

GC – MS Analysis of Aqueous Extract of Unripe Fruit of *Carica papaya*

Sunday Ahamefula Ezekwe¹ and Paul Chidoka Chikezie²

¹Department of Medical Biochemistry, Imo State University, Owerri, Nigeria

²Department of Biochemistry, Imo State University, Owerri, Nigeria

Abstract

The present study was aimed to identify the likely phytochemicals present in Aqueous Extract of Unripe Fruit (AqEUF) of *Carica papaya*. The phytochemicals present in AqEUF of *C. papaya* was identified using GC–MS detection system. The results showed the presence of fifteen phytochemicals in AqEUF of *C. papaya*. The major phytochemicals present in AqEUF of *C. papaya* in terms of their relative abundance were octadecanoic acid, hexadecenoic acid, Z-11 and hexadecanoic acid, methyl ester, which corresponded to 23.84%, 19.17% and 18.25% respectively. The relative abundance of minor phytochemicals present in AqEUF of *C. papaya* was within a narrow range of 0.78-5.38%. The present investigation revealed that AqEUF of *C. papaya* was composed of variety of metabolites and therapeutic active substances as well as novel substances. These substances could be isolated and further empirically evaluated to confirm their biologic and medicinal activities as well as investigate their mechanism of action.

Keywords: Aqueous extract; *Carica papaya*; Fruit; GC–MS analysis; Phytochemicals

Introduction

Carica papaya (papaya) originated from the tropical regions of Mexico and America but is now widely distributed throughout the tropics and subtropics of the world [1]. The *C. papaya* is a fast growing, erect and typically unbranched herbaceous tree, with hollow trunk of about 20 cm in diameter [2]. The plant is cultivated mainly for its fruit as a foodstuff, which is composed of internal juicy and succulent edible pulp when ripe. The nutraceutical and medicinal activities of different parts of the papaya plant have been exhaustively described elsewhere [3-5].

Profiling of bioactive principles from edible and medicinal plants provides useful insights into their chemical diversities, medicinal potentials and toxicity concerns that are of relevance to the clinician, nutritionist, pharmacist or toxicologist [6]. Gas Chromatography–Mass Spectrometry (GC–MS), among other hyphenated techniques described elsewhere [7-9], offers a reliable and reproducible analytical protocol for the identification, quantitation and characterization of bioactive principles from herbal extracts [10-13].

Bioactive principles from plant materials provide unlimited opportunities for new drug discoveries [14-16] and their presence in foods and dietary supplements have been implicated in the prevention of pathologic conditions as well as promoting general wellness [6,17, 18]. Nevertheless, there are also inherent toxicity concerns associated with the use of bioactive principles from plant materials [6].

The ripe or unripe fruit as well as other different parts of *C. papaya* have been reported to possessing wide range of biological applications [1,19-22]. The present study was aimed to identify the phytochemicals of Aqueous Extract of Unripe Fruit (AqEUF) of *C. papaya* that are likely responsible for its biologic activity using GC–MS analytical protocols.

Materials and Methods

Collection of plant specimen

Unripe but matured fruits of *C. papaya* were harvested during the wet season, on the 6th of July, 2015 from a botanical garden in Imo State University, Owerri, which lies on the rainforest belt of Nigeria (Latitude 5° 30.2237'N; Longitude 7° 2.6277'E). The unripe fruits were

identified and authenticated by Dr. F.N. Mbagwu at the Herbarium (Sample Voucher Number: IMSUH 203), Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The unripe fruits were subsequently washed under continuous current of tap water for 5 min and air-dried at ambient room temperature ($T=25 \pm 5^\circ\text{C}$) for 30 min.

Preparation and extraction of sample

Protocol for preparation of sample of unripe fruit of *C. papaya* for extraction was according to the methods previously described by Eleyinmi [23], but with minor modifications with respect to the temperature and duration of drying the sample. A 100 g part of the unripe fruit pulp was weighed using a triple beam balance (OHAU 750-50; OHAUS Triple Beam Balance, Model TJ611, Burlington, NC, USA) and dried in an oven (WTC BINDER; 7200 Tuttlingen, Germany) at 60°C until it becomes crispy. The dried sample was ground into powder using the Thomas-Willey milling machine (ASTM D-3182; India) and sieved on a wire mesh screen (3 × 3 mm²). Finally, the ground sample was stored at refrigerated temperature of 4°C in air-tight plastic bottles with screw caps pending extraction.

The AqEUF of *C. papaya* was prepared according to the methods previously described [24]. Twenty-five grams (25 g) of the ground sample was suspended in 250 mL of distilled water in stoppered flasks and allowed to stand for 24 h. The suspension was filtered with Whatman No 24 filter paper. The filtrate was concentrated in a rotary evaporator (Büch Rotavapor R-200) for 12 h at 50°C under reduced pressure and dried in vacuum desiccator. The yield was calculated to be 6.06% w/w. Portion of the extract was finally suspended in ethyl acetate and subjected to GC-MS analysis.

*Corresponding author: Paul C Chikezie, Department of Biochemistry, Imo State University, Owerri, Nigeria, Tel: +2348038935327; E-mail: p_chikezie@yahoo.com

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GC-MS system

The main phytochemicals of AqEUF of *C. papaya* was identified using GC–MS detection system as previously described [25] but with minor modification, whereby portion of the extract was analyzed directly by headspace sampling. GC–MS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VLMSD (Agilent Technologies, CA, and USA). The capillary column used was DB-5MS (30 m × 0.25 mm, film thickness of 0.25 μm; J&W Scientific, CA, USA). The temperature program was set as follows: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 0.811851 mL/min. The MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadrupole temperature were set at 230°C and 150°C, respectively. Identification of phytochemicals was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST08.L and Wiley7n.l libraries.

Results

The phytochemicals present in AqEUF of *C. papaya* with their corresponding retention time, molecular formula and molecular weight as well as their relative abundance, which was expressed in terms of peak area% are presented in Table 1 and depicted in Figure 1.

Figure 2A-2O showed the mass spectra and molecular structures of the detected fifteen phytochemicals present in AqEUF of *C. papaya*.

Table 1 showed the presence of fifteen phytochemicals in AqEUF of *C. papaya*. By comparative inspection, the major phytochemicals present in AqEUF of *C. papaya* in terms of their relative abundance were octadecanoic acid, hexadecenoic acid, Z-11 and hexadecanoic acid, methyl ester, which corresponded to 23.84%, 19.17% and 18.25% respectively. In the same context described above, the minor phytochemicals present in AqEUF of *C. papaya* were within a narrow range of 0.78-5.38%. Specifically, n-hexadecanoic acid represented the phytochemical with the lowest concentration among the entire phytochemicals present in AqEUF of *C. papaya*.

S. No.	RT (min)	Phytochemicals	M/F	MW	%PA
1.	25.183	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C ₁₉ H ₃₈ O ₄	330	4.72
2.	19.702	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	23.84
3.	18.709	12,15-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	5.38
4.	16.568	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	0.78
5.	16.269	Hexadecenoic acid, Z-11	C ₁₆ H ₃₀ O ₂	254	19.17
6.	27.983	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyloxy)-1-[(trimethylsilyloxy)methyl]ethyl]	C ₂₇ H ₅₂ O ₄ Si ₂	496	3.58
7.	4.614	4(1H)-Pyrimidinone,2,6,-diamino	C ₄ H ₆ H ₄ O	126	2.27
8.	4.764	1,2,3-Propanetriol, monoacetate	C ₅ H ₁₀ O ₄	134	2.19
9.	5.517	4H-Pyranone-2,3-dihydro-3,5-dihydroxy-dihydroxy-6-methyl	C ₆ H ₈ O ₄	144	4.25
10.	6.451	6-Acetyl-β-D-mannose	C ₈ H ₁₄ O ₇	222	3.31
11.	8.588	2,4-Hexadienedioic acid	C ₆ H ₆ O ₄	142	4.06
12.	13.05	1-Gala-1-ido-octose	C ₈ H ₁₆ O ₆	240	3.19
13.	13.58	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	223	3.88
14.	15.52	Benzoic acid, 4-hydroxy-3,5-dimethoxy	C ₉ H ₁₀ O ₅	198	1.13
15.	16.52	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	18.25

RT: Retention Time; M/F: Molecular Formula; MW: Molecular Weight; PA: peak Area

Table 1: Phytochemicals detected in AqEUF of *Carica papaya*.

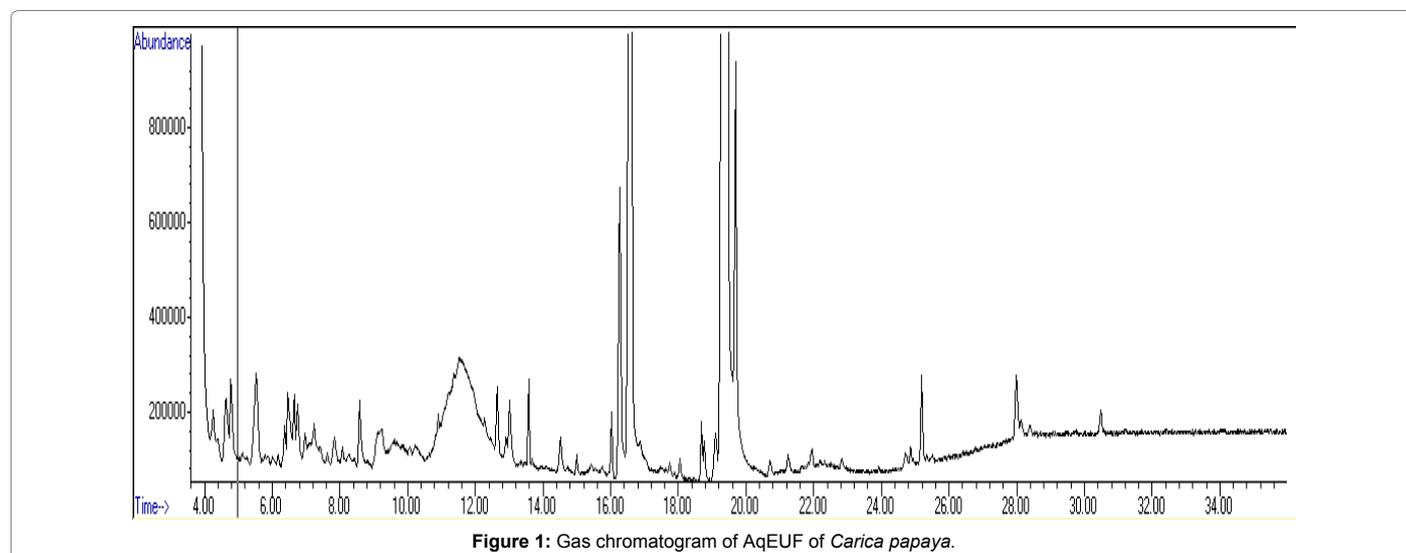
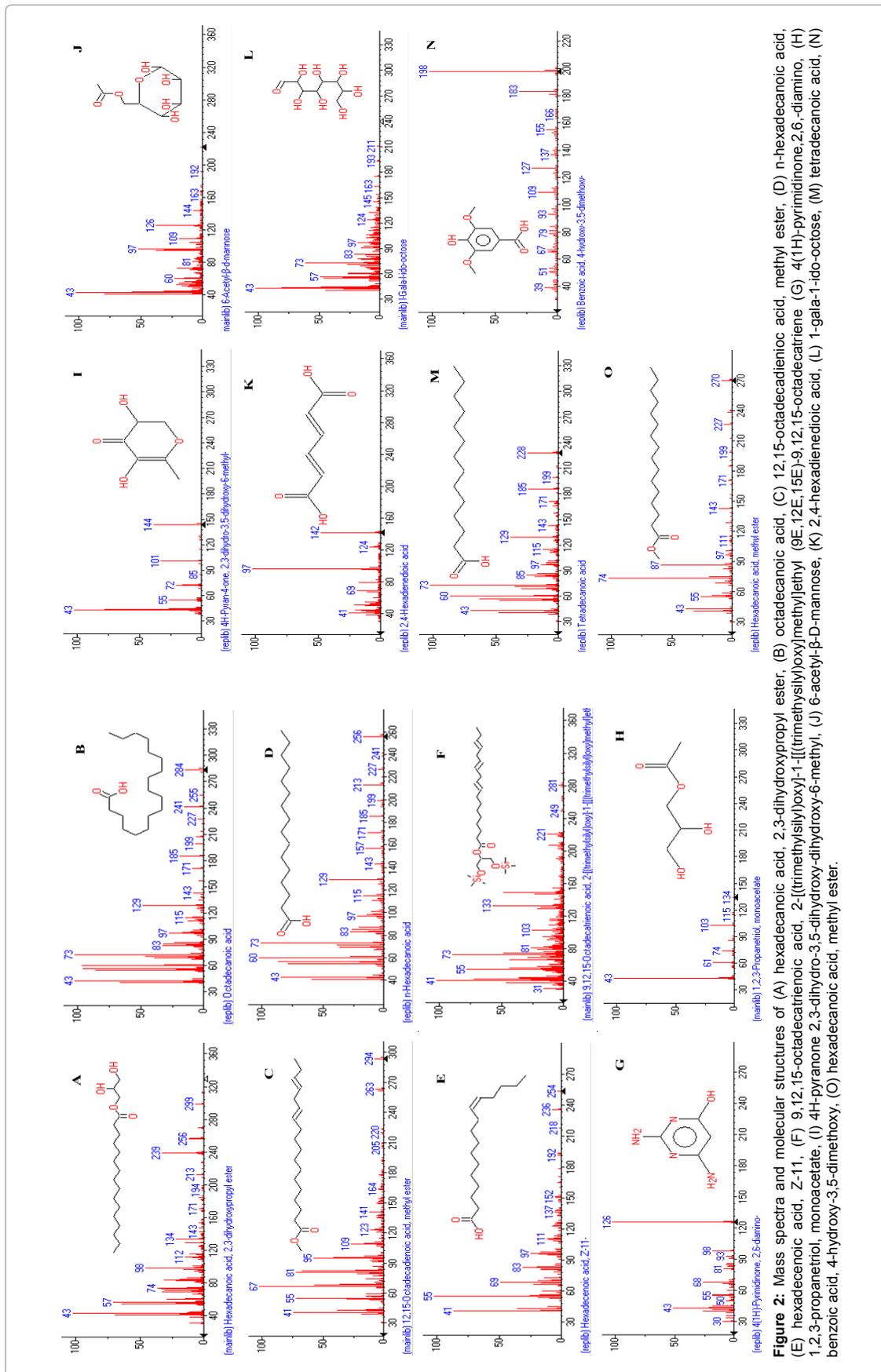


Figure 1: Gas chromatogram of AqEUF of *Carica papaya*.



Overall, elution of 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl (9E,12E,15E)-9,12,15-octadecatriene fraction of AqEUF of *C. papaya* gave the highest retention time of 27.983 min, whereas that of 4(1H)-pyrimidinone,2,6-diamino represented the lowest retention time of 4.614 min (Table 1).

Discussion

Findings from previous studies on GC–MS analyses of vast array of plant extracts revealed that most of the herbal extracts with medicinal attributes contained some of the phytochemicals or analogs of the phytochemicals present in AqEUF of *C. papaya*. For instance, octadecanoic acid, 2,3-dihydroxypropyl ester, which is an analogue of hexadecanoic acid, 2,3-dihydroxypropyl ester, is present in marine red seaweeds such as *Pterochadia capillacea* and was noted to exhibit antibacterial activity [26]. Additionally, hexadecanoic acid, 2,3-dihydroxypropyl ester is present in methanolic leaf extract of *Spermacoce articularis*, which is an important medicinal plant used in Indian folk medicine [27], as well as in chloroform extracts of mosses (*Polytrichum commune* and *Rhytidiadelphus triquetrus*), noted for their antimicrobial activity and anticancer activity on cancer cell lines [28]. The reports of Klavina et al. [28] revealed that mosses that exhibited antimicrobial activity and anticancer activity contained analogs of hexadecanoic acid, Z-11. The present study showed that hexadecanoic acid, Z-11 was a major phytochemical present in AqEUF of *C. papaya*, which also contained appreciable quantity of hexadecanoic acid, 2,3-dihydroxypropyl ester.

Like AqEUF of *C. papaya*, octadecanoic acid is a major phytochemical of hexane leaf extract of *Mesembryanthemum edule* used for the treatment of respiratory tract infections, tuberculosis, dysentery, diabetic mellitus, laryngitis and vaginal infections by Eastern Cape traditional healers [29]. Octadecanoic acid from *Azadirachta indica* (neem) extracts [30] and marine red seaweeds, namely, *Jania rubens*, *Corallina mediterranea* and *P. capillacea* exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella spp* [26]. Previous report showed that 9,12-octadecadienoic acid (Z,Z)-, methyl ester from chloroform extract of *Albizia adianthifolia* (Schumacher) [31,32] and methanol extract of *Jatropha curcas* [33], which is a closely related compound to 12,15-octadecadienoic acid, methyl ester present in AqEUF of *C. papaya*, impeded cell proliferation and microbial growth.

Previous studies revealed the presence of n-hexadecanoic acid in methanolic leaf extracts of *Justicia adhatoda* [34] and *Clerodendrum viscosum* [13]. The n-hexadecanoic acid, like its ester derivative, exhibits antioxidant activity and may serve as anti-cancer, anti-microbial, anti-haemolytic, anti-diabetic agents in addition to causing pesticidal inhibitory action to 5- α reductase activity [12,27,35]. Unfortunately, the relative abundance of n-hexadecanoic acid present in AqEUF of *C. papaya* was comparatively low.

GC–MS profiling revealed the presence of 4H-Pyranone 2,3-dihydro-3,5-dihydroxy-dihydroxy-6-methyl in methanolic leaf extracts of *Lawsonia inermis* [36] and *C. viscosum* [13] as well as in methanolic dry fruit extract of *Prunus armenicus* (apricot) [37]. Evidence showed that methanolic fruit extract of apricot exhibited antioxidant and antimicrobial activities [37]. Specifically, isolated fraction of 4H-pyranone 2,3-dihydro-3,5-dihydroxy-dihydroxy-6-methyl from methanolic leaf extract of *Dolichandrone atrovirens* possessed anti-inflammatory and antimicrobial activities [38]. In the same vein, AqEUF of *C. papaya* contained noticeable quantity of 4H-pyranone 2,3-dihydro-3,5-dihydroxy-dihydroxy-6-methyl.

Tetradecanoic acid (myristic acid) has been detected in methanolic leaf extracts of *J. rubens*, *C. mediterranea* and *P. capillacea* [26], *L. inermis* [36] and hexane/acetone leaf extracts of *M. edule* [29]. Previous studies showed that tetradecanoic acid was the major antibacterial and antioxidant principles isolated from *Myristica fragrans* (nutmeg) [39,40] as well as antibacterial agent from *P. capillacea* [41]. Furthermore, tetradecanoic acid impedes cell proliferation and exhibits nematocidal and hypocholesterolemic activities [42]. Although the result of the present study showed that AqEUF of *C. papaya* contained tetradecanoic acid, its relative abundance was comparatively low.

Studies according to Sudha et al. [43], reported that hexadecanoic acid, methyl ester possessed hypocholesterolemic and anti-coronary activities. Interestingly, the findings from the present study showed that hexadecanoic acid, methyl ester, in terms of relative abundance, was a major phytochemical present in AqEUF of *C. papaya*. Previous findings showed that 6-Acetyl- β -D-mannose and 2,4-hexadienedioic acid, which were present in noticeable quantities in AqEUF of *C. papaya*, were likely antimicrobial agents [44-46]. It is worthwhile to note that some phytochemicals present in AqEUF of *C. papaya*, namely, (a) 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl (9E,12E,15E)-9,12,15-octadecatriene, (b) benzoic acid, 4-hydroxy-3,5-dimethoxy, (c) 4(1H)-pyrimidinone,2,6-diamino, (d) 1-gala-1-ido-octose and (e) 6-acetyl- β -D-mannose are novel substances in that their therapeutic attributes and biologic activities have not been previously reported elsewhere.

Conclusion

The present investigation revealed that AqEUF of *Carica papaya* was composed of variety of metabolites and therapeutic active substances. These substances could be isolated and further empirically evaluated to confirm their biologic and medicinal activities as well as investigate their mechanism of action. Additionally, the novel phytochemicals present in AqEUF of *C. papaya* could be isolated and their therapeutic attributes and biologic activities elucidated. Finally, a corresponding GC–MS assisted phytochemical profiling should be carried out using non-polar extractants of unripe fruit of *C. papaya* to identify other constituents not mentioned here.

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