities in traditional phytotherapy.

Keywords: Chemical constituents; nutrients composition; Carica papaya; Vernonia amygdalina; GC-MS analysis.

1. INTRODUCTION

Many traditional medicinal plants have played a key role in the world healthcare, having about 80% of Africans depending on it [1]. Arising from their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites, plants have from ancient history been used as sources of food and medication against various ailments. In rural areas where access to modern health facilities is limited by the level of development, plants/ herbs remain the source of the healthcare system [2]. It has shown a wide range of its use in the treatment of diseases especially priority diseases of Africa such as malaria, sickle cell anaemia, diabetes, hypertension amongst others [3].

There are African phytomedicines which are well known in the international market, and they contribute to the economy of the producing countries [1]. Hence, the need for more scientific studies on more plants.

The diverse medicinal plants at present have always been considered to be a possible alternative and rich source of new drugs either in crude or purified form. More so, most of the antimalaria drugs in use today (artemisinin and quinine) were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates [4].

Vernonia amygdalina which is regarded as a medicinal herb is a multi-purpose and rapid regenerating soft wooded shrub of 2-10 m tall, with petiolate leaves of around 6mm in diameter [5]. It is widely used as a daily green vegetable or herb to treat malaria, diabetes and other diseases [5]. *V. amygdalina* can be commonly found along drainage lines and in natural forest, or at home and commercial plantation in Nigeria [6,7]. On the other hand, *Carica papaya* belongs to the family *Caricaceae,* which is recognised by its weak and unbranched soft stem, yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves. The leaves have been used traditionally for the treatment of wide range of diseases such as malaria, jaundice and antiviral activity [8].

It has been scientifically shown that *C. papaya* and *V. amygdalina* has many phenolic groups which may scavenge free radicals, functioning as antioxidant and antibacterial [9-11]. There is a growing awareness in correlating phytochemical compounds present in medicinal plants to their biological activities [12,13]. *C. papaya* and *V. amygdalina* plant are used in the Nigerian traditional healthcare practice. Traditional medicine practitioners use both plants as anti-helminthic, anti-malaria, digestive tonic, appetizer, anti-haemolytic, anti-fungal, for urinary complaints and gonorrhoea, (infusion), dressing of wounds (fresh leaves), anti-dysentery, and chronic diarrhoea, as sedative and tonic, as cure for pile and wounds of the urinary tracts, and several other diseases [14]. The plant extracts have been scientifically proven to function as: antibacterial agent [15], antifungal [16,15], anti-cancer/ tumor [17], anti-plasmodial [18,19].

Plant-based natural constituents are considered one of the main sources of biologically active compounds [20]. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino amongst others [21]. Therefore, screening for active compounds from *C. papaya* and *V. amygdalina* plants may lead to the discovery of new medicinal drugs which can confer protection and treatment roles against various diseases.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *Carica papaya and Vernonia amygdalina* were collected from Federal Low Cost Housing Estate, North Bank, Makurdi. The plant materials were identified by taxonomist (Mr.

Ojobo O.) in the Department of Biological Sciences, University of Agriculture, Makurdi where a voucher specimen number (800079) is deposited. The plant materials were shade dried to constant weight at room temperature and ground into powdered form using a grinder-mixer. The powdered sample was filtered using sieve, and subjected to extraction.

2.2 Extraction Procedure

Exactly 100 g of the powered sample was weighed into thimble, which was fitted into soxhlet extractor and was extracted using n-hexane and methanol (60:40% ratios) at the temperature of 50℃. The process of heating and cooling continues until sufficient level of soaking and cooling was obtained in about 4 hours. Then, the extracts were cold macerated at room temperature with frequent shaking. Whatman filter paper No 8 µm was used to fitter the extract. The filtrate was further concentrated with water bath set at 30℃ in reduced pressure. The dried greenish/ reddish brown extract obtained was used for analysis.

2.3 Proximate Analysis

The moisture contents of the sample were determined according to the method of Induhara Swamy et al. [22]. The crude protein, ash, crude fibre and crude lipids were assay by method described by AOAC, [23]. Total carbohydrate was determined by the difference (the sum of the percentage of moisture, ash crude lipid, crude protein and crude fibre was subtracted from 100) [24].

2.4 Mineral Analysis

Calcium, zinc, magnesium, iron, copper, cobalt and cadmium in the samples were determined by X-ray spectrometric method. The mini pal 4 version PW 4030 X-ray Spectrometer (Perkin Elmer, Inc., USA) was used to determine the concentration of the elements in the samples. The mini pal 4 version PW4030 X-ray Spectrometer is an energy dispersive microprocessor controlled analytical instrument designed for the detection and measurement of elements in a sample (solids, powders, and liquids), from sodium to uranium [25].

2.5 Antinutrient Analysis

Alkaloid content was estimated by method described by Harborn, [26], Flavonoid determination was by the method of Boham and Kocipaia, [27]. Phytic acid was determined using the methods of Reddy et al. [28]. Tannin content was determined as described by Doss et al. [29]. The determination of Oxalate was done using the titration method described by Leyva et al. [30]. Saponin content was determined by the method described by Obadoni and Ochuko, [31].

2.5.1 Mass condition for sample *C. papaya* and *V. amygdalina* analysis

Exactly 0.2 mL of the sample was dissolved in 0.8 mL n-Hexane in a 1 mL GC vial and subjected to analysis at suitable conditions.

Instrument- GCMS-QP2010 PLUS SHIMADZU, JAPAN
Purge flow-3.0mL/min
Column temperature- 80℃
Injection temperature-250℃
Mass range- 50-600
Injected volume- 0.2µL

Temperature program:

Rate	Temperature (℃)	Time (min)
-	80.00	1.00
10.00	200.00	4.00
10.00	280.00	5.00

2.5.2 Mass condition for sample *C. papaya* and *V. amygdalina* analysis

Temperature program:

Rate	Temperature (℃)	Time (min)
-	40.00	3.00
10.00	200.00	28.00

2.6 Statistical Analysis

The analysis was carried out in duplicates for all determinations and the results were expressed as mean ± SEM. The SPSS 17.0 for Windows Computer Software Package was used for the Analysis of Variance (ANOVA). The differences in mean values were compared using the Duncan's new multiple range test and the significance difference was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 presents the proximate analysis of the plants samples. Amongst the macro-nutrients present in both samples were moisture, ash, crude lipid, crude protein, crude fibre and carbohydrate. *C. papaya* has significantly (*P<0.05*) higher quantities of ash, crude lipid and crude protein when compared with *V. amygdalina* except in moisture content, which the latter was significantly higher than the *C. papaya.* The mineral composition of *C. papaya* and *V. amygdalina* as presented in Table 2 showed no significant (*P>0.05*) change in the amount of copper, cadmium, iron and cobalt. However, a significantly (*P<0.05*) higher amount of magnesium and zinc is observed in *V. amygdalina* when compared with *C. papaya.* The anti-nutrient content of the samples as shown in Table 3 showed that, *C. papaya* had significant (*P<0.05*) higher amount of oxalate, phytate and flavonoid as compared with *V. amygdalina*. However, no significant (*P>0.05*) difference in tannin, saponin and alkaloids contents was observed in both samples.

The identification of the compounds was confirmed by comparison of the retention indices with those of authentic compounds and with the NIST 02 library. Percentage composition was computed from GC peak areas on DB-5 MS column without applying correction factors. The constituents of *C. papaya* leaf extract showed twenty constituents, which was found to be dominated by Oleic acid (28.98%) with molecular weight of 282, the least compound present was Trans-geranylacetone (0.17%), with molecular weight of 194 (Table 4). Table 5, presents the constituents of *V. amygdalina* leaf extracts. Sixteen constituents were identified and found to be dominated with 9-octadecenoic acid (35.18%), with molecular weight of 282. The least constituent was Methyl tridecanoate (0.45%) with molecular weight of 228.

In the present study, the GC-MS analysis of leaf extract of *C. papaya* showed the presence of twenty compounds. The compounds namely oleic acid (28.98%) and hexadecanoic acid were found to be dominant with 28.98% and 16.18% peak area respectively. The *V. amygdalina* analysis presents sixteen compounds, which was found to be abundant in 9-octadecenoic acid and hexadecanoic acid having a percentage peak area of 35.18% and 16.20% respectively. Many minor components were also identified as shown in the Tables 4 and 5. The major compounds identified have been reported to possess interesting biological activities. Carrillo et al. [32] have shown that oleic acid has an anti-inflammatory potential through the activation of different pathways of immune competent cells**,** they also lowers heart attack risk, artherosclerosis, and improved the effectiveness of herceptin which aids in cancer prevention [33,34]. Studies have shown that oleic acid produces a suppression of lymphocyte proliferation, an inhibition of cytokine production and a reduction in Jurkat cells activity [35,36]. Vasudevan et al. [37] reported the inhibition of phospholipase A2 by hexadecanoic acid, hence, an anti-inflammatory compound used for the treatment of rheumatic symptoms in the traditional medical system. Hexadecanoic acid

Table 1. Proximate composition of samples

Samples	Moisture	Ash	C. lipid	C. protein	C. fibre	CHO
C. Papaya	59.50 ± 0.95^{a}	3.65 ± 0.00^{a}	6.13 ± 0.26^{a}	18.68 ± 0.05^{a}	2.13 ± 0.50^{a}	10.62 ± 0.51^{a}
V. amygdalina	64.00 ± 1.00^{b}	1.97 ± 0.5^{b}	6.31 ± 0.25^{a}	16.08 ± 0.50^{b}	1.27 ± 0.25^{b}	10.37 ± 0.02^{a}

Values are expressed as mean ± SEM. Different superscripts (a and b) down the column are significantly different from each other at $P < 0.05$. CHO = Carbohydrate

Table 2. Mineral composition of samples

Parameters	*C. papaya*	*V. amygdalina*	Recommended Dietary Allowance (RDA) In mg/day
Mn	0.6585 ± 0.0013^{a}	0.8170 ± 0.1549^{b}	0.003-15∗, 800∗∗,1200∗∗∗
Cu	0.0060 ± 0.0013^{a}	0.0046 ± 0.0008^{a}	0.2-0.44∗, 0.7-0.89∗∗, 0.9∗∗∗
Cad	0.0009 ± 0.0006^{a}	0.0025 ± 0.0002^{a}	Toxic to health
Fe	1.8369 ± 0.0076^{a}	1.9275 ± 0.0032^{a}	0.27-11∗, 8-15∗∗, 10∗∗∗
Ca	4.7972 ± 0.0009^{a}	4.2755 ± 0.0006^{b}	210-800∗, 800∗∗, 1200∗∗∗
Co	0.0712 ± 0.0010^{a}	0.0314 ± 0.0004^{a}	0.6×10^{5}∗, 0.01-0.02∗∗, 0.01-0.02∗∗∗
Zn	0.0373 ± 0.0001^{a}	0.0472 ± 0.0031^{b}	2-5∗, 11∗∗, 15∗∗∗

Values are expressed as mean ± SEM. Different superscripts (a and b) along the rows are significantly different from each other at $P < 0.05$.

 # Culled from the United States Department of Agriculture (assessed via http://www.nap.edu)

∗Infants; ∗∗children; ∗∗∗adults

and oleic acid have been shown to possess an antimicrobial and antioxidant potential [38,39], hence, the administration of olive oil containing diets improve the immune response by elimination of pathogens (such as bacteria and fungi) through interference with microphages, lymphocytes and neutrophils [40].

The present study revealed that *V. amygdalina* and *C. papaya* contains an appreciable amount of alkaloids, saponins, phytate, flavonoid, tannin and glycosides, as reported by other researchers [41]. The presence of crude protein and other nutrients content in the plant samples suggested that they are a good source of protein, as high

Table 3. Anti-nutrient composition of the samples

Samples	Tannin	Oxalate	Phytate	Saponin	Alkaloid	Flavonoid
C. papaya	$0.55±0.01^{a}$	$0.18±0.01^{a}$	$6.98±0.13^{a}$	$3.84±0.36^{a}$	$8.09±0.11^{a}$	$2.89±0.04^{a}$
V. amygdalina	$0.51±0.02^{a}$	$0.07±0.01^{b}$	$3.21±0.20^{b}$	$4.29±0.04^{a}$	$8.12±0.09^{a}$	$4.14±0.07^{b}$

Values are expressed as mean ± SEM. Different superscripts (a and b) down the column are significantly different from each other at $P < 0.05$.

Table 4. Chemical constituents identified from the fraction of *Carica papaya*

Peak no	Chemical name	Peak area (%)	Molecular weight	Molecular formula
1	Decylene	0.36	140	$C_{10}H_2O$
2	Trans-Geranylacetone	0.17	194	$C_{13}H_{22}O$
3	Methyl tridecanoate	0.34	228	$C_{14}H_{28}O_2$
4	Palmitic acid	0.91	256	$C_{16}H_{32}O_2$
5	Myristic acid, methyl ester	0.73	242	$C_{15}H_{30}O_2$
6	Myristic acid	2.06	228	$C_{14}H_{28}O_2$
7	Palmitic acid, methyl ester	7.62	270	$C_{17}H_{34}O_2$
8	Hexadecanoic acid	16.18	256	$C_{16}H32O_2$
9	Linolelaidic acid, methyl ester	5.07	294	$C_{19}H_{34}O_2$
10	Methyl cis-6-octadecenoate	7.98	296	$C_{19}H_{36}O_2$
11	Stearic acid, methyl ester	3.54	298	$C_{19}H_{38}O_2$
12	Oleic acid	28.98	282	$C_{18}H_{34}O_2$
13	Stearic acid	7.29	284	$C_{18}H_{36}O_2$
14	15-Tetracosenoic acid	3.53	380	$C_{25}H_{48}O_2$
15	Methyl heptacosanoate	2.44	424	$C_{28}H_{56}O_2$
16	trans-13-Docosenoic acid	4.05	338	$C_{22}H_{42}O_2$
17	Methyl erucate	3.74	352	$C_{23}H_{44}O_2$
18	Methyl behenate	1.15	354	$C_{23}H_{46}O_2$
19	Heneicosanoic acid, methyl ester	2.08	340	$C_{22}H_{44}O_2$
20	Farnesyl cyanide	1.78	410	$C_{30}H_{50}$

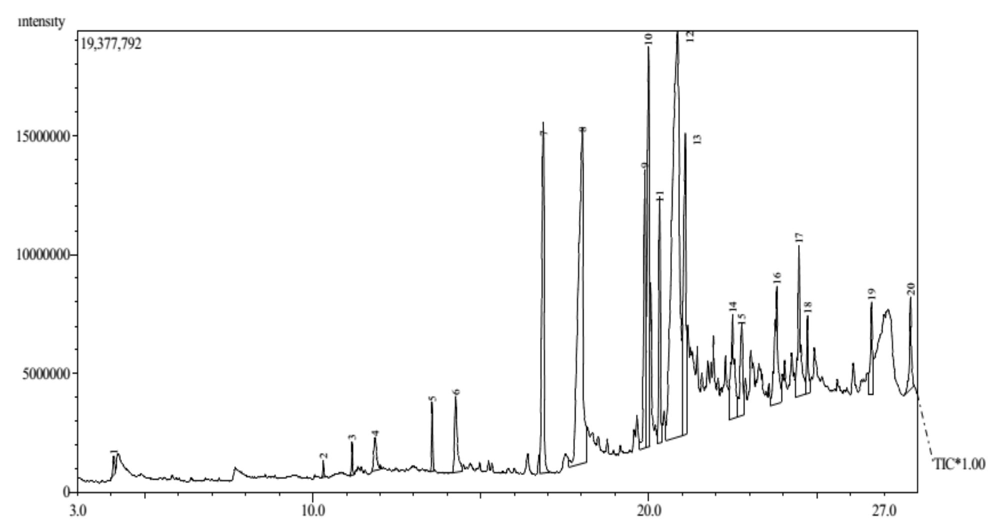

Fig. 1. Gas chromatogram for *Carica papaya*

Table 5. Chemical constituents identified from the fraction of *Vernonia amygdalina*

Peak no	Chemical name	Peak area (%)	Molecular weight	Molecular formula
1.	Dec-1-ene	0.72	140	$C_{10}H_2O$
2.	Methyl tridecanoate	0.45	228	$C_{14}H_{28}O_2$
3.	1- octadecyne	2.15	250	$C_{18}H_{34}$
4.	1- hexadecyne	0.61	222	$C_{16}H_3O$
5.	3- icosyne	1.09	278	$C_{20}H_{38}$
6.	Metyl-14 methylpentadecanoate	5.95	270	$C_{17}H_{34}O_2$
7.	Hexadecanoic acid	16.20	256	$C_{16}H_{32}O_2$
8.	Linolelaidic acid, methyl ester	4.49	294	$C_{19}H_{34}O_2$
9.	Methyl 11-octadecenoate	8.03	296	$C_{19}H_{36}O_2$
10.	Stearic acid, methyl ester	8.88	298	$C_{19}H_{38}O_2$
11.	9- octadecenoic acid	35.18	282	$C_{18}H_{34}O_2$
12.	Stearic acid	11.30	284	$C_{18}H_{36}O_2$
13.	15- tetracosanoic acid, methyl ester	1.18	380	$C_{25}H_{48}O_2$
14.	Methyl isoheptadecanoate	0.54	284	$C_{18}H_{36}O_2$
15.	Methyl 13-docosenoate	1.99	352	$C_{23}H_{44}O_2$
16.	Heneicosanoic acid	1.26	340	$C_{22}H_{44}O_2$

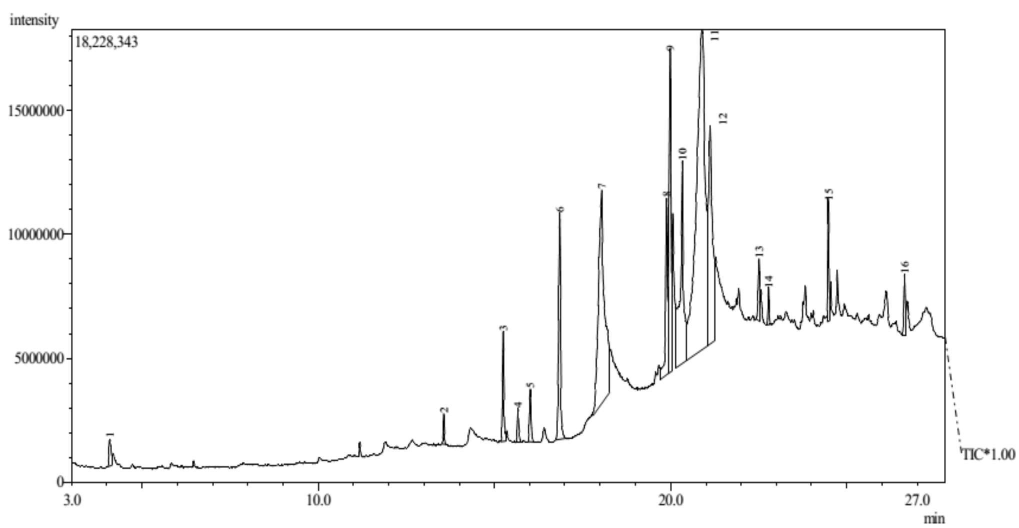

Fig. 2. Gas chromatogram for *Vernonia amygdalina*

amount of protein is essential for animal growth and increased milk production [42]. Both extracts were found to contain an appreciable amount of minerals, which can be exploited in nutraceutical industry. Oche et al. [43] have established the *in-vivo* activity of the crude extracts as antimalarial agent. However, some *in-vivo* work is on-going to ascertain the antioxidant and anti-trypanosomiasis potential of both plants, as most of the identified compounds are cited in the literature for their antimicrobial, anti-trypanosomiasis, antioxidant and anti-inflammatory properties.

4. CONCLUSION

We report here, the presence of the important components of *C. papaya* and *V. amygdalina* resolved by GC-MS analysis and their nutrients compositions, which reveals the bioactive chemical components in the medicinal plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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