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

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Abstract

The present study was aimed to identify the likely phytocomponents present in Aqueous Extract of Unripe Fruit (AqEUF) of *Carica papaya*. The phytocomponents present in AqEUF of *C. papaya* was identified using GC–MS detection system. The results showed the presence of fifteen phytocomponents in AqEUF of *C. papaya*. The major phytocomponents 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 phytocomponents 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; Phytocomponents

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 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 phytocomponents 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 ± 5°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 mm^2). 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.

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GC-MS system

The main phytocomponents 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 VL MSD (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 quadruple temperature were set at 230°C and 150°C, respectively. Identification of phytocomponents 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 phytocomponents 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 phytocomponents present in AqEUF of *C. papaya*.

Table 1 showed the presence of fifteen phytocomponents in AqEUF of *C. papaya*. By comparative inspection, the major phytocomponents 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 phytocomponents present in AqEUF of *C. papaya* were within a narrow range of 0.78-5.38%. Specifically, n-hexadecanoic acid represented the phytocomponent with the lowest concentration among the entire phytocomponents present in AqEUF of *C. papaya*.

S. No.	RT (min)	Phytocomponents	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-Octadecadienioc 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-[(trimethylsilyl)oxy]-1-[[(trimethysilyl)oxy]methyl]ethyl (9E,12E,15E)-9,12,15-octadecatriene	$C_{27}H_{52}O_4Si_2$	496	3.58
7.	4.614	4(1H)-Pyrimidinone,2,6,-diamino	$C_4H_6H_4O$	126	2.27
8.	4.764	1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	134	2.19
9.	5.517	4H-Pyranone-2,3-dihydro-3,5-dihydroxy-dihydroxy-6-methyl	$C_6H_8O_4$	144	4.25
10.	6.451	6-Acetyl-β-D-mannose	$C_8H_{14}O_7$	222	3.31
11.	8.588	2,4-Hexadienedioic acid	$C_6H_6O_4$	142	4.06
12.	13.05	1-Gala-1-ido-octose	$C_8H_{16}O_6$	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	$C_9H_{10}O_5$	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: Phytocomponents detected in AqEUF of *Carica papaya*.

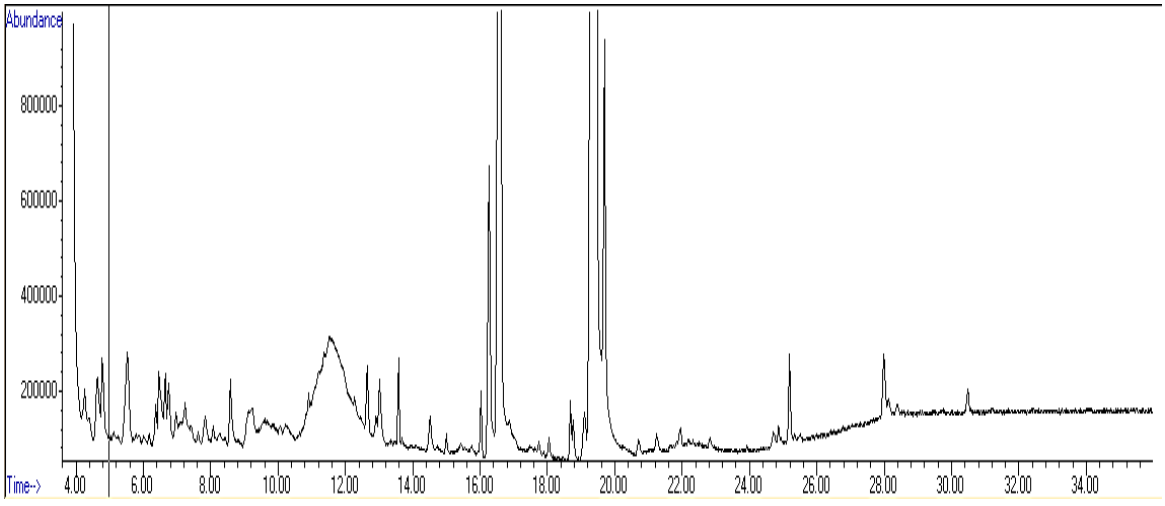

Figure 1: Gas chromatogram of AqEUF of *Carica papaya*.

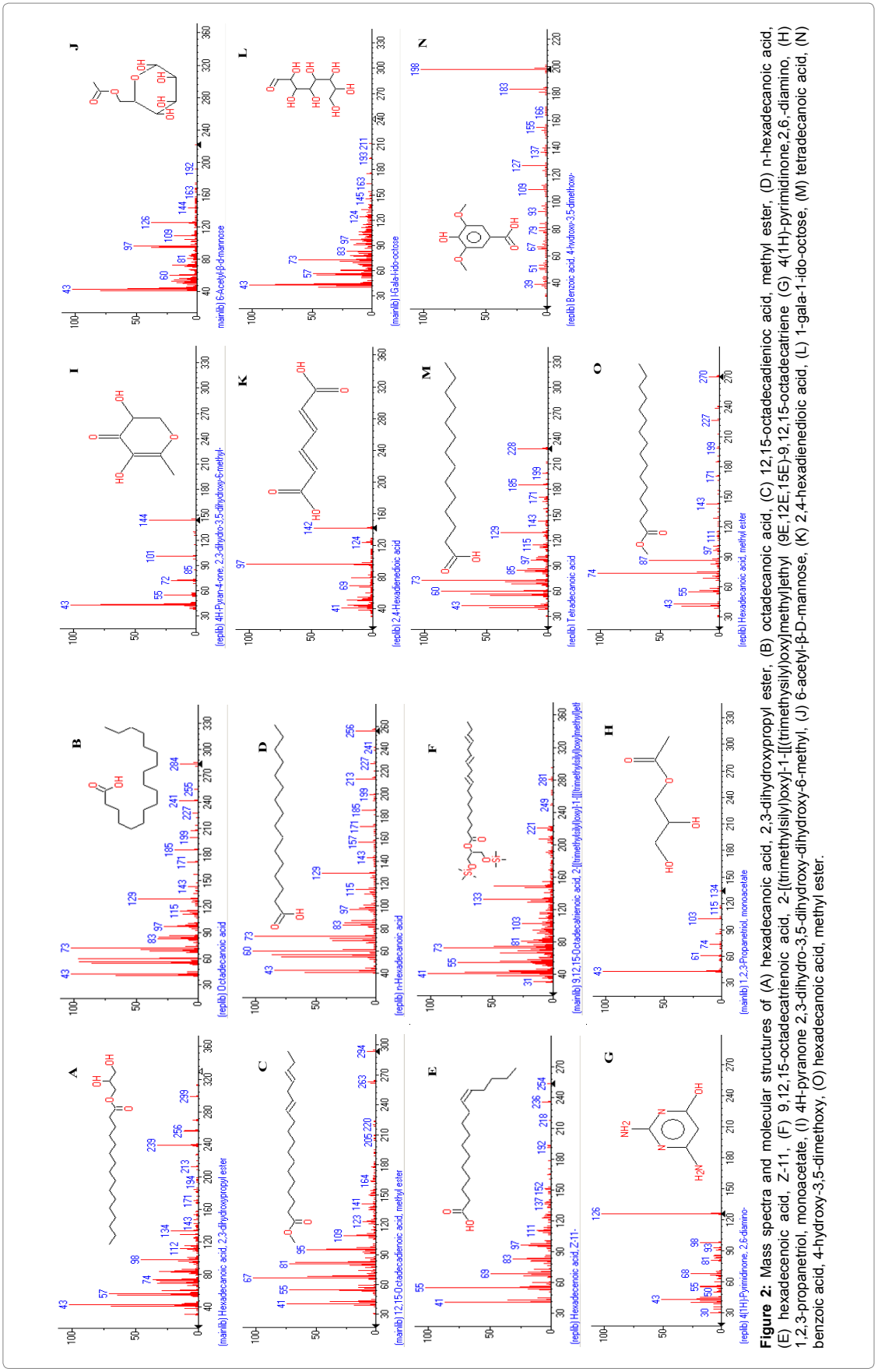

Figure 2: Mass spectra and molecular structures of (A) hexadecanoic acid, 2,3-dihydroxypropyl ester, (B) octadecanoic acid, (C) 12,15-octadecadienioc acid, methyl ester, (D) n-hexadecanoic acid, (E) hexadecenoic acid, Z-11, (F) 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethysilyl)oxy]methyl]ethyl (9E,12E,15E)-9,12,15-octadecatriene (G) 4(1H)-pyrimidinone,2,6.-diamino, (H) 1,2,3-propanetriol, monoacetate, (I) 4H-pyranone 2,3-dihydro-3,5-dihydroxy-6-methyl, (J) 6-acetyl-β-D-mannose, (K) 2,4-hexadienedioic acid, (L) 1-gala-1-ido-octose, (M) tetradecanoic acid, (N) benzoic acid, 4-hydroxy-3,5-dimethoxy, (O) hexadecanoic acid, methyl ester.

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 phytocomponents or analogs of the phytocomponents 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 hexadecenoic acid, Z-11. The present study showed that hexadecenoic acid, Z-11 was a major phytocomponent 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 phytocomponent 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* (Schumach) [31,32] and methanol extract of *Jatropha curcas* [33], which is a closely related compound to 12,15-octadecadienioc 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 phytocomponent 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 phytocomponents present in AqEUF of *C. papaya*, namely, (a) 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethysilyl) 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 phytocomponents present in AqEUF of *C. papaya* could be isolated and their therapeutic attributes and biologic activities elucidated. Finally, a corresponding GC–MS assisted phytocomponent profiling should be carried out using non-polar extractants of unripe fruit of *C. papaya* to identify other constituents not mentioned here.

References

1. Juárez-Rojop IE, Tovilla-Zárate CA, Aguilar-Domínguez DE, Roa-de la Fuente LF, Lobato-García CE, et al. (2014) Phytochemical screening and hypoglycemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats. Rev Bras Farmacogn 24: 341-347.
2. Aminul I, Al-Mamun MA, Parvin S, Sarker MEH, Zaman MK, et al. (2015) Evaluation of antibacterial activities of latex of Caricaceae (*Carica papaya* L.). Asian J Pharm Clin Res 8: 308-311.
3. Nguyen HP, Kimaru IW (2014) GC–MS analysis of an herbal medicinal remedy to identify potential toxic compounds. Adv Pharmaceut Anal 12: 14-18.
4. Tewari BB, Gomathinayagam S (2014) A critical review on *Ocimum tenuflorum*, *Carica papaya* and *Syzygium cumini*: the medicinal flora of Guyana. Bolivian J Chem 31: 28-41.
5. Nguyen TT, Parat MO, Hodson MP, Pan J, Shaw PN, et al. (2015) Chemical characterisation and in vitro cytotoxicity on squamous cell carcinoma cells of *Carica papaya* leaf extracts. Toxins 8: 7.1-7.11.
6. Chikezie PC, Ibegbulem CO, Mbagwu FN (2015) Bioactive principles from medicinal plants. Res J Phytochem 9: 88-115.
7. Sasidharan S, Sumathi V, Jegathambigai NR, Latha LY (2011) Antihyperglycaemic effects of ethanol extracts of *Carica papaya* and *Pandanus amaryfollius* leaf in streptozotocin-induced diabetic mice. Nat Prod Res 25: 1982-1987.
8. Ejele AE, Akalezi CI, Iwu IC, Ukiwe LN, Enenebaku CK, et al. (2014) Bioassay-guided isolation, purification and charaterization of antimicrobial compound from acidic metabolite of *Piper umbellatum* seed extract. Int J Chem 6: 61-70.

9. Karayil S, Chandran KPS, Sudeesh PS, Veraiah K (2014) Isolation and Structural elucidation of novel bioactive molecule-Coumarin from traditionally used medicinal plant-*Ceropegia juncea* (Roxb.). IOSR J Pharm Biol Sci 9: 19-22.

10. Ezhilan BP, Neelamegam R (2012) GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Pharmacogn Res 4: 11-14.

11. Kanthal LK, Dey A, Satyavathi K, Bhojaraju P (2014) GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. Pharmacogn Res 6: 58-61.

12. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C (2015) GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci Technol 52: 1212-1217.

13. Ghosh G, Panda P, Rath M, Pal A, Sharma T, et al. (2015) GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. Pharmacogn Res 7: 110-113.

14. Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 66: 1022-1032.

15. Gueritte F, Fahy J (2005) The vinca alkaloids. In anticancer agents from natural products. Cragg GM, Kingston DGI, Newman DJ. (Eds). Boca Raton Florida: Taylor & Francis Group pp: 123-136.

16. Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C, Myles EL (2013) *In vitro* anticancer screening of 24 locally used Nigerian medicinal plants. BMC Compl Altern Med 13: 79.

17. Galati G, O'Brien PJ (2004) Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radic Biol Med 37: 287-303.

18. Rabi T, Bishayee A (2009) Terpenoids and breast cancer chemoprevention. Breast Cancer Res Treat 115: 223-239.

19. Hewitt H, Whittle S, Lopez S, Bailey E, Weaver S (2000) Topical use of papaya in chronic skin ulcer therapy in Jamaica. West Indian Med J 49: 32-33.

20. Rahmat A, Rosli R, Wan Nor IW, Endrini S, Sani HA (2002) Antiproliferative activity of pure lycopene compared to both extracted lycopene and juices from watermelon (*Citrullus vulgaris*) and papaya (*Carica papaya*) on human breast and liver cancer cell lines. J Med Sci 2: 55-58.

21. Nayak BS, Pereira LP, Maharaj D (2007) Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats. Indian J Exp Biol 45: 739-743.

22. Lim TK (2012) Edible medicinal and non-medicinal plants.

23. Eleyinmi AF (2007) Chemical composition and antibacterial activity of *Gongronema latifolium*. J Zhejiang Univ Sci 8: 352-358.

24. Ibegbulem CO, Chikezie PC (2013) Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium*, *Aloe perryi, Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (*Rattus norvegicus*). Pharmacogn Commun 3: 12-16.

25. Rašković A, Pavlović N, Kvrgić M, Sudji J, Mitić G, et al. (2015) Effects of pharmaceutical formulations containing thyme on carbon tetrachloride-induced liver injury in rats. BMC Compl Altern Med 15: 442.

26. Mohy El-Din SM, El-Ahwany AMD (2016) Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens*, *Corallina mediterranea* and *Pterocladia capillacea*). J Taibah Univ Sci 10: 471-484.

27. Sheeba GD, Viswanathan P (2016) GC-MS Analysis of Phytocomponents in *Spermacoce articularis* L. *f.* leaf. Res Pharm 4: 1-7.

28. Klavina L, Springe G, Nikolajeva V, Martsinkevich I, Nakurte I, et al. (2015) Chemical composition analysis, antimicrobial activity and cytotoxicity screening of moss extracts (moss phytochemistry). Mol 20: 17221-17243.

29. Omoruyi BE, Afolayan AJ, Bradley G (2014) Chemical composition profiling and antifungal activity of the essential oil and plant extracts of *Mesembryanthemum edule* (L.) bolus leaves. Afr J Tradit Compl Altern Med 11: 19-30.

30. Zhong PU, Yu-qun Z, Zhong-qiong Y, Jiao X, Ren-yong J (2010) Antibacterial activity of 9-octadecanoic acid, hexadecanoicacid–tetrahydrofuran-3,4-diyl ester from Neem oil. Agric Sci China 9: 1236-1240.

31. Yu FR, Lian XZ, Guo HY, McGuire PM, Li RD, et al. (2005) Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. J Pharm Pharm Sci 8: 528-535.

32. Abubakar MN, Majinda RRT (2016) GC-MS Analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Med 3: 9.

33. Rahman MM, Ahmad SH, Mohamed MTM, Ab Rahman MZ (2014) Antimicrobial Compounds from Leaf Extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. Sci World J 1012: 593-598.

34. Jayapriya G, Shoba FG (2015) GC-MS analysis of bio-active compounds in methanolic leaf extracts of *Justicia adhatoda* (Linn.). J Pharmacogn Phytochem 4: 113-117.

35. Gnanavel V, Saral AM (2013) GC-MS analysis of petroleum ether and ethanol leaf extracts from *Abrus precatorius Linn*. Int J Pharm Biosci 4: 37-44.

36. Dev SNC, De K, Khan MW (2016) GC-MS analysis of phytochemicals of methanolic extract of leaves of *Lawsonia inermis* Linn. Indian J Med Res Pharmaceut Sci 3: 77-82.

37. Sharma S, Satpathy G, Gupta RK (2014) Nutritional, phytochemical, antioxidant and antimicrobial activity of *Prunus armenicus*. J Pharmacogn Phytochem 3: 23-28.

38. Deepa P, Murugesh S (2013) GC-MS determination of bioactive compounds of *Dolichandrone atrovirens* (Sprague) bark. Int J Biol Pharm Allied Sci 2: 1644-1657.

39. Narasimhan B, Dhake AS (2006) Antibacterial principles from *Myristica fragrans* seeds. J Med Food 9: 395-399.

40. Gupta AD, Bansal VK, Babu V, Maithil N (2013) Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). J Genet Eng Biotechnol 11: 25-31.

41. Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ (2007) Antibacterial and antifungal activities of fatty acid methyl estersof the blind your eye mangrove from India. Braz J Microbiol 38: 739-742.

42. Sharma P, Vijayvergia R (2015) *In vitro* α-amylase inhibitory activity and GC-MS analysis of *Petrea volubilis*. Int J Sci Res 4: 190-194.

43. Sudha T, Chidambarampillai S, Mohan VR (2013) GC-MS analysis of bioactive components of aerial parts of *Fluggealucopyrus willd* (Euphorbiaceae). J Appl Pharm Sci 3: 126-130.

44. Narasimhan B, Judge V, Narang R, Ohlan R, Ohlan S (2007) Quantitative structure–activity relationship studies for prediction of antimicrobial activity of synthesized 2,4-hexadienoic acid derivatives. Bioorg Med Chem Lett 17: 5836-5845.

45. Mohammed GJ, Kadhim MJ, Hussein HM (2016) Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. Int J Pharmacogn Phytochem Res 8: 889-905.

46. Saad AM (2016) Determination of secondary metabolites products by *Trichoderma horzianum* and evaluate antimicobial activity. Res J Pharmaceut Biol Chem Sci 7: 105-122.