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IDENTIFICATION AND QUANTIFICATION OF PHYTOCHEMICALS FROM *Carica papaya* Linn (Caricaceae) ROOT EXTRACT USING GC-FID

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Abstract
Phytochemicals in the ethanolic extract from the root of *Carica papaya* Linn partitioned in chloroform-water system were identified and quantified using gas chromatography with flame ionization detection. The identified constituents were catechin, sapogenin, sparteine, quinine, naringenin, naringin, anthocyanidine and flavone with concentration 77.14 µg/g, 25.84 µg/g, 15.12 µg/g, 13.36 µg/g, 8.10 µg/g, 6.20 µg/g, 3.78 µg/g and 0.06 µg/g respectively. The reported diuretic, arbotifacient, antifungal, antityphoid and antimalarial properties of the root extract of this plant could be attributed to the presence of sparteine, catechin, flavone and quinine components. The presence of naringin, sparteine and catechin suggested that the extract could serve as an anti-inflammatory agent and also be used in the treatment of cardiovascular diseases.

Introduction

Carica papaya commonly known as paw-paw belongs to the genus Carica of the family Caricaceae. The plant is native to tropical America possibly from Southern Mexico and neighboring Central America [1]. It is a large tree-like herbaceous perennial plant with soft single stem that grows up to 5-10 m height, carrying sparely arranged leaves at the top of the trunk. The lower trunk is scarred where leaves and fruits are born [2]. The plant is recognized by its weak and usually unbranched soft stem yielding copious white latex [3]. Its food and nutritional values are well known throughout the world. In 2016, global production of papayas was 13.05 million tonnes, led by India, with Nigeria at the fifth position [4].

The different parts of *C. papaya* plant have been applied in traditional medicine. Its fruit, leaf, peel, pulp, bark, seed and root possess excellent medicinal properties for treatment of different ailments. The medicinal and pharmacological properties of the parts of *C. papaya* plant have been reported in various literatures. The leaves have shown anti-dengue, anti-plasmodial, anti-cancer, antibacterial, hepatoprotection, anti-inflammatory and antioxidant properties in *in-vitro* and *in-vivo* studies [5-6]. Its flower extract is used in the treatment of jaundice [7]. The ripe fruit is used as topical ulcer dressing to promote granulation, healing and reducing odour in chronic skin ulcers. The green fruit is used for treating malaria, hypertension, diabetes mellitus, jaundice and intestinal helminthiasis [8]. The seeds have pharmacological activities like anthelmintic, antifertility, contraceptive, anti-inflammatory, analgesic and antimicrobial property. They are also used in treatment of sickle cell disease and poison related problems [9]. Papaya peel extracts have shown antibacterial properties, antioxidant activity, anti-cancer activity and induced apoptosis [10-11]. This extract is also used in the preparation of insecticidal formulations [12]. The bark and twig tissues are found to possess antitumour and pesticidal properties [13]. The root extracts can be used as medicine for renal and urinary bladder problems, abortifacient, diuretic, antifungals and also in checking irregular bleeding from the uterus. It also helps in treating gastroenteritis, urethritis, otitis media, typhoid fever, infectious wound, pneumonia internal heat, stomach noise and abdominal pains [14-15].

Most of the medicinal studies on the different parts of *C. papaya* plant make use of the crude extract from the part of interest. Reports on the particular chemical compounds responsible for these observed properties are scarce in literature. In this study, the phytochemical components of the ethanolic extract of *C. papaya* root were identified and quantified. The identified compounds were linked to some of the medicinal properties reported in literature and the unreported properties based on the identified chemicals were suggested.

Methodology

Collection and Preparation of Plant Material

Fresh roots of *C. papaya* were collected from the botanical garden of the Plant Science and Biotechnology Department, Imo State University, Owerri. The root samples were placed on newsprint paper and air-dried at room temperature. Thereafter, they were blended using a manual blender and the powdered sample was stored in a polythene bag prior to use.

Extraction of Phytochemicals from C. papaya Root

Powdered root sample of weight 100 g was weighed and transferred into a 500 mL beaker. 300 mL of absolute ethanol (98 %) was added to the beaker and the mixture was left to stand for 48 h at room temperature. Thereafter, the mixture was filtered with a white cotton cloth, and then refiltered using Whatman No. 1 filter paper. The filtrate was concentrated by rotary evaporation. The concentrated pale green extract was partitioned in 1:1 chloroform-water mixture. The lower chloroform layer was collected and allowed to dry at room temperature, then stored in a refrigerator until required.

Identification and Quantification of Phytochemicals by GC-FID

The identification and quantification of the chloroform soluble phytochemicals from *C. papaya* root was performed on a Buck M910 Gas Chromatography equipped with a flame ionization detector (GC-FID). A RESTEK 15 m MXT-1 column (15 m × 250 µm × 0.15 µm) was used. The injector temperature was 280 ^{o}C with splitless injection of 2 µL of sample and a linear velocity of 30 cms^{-1}. Helium 5.0 Pa.s was the carrier gas with a flow rate of 40 mL/min. The oven operated initially at 200 ^{o}C and then heated to 330 ^{o}C at a rate of 3 ^{o}C/min, and kept at this temperature for 5 minutes. The detector was operated at a temperature of 320 ^{o}C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in µg/g.

Results and Discussion

The GC-FID scan of *C. papaya* root extract partitioned in chloroform is shown in Figure 1 and the concentrations of the identified compounds and the structures assigned to them are shown in Table 1.

The concentration of these compounds were in the order catechin (77.14 µg/g) > sapogenin (25.84 µg/g) > sparteine (15.12 µg/g) > quinine (13.36 µg/g) > naringenin (8.10 µg/g) > naringin (6.20 µg/g) > anthocyanidine (3.78 µg/g) > flavone (0.06 µg/g).

Catechin is a polyphenolic secondary metabolite found in plants. It is a natural antioxidant that belongs to the flavonoid family. Its presence has been reported in apples, blackberries, dark chocolate, red wine, cherries, guava, pears, sweet and purple potatoes, green tea, pome fruit, cocoa and acai oil from the fruit of acai palm [16-17]. The green tea catechins have been shown to be more effective antioxidants than Vitamins C and E [18]. Several diseases such as cancer, Parkinson's disease, Alzheimer's disease, cardiovascular diseases and diabetes have their etiologies linked to changes in oxidant/antioxidant balance and free radical damage. Catechins in natural products have been shown to be efficient in the reduction of these processes [19-20]. Catechins in green tea account for about 30 % of the dry weight of fresh tea leaves, and the extracts from these leaves have also been shown to have antibacterial and antifungal activities [21]. *C. papaya* root extract has been reported to have good antibacterial and antifungal activities [22-23]. Ojo *et al.* [24] also reported the good antioxidant activity of the aqueous extract from the root of this plant.

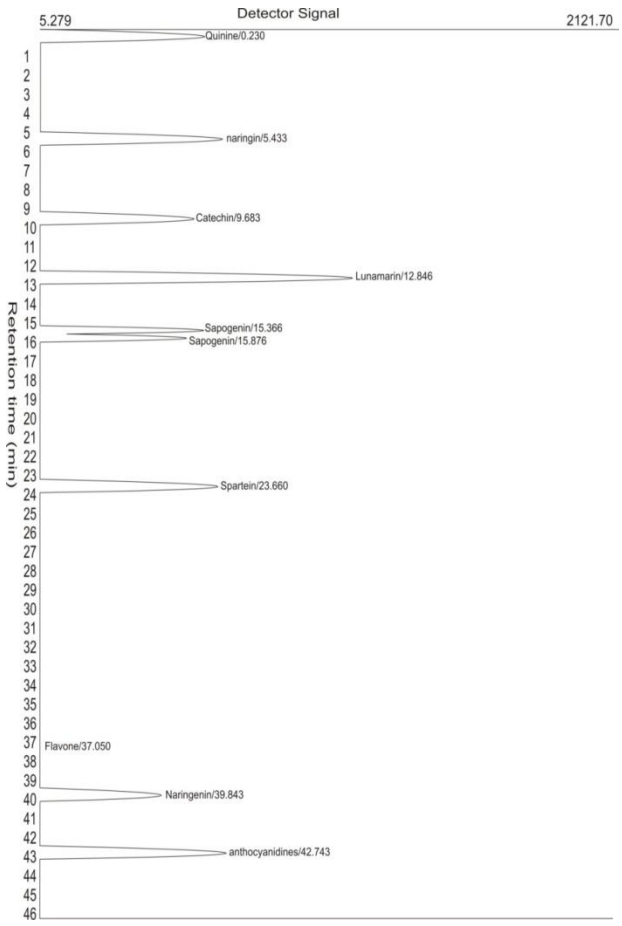


Figure 1: GC-FID scan of *C. papaya* root extract

Sapogenin belong to the family of saponins and comprise of structurally related compounds containing a steroid or triterpenoid aglycone linked to one or more oligosaccharide moieties. Saponins are often bitter to taste which reduce the plants palatability and serve as anti-feedants which protect the plant against microbes and fungi [25].

Sparteine is a quinolizidine alkaloid and was first extracted from *Cytisus scoparius*. It can also be isolated from several Fabaceae species including Lupinus and Spartium. This compound has been used to induce uterine contraction and has been shown to exhibit diuretic and anti-inflammatory activities [26]. It also has bactericidal activity against *Staphyloccoccus aureus*, *Bacillus subtilis* and *Bacillus thuringienis* [27]. Iwu *et al.* [15] have reported that *Proteus spps* were sensitive to the ethanolic root extract. The diuretic and abortifacient properties of the root extract of *C. papaya* have been reported [28-29], and this correlates with the known activities of this alkaloid.

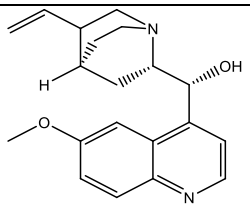
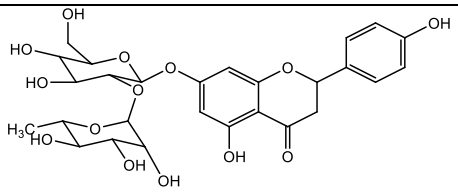
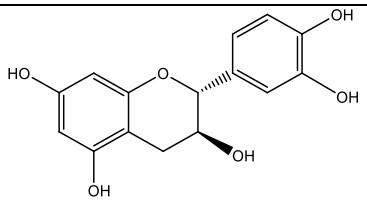
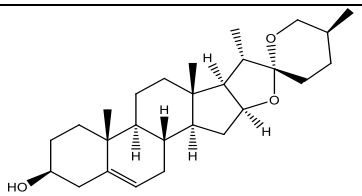
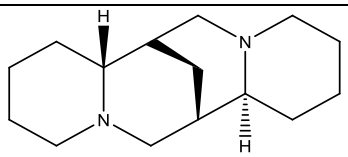
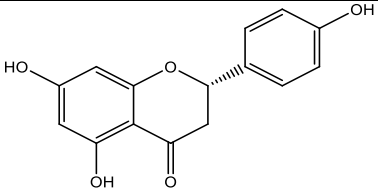
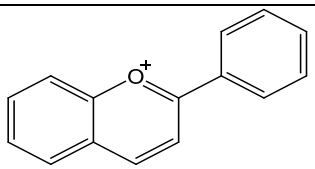
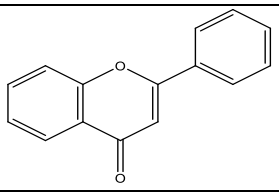
Quinine is a naturally occurring alkaloid. It was first isolated from the bark of Cinchona. It was the first drug used in the treatment of malaria [30]. *C. papaya* root has been reported to possess moderate antiplasmodial effect [31], indicating the activity of this compound on the microbes.

Naringin and its aglycone naringenin are important flavonoids that have been isolated from citrus plants. They have been found to display strong anti-inflammatory and antioxidant activities.

Several lines of investigation suggest that naringin supplementation is beneficial for the treatment of obesity, diabetes, hypertension, and metabolic syndrome [32-34].

Anthocyanidine and flavone are common plant pigments. Flavones are the primary pigments in white- and cream-colored flowers and act as copigments with anthocyanins in blue flowers [35]. Flavone and other flavonoids can also act as UV protectants in plants, because they absorb in the 280-315 nm range [36]. Flavones can also act as natural pesticides in plants, providing protection against insects and fungal diseases [37]. Adejuwon *et al.* [38] have reported the good antifungal activities of the methanolic root extract of *C. papaya*, which might be connected to the presence of this group of compounds.

Table 1: Phytochemicals in C. papaya root and their concentrations

Phytochemical	Structure	CAS number	µg/g
Quinine		6119-47-7	13.36
Naringin		10236-47-2	6.20
Catechin		18829-70-4	77.14
Sapogenin		8047-15-2	25.84
Sparteine		90-39-1	15.12
Naringenin		67604-48-2	8.10
Anthocyanidines		11029-12-2	3.78
Flavones		525-82-6	0.06

Conclusion

The phytoconstituents identified in the ethanolic extract of *C. papaya* root were catechin, sapogenin, sparteine, quinine, naringenin, naringin, anthocyanidine and flavone. The reported diuretic and arbotifacient properties of the root of this plant could be attributed to the presence of the alkaloid sparteine. Catechin and flavone components could be responsible for the observed antifungal and antityphoid activities. The antimalarial activity could be linked to its quinine content. The presence of naringin, sparteine and catechin in the extract suggested that it could be applied as an anti-inflammatory agent and used in the treatment of cardiovascular diseases.

References

1. J. Morton (1987), Papaya. In: Fruits of warm climates. Julia F. Morton, Miami, FL., 336–346.
2. A.K. Srivastava and V.K. Singh (2016), *Carica Papaya*- a herbal medicine. *International Journal of Research Studies in Biosciences*, **4**(11), 19-25.
3. V. Anjum, S.H. Ansari, K.J. Naquvi, P. Arora and A. Ahmad (2013), Development of quality standards of *Carica papaya* Linn. leaves, *Sch. Res. Lib.*, **5**(2):370-376.
4. UN Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (2017), Papaya production in 2016; Crops/Regions/World list/Production Quantity (pick lists).
5. A. Nugroho, H. Heryani, J.S. Choi and H.J. Park (2017), Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite scavenging activity, *Asian Pacific Journal Tropical Biomedicine*, **7**(3), 208-213.
6. G. Arvind, D. Bhowmik, S. Duraivel and G. Harish (2013), Traditional and medicinal uses of Carica papaya, *J. Med. Car. Pap.*, **1**(1), 2320-3862.
7. A. Roshan, N.K. Verma and A. Gupta (2014), A brief study on *Carica Papaya*-a review, *International Journal of Current Trends in Pharmaceutical Research*, **2**(4), 541-550.
8. A.M. Maisarah, R. Asmah and O. Fauziah (2014), Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica Papaya*, *Journal of Nutrition & Food Sciences*, **4**(2), 1-7.
9. B. Parni and Y. Verma (2014), Biochemical properties in peel, pulp and seeds of *Carica papaya*, *Plant Archives*, **14**(1), 565-568.
10. F.A.B. Dada, F.O.Z. Nzewuji, A.M. Esan and S.I. Oyeleye (2016), Phytochemical and antioxidant analysis of aqueous extracts of unripe papaw (*Carica papaya* Linn.) fruit's peel and seed, *IJRRAS*, **127**(3), 68-71.
11. S.A.S. Muhamad, B. Jamilah, A.R. Russly and A. Faridah (2017), In vitro antibacterial activities and composition of *Carica papaya* cv Sekaki/Hong Kong peel extracts, *International Food Research Journal*, **24**(3), 976-984.
12. L. Hayatie, A. Biworo and E. Suhartono (2015), Aqueous extracts of seed and peel of *Carica Papaya* against *Aedes aegypti*, *Journal of Medical and Bioengineering*, **4**(5):417-421.
13. K.B. Bergonio and M.A. Perez (2016), The potential of male papaya (*Carica papaya* L.) flower as a functional ingredient for herbal tea production, *Indian Journal of Traditional Knowledge*, **15**(1), 41-49.
14. J.H. Doughari, A.M. Elmahmood and S. Manzara (2007), Studies on the antibacterial activity of root extracts of *Carica papaya* L., *African Journal Microbiology Research*, 37-41.
15. C.I. Iwu, C. Ogukwe, M. Akah, M. Chijioke-Okere, U.L. Onu and J.O. Iwu (2016), Phytochemicals and Antimicrobial Properties of the Root and Leaf Extract of *Carica Papaya*, *International Journal of Innovative Research and Development*, **5**(8):175-179.
16. L.A. Pacheco-Palencia, S. Mertens-S. Talcott and S.T. Talcott (2008), Chemical composition, antioxidant properties, and thermal stability of a phytochemical enriched oil from Acai

(*Euterpe oleracea* Mart.), *J. Agric. Food Chem.*, **56**(12), 463-4636.
17. C. Kwik-Uribe and R.M. Bektash (2008), Cocoa flavanols- measurement, bioavailability and bioactivity, *Asia Pac. J. Clin. Nutr.*, **17**(1), 280-283.
18. C.A. Rice-Evans, N.J. Miller, P.G. Bolwell, P.M. Bramley and J.B. Pridham (1995), The relative antioxidant activities of plant-derived polyphenolic flavonoids, *Free Radical Research*, **22**(4), 375-383.
19. M.C. Polidori (2003), Antioxidant micronutrients in the prevention of agerelated diseases, *Journal of Postgraduate Medicine*, **49**(3), 229-235.
20. V.B. Junqueira, S.B. Barros, S.S Chan, L. Rodrigues, L. Giavarotti, R.L. Abud and G.P. Deucher (2004), Aging and oxidative stress, *Molecular Aspects of Medicine*, **25**(1-2):5-16.
21. S. Archana and A. Jayanthi (2011), Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens, *Journal of Applied Pharmaceutical Science*, **1**(8), 149-152.
22. I.I. Wemambu, D.J. Ajose and C.C. Eni (2018), Antibacterial effect of *Carica papaya* root extract on some selected pathogens from clinical isolates, *Acta Scientific Microbiology*, **1**(7):6-10.
23. M. Kumar, M. Faheem, S. Singh, A. Shahzad and A.K. Bhargava (2013), Antifungal activity of the *Carica papaya* important food and drug plant, *Asian Journal of Plant Science and Research*, **3**(1), 83-86.
24. O.A. Ojo, A.B. Ojo, O. Awoyinka, B.O. Ajiboye, B.E. Oyinloye, O.A. Osukoya, I.I. Olayide and A. Ibitayo (2018), Aqueous extract of *Carica papaya* Linn. roots potentially attenuates arsenic induced biochemical and genotoxic effects in Wistar rats, *Journal of Traditional and Complementary Medicine*, **8**(2), 324-334.
25. R. Xu, W. Zhao, J. Xu, B. Shao and G. Qin (1996), Studies on bioactive saponins from Chinese medicinal plants, *Advances in Experimental Medicine and Biology*, **404**, 371-382.
26. T. Schmeller and M. Wink (1998), Utilization of alkaloids in modern medicine. In Alkaloids, M.F. Roberts and M. Wink, eds. New York, Plenum, 435-459.
27. R. De la Vega, M. Gutierrez, C. Sanz, R. Calvo, L. Robredo, C. De la Cuadra and M. Muzquiz (1996), Bactericide-like effect of Lupinus alkaloids, *Industrial Crops and Products*, **5**(2), 141-148.
28. B. Sripanidkulchai, V. Wongpanich, P. Laupattarakasem, J. Suwansaksri and D. Jirakulsomchok (2001), Diuretic effects of selected Thai indigenous medicinal plants in rats, *J. Ethnopharmacol*., **75**(2-3), 185-190.
29. H.N. Sharma and H.C. Mahanta (2000), Modulation of morphological changes of endometrial surface epithelium by administration of composite root extract in albino rat Contraception, **62**(1), 51-54.
30. J. Achan, A.O. Talisuna, A. Erhart, A. Yeka, J.K. Tibenderana, F.N. Baliraine, P.J. Rosenthal and U. D'Alessandro (2011), Quinine, an old anti-malaria drug in a modern world: role in the treatment of malaria, *Malaria Journal*, **10**, 144.
31. G. Zeleke, D. Kebebe, E. Mulisa and F. Gashe (2017), In Vivo anti-malarial activity of the solvent fractions of fruit rind and root of *Carica papaya* Linn (Caricaceae) against *Plasmodium berghei* in Mice, *Journal of Parasitology Research*, 1-9.
32. M.A. Alam, N. Subhan, M.M. Rahman, S.J. Uddin, H.M. Reza and S.D. Sarker (2014), Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action, *Advances in Nutrition*, **5**(4), 404-417.
33. E. Tripoli, M.L. Guardia, S. Giammanco, D.D. Majo and M. Giammanco (2007), Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review, *Food Chem*, **104**, 466-479.

34. C.E. Duru (2020), Mineral and phytochemical evaluation of Zea mays husk, *Scientific African*, **7**, e00224.
35. J.B. Harborne and R.J. Grayer (1994), Flavonoids and insects. In: Harborne J.B., Editor. The flavonoids: advances in research since 1986. 1st ed. London: Chapman & Hall, 589-618.
36. J.B. Harborne and C.A. Williams (2000). Advances in flavonoid research since 1992, *Phytochemistry*, **55**, 481-504.
37. V. Lattanzio, V. De Cicco, D. Di Venere, G. Lima and M. Salerno (1994), Antifungal activity of phenolics against fungi commonly encountered during storage, *Ital. J. Food Sci*., **6**, 23-30.
38. A.O. Adejuwon, E.O. Agbaje and N. Idika (2011), Antifungal and antibacterial activities of aqueous and methanolic root extracts of *Carica papaya* linn. (Caricaceae), *International Research Journal of Microbiology*, **2**(8), 270-277.