Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2019.26.31

Research Article
GC-MS and FT-IR Analyses of Phytocomponents From petroleum ether Fraction of Leaf Extract of *Psidium guajava*

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Abstract

Background and Objectives: Phytocomponents from plant materials are largely responsible for their biologic activities. The phytocomponents from petroleum ether fraction of the leaf extract of *P. guajava* was identified, quantified and characterized. **Materials and Methods:** Chromatographic and spectrophotometric methods, namely, Gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared spectrometry (FT-IR) were used for the identification, quantification and characterization of the phytocomponents. **Results:** The major phytocomponent from petroleum ether fraction of the leaf extract of *P. guajava* was bis (2-ethylhexyl) phthalate, whereas the minor phytocomponents were 2-pentadecanol and 14-pentadecenoic acid. Petroleum ether fraction of leaf extract of *P. guajava* gave a characteristic broad peak around 3328.5 cm^{-1}, which was indicative of the presence of an alcohol functional group. **Conclusion:** GC-MS and FT-IR analyses of petroleum ether fraction of leaf extract of *P. guajava* identified five phytocomponents viz., 2-pentadecanol, carbonic acid, eicosyl vinyl ester, bis (2-ethylhexyl) phthalate, 14-pentadecenoic acid and 2-methyltetracosane.

Key words: Leaf extract, petroleum ether, *Psidium guajava,* phytocomponents, gas chromatography, mass spectrometry

Citation: Paul C. Chikezie, Raphael C. Ekeanyanwu and Adaeze B. Chile-Agada, 2019. GC-MS and FT-IR analyses of phytocomponents from petroleum ether fraction of leaf extract of *Psidium guajava*. Res. J. Med. Plants, 13: 26-31.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Guava (*Psidium guajava* L.), belongs to the family Myrtaceae, is a native of the American tropics from where it spread to the tropical and subtropical regions of the world. Guava tree bears fruits that are rich in ascorbic acid[1]. The plant is composed of medicinally relevant phytocomponents with variable pharmacological and clinical potentials[2]. According to Gutierrez *et al*.[2] the biological activities of *P. guajava* leaf extract are consistent with the presence of notable phytocomponents such as the carotenoids, terpenoids, flavonoids, phenolics and triterpenes. Previous reports showed that aqueous extract of *P. guajava* was hepatoprotective[3] and served as an expectorant[4], whereas, the chloroform extract exhibited antimicrobial activities[4].

Phytocomponents or bioactive principles are biomolecules that play major roles in therapeutic activities of herbs and are used in the pharmaceutical industries for the production of medicinal agents. Paradoxically, bioactive principles are also responsible for the toxic outcomes of some of these plants that are of concern and interest to the toxicologist[5,6]. Chromatographic and spectrophotometric protocols provide reproducible and dependable methods for the qualitative and quantitative evaluation as well as characterization of phytocomponents from medicinal plants[7]. Previous studies have reported the therapeutic potentials of several fractions of leaf extracts of *P. guajava*[8] as well as toxic activities of petroleum ether fractions of plant extracts[9]. Because phytocomponents from plant materials are largely responsible for their biologic activities, the present investigation validates the identities, quantities and characteristics of phytocomponents from petroleum ether fraction of leaf extract of *P. guajava*. The phytocomponents from petroleum ether fraction of leaf extract of *P. guajava* were identified, quantified and characterized using chromatographic and spectrophotometric methods, namely, Gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared spectrometry (FT-IR).

MATERIALS AND METHODS

Study area: The study was carried out at the Medical Biochemistry Laboratory, Department of Biochemistry, Imo State University, Owerri, Nigeria from April to September, 2019.

Collection of leaf samples: Healthy and matured leaves of *P. guajava* found growing within the location (Latitude 5°30.2237'N, Longitude 7°2.6277'E) were harvested during the wet season of 2nd and 7th April, 2019. The leaves were authenticated by a taxonomist. The voucher number of the leaves was IMSUH 010. For future reference purposes, a specimen of the leaves was deposited in the herbarium.

Extraction and preparation of fractionated of leaf extract: The extraction protocol, using the soxhlet extractor, was carried out within 24 h of collection of the leaf samples of *P. guajava* according to the methods previously described by Chikezie *et al*.[10]. Fractionation of the leaf extract was according to the methods of Okoye *et al*.[11] whereby the crude hydro-ethanolic leaf extract was partitioned with an equal volume of petroleum ether. The petroleum ether fraction of the leaf extract was finally concentrated under reduced pressure for 24 h at 50°C in a rotary evaporator (BüchRotavapor R-200). The petroleum ether fraction was subjected to GC-MS and FT-IR analyses.

GC-MS and FT-IR analyses: Chromatographic-spectrophotometric protocols were carried out using GC-MS systems {Agilent 7890A GC system set up with 5975C VL MSD, Agilent Technologies, Inc., Santa Clara, CA, USA; The MS system was accomplished in electron ionization (EI) mode with Selected Ion Monitoring (SIM)}[12]. FT-IR instruments (PerkinElmer Spectrophotometer, USA) protocols were according to the methods previously described by Ighodaro *et al*.[13].

RESULTS

Phytocomponent composition of *P. guajava* extract by GC-MS chromatogram: Phytocomponents from petroleum ether fraction of leaf extract of *P. guajava* are presented in Table 1. The major phytocomponent from petroleum ether fraction of the leaf extract was bis (2-ethylhexyl) phthalate, which constituted 71.49% in relative abundance compared to other phytocomponents (Table 1). Additionally, the minor phytocomponents, in terms of their relative abundance, were 2-pentadecanol (2.52%) and 14-pentadecenoic acid (2.35%) (Table 1).

Peak values of FT-IR spectra of *P. guajava* extract: The characteristic peak values of FT-IR spectra of petroleum ether fraction of leaf extract of *P. guajava* are summarized in Table 2. Petroleum ether fraction of leaf extract of *P. guajava* gave a characteristic broad peak around 3328.5 cm^{-1}, which was indicative of the presence of an alcohol functional group. The weak band within the region of 2855.1-2922.2 cm^{-1} and

Table 1: Phytocomponent composition of petroleum ether fraction of leaf extract of *P. guajava* by GC-MS chromatogram

R_T (min)	Phytocomponents	MF	MW (g moL^{-1})	PA (%)
19.618	2-Pentadecanol	$C_{15}H_{32}O$	228.414	02.52
22.143	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	214.300	10.29
24.191	Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.560	71.49
27.621	14-Pentadecenoic acid	$C_{16}H_{32}O_2$	240.380	02.35
29.614	2-Methyltetracosane	$C_{25}H_{52}$	352.680	13.35

R_T: Retention time, MF: Molecular formula, MW: Molecular weight, PA: Peak area

Table 2: Peak values of FT-IR spectra of petroleum ether fraction of leaf extract of *P. guajava*

Peak/band (cm^{-1})	T (%)	Functional groups/assignment	Origin
3328.5	95.782	Hydroxy group, H-bonded O-H stretch	O-H
2922.2	93.277	Alkanes sp3 C-H bend	C-H
2855.1	95.041	Alkanes sp3 C-H stretch	C-H
2292.3	99.510	C-N stretch	C≡N
2117.1	99.933	Isothiocyanate	-NCS
1871.1	99.652	Five-membered ring anhydride	C = O
1718.3	96.925	Carboxyl C-O stretch	C = O
1613.9	95.675	Aromatic ring stretch	C = C
1453.7	95.252	Methyl C-H asym./sym. bend	C-H
1364.2	95.249	Alkanes sp3 C-H bend	C-H
1315.8	95.222	Alkanes sp3 C-H bend	C-H
1233.7	94.847	Acyl C-O, phenol C-O stretch	C = O, C-O
1162.9	94.972	Secondary amine, C-N stretch	>N-H, C≡N
1028.7	91.640	Alkoxy C-O stretch	X-O-C

T: Transmittance

1315.8-1453.7 cm^{-1} were characteristic of alkane sp3 C-H bend. The presence of nitrogen-containing functional groups, namely, the cyanates (C≡N) and isothiocyanates (-NCS) were characterized by weak bands around the regions of 2117.1-2292.3 cm^{-1}.

The presence of carbonyl and anhydride functional groups were typified by weak bands around the regions of 1718.3-1871.1 cm^{-1}. The peak at 1613.9 cm^{-1} was indicative of the presence of aromatic compounds in petroleum ether fraction of *P. guajava*. Finally, other notable phytocomponents from petroleum ether fraction of leaf extract of *P. guajava* were acyl and phenol (1233.7 cm^{-1}), secondary amines and cyanates (1162.9 cm^{-1}) and alkoxyl (1028.7 cm^{-1}) functional group-containing compounds.

DISCUSSION

Phytocomponents exhibit medicinal and toxic activities that are consistent with their sources, molecular and chemical peculiarities[5]. The phytocomponents from petroleum ether fraction of leaf extract of *P. guajava* appeared to justify its biological and medicinal activities reported elsewhere[2,14-18]. Empirical investigations had revealed a connection between the length of the carbon chain of aliphatic alcohols and their anti-bacterial activities[19,20]. FT-IR analysis of petroleum ether fraction of leaf extract of *P. guajava* confirmed the presence of aliphatic alcohols such as 2-pentadecanol. Previous reports precisely showed that n-pentadecanol, an analog of 2-pentadecanol, was the phytocomponent from methanolic flower extract of *Saussurea obvallata* that exhibited anti-oxidant and anti-microbial properties *in vitro*[21].

Likewise, bis (2-ethylhexyl) phthalate from seed extract of *Buchholzia Coriacea* Engler (Capparaceae)[22], flower extract of *Calotropis gigantea* (Linn)[23] and *Penicillium janthinellum*[24] 62 exhibited antioxidant, antitumor, antiviral, anti-fungal and anti-bacterial properties. The present study revealed that bis (2-ethylhexyl) phthalate was a major phytocomponent from petroleum ether fraction of leaf extract of *P. guajava*, which validates its antioxidant, anti-fungal and anti-bacterial properties when juxtaposed with previous research findings[14,15,17,18,23,25]. In addition, reports showed that bis (2-ethylhexyl) phthalate (di-2-ethylhexyl phthalate) including structurally similar molecular analogs such as the dicarboxylic acids viz. 1, 2-cyclohexanedicarboxylic acid and phthalic acid analogs stabilized erythrocyte membrane against osmotic stress *in vitro*[26,27].

The aforementioned beneficial properties notwithstanding, a wide range of toxic outcomes of bis (2-ethylhexyl) phthalate following continuous exposure of human population to environmental products and contaminants has been extensively reviewed[28]. From a generalized viewpoint, the issues of beneficial and toxic properties as well as the nature and origin of bis (2-ethylhexyl) phthalate in biologic systems are still controversially discussed[23,28,29].

The presence of 14-pentadecenoic acid analogs, pentadecenoic acid and its various isomers, in folklore medicinal plant (*Exacum lawii*) has been reported by Sharma and Hemalatha[30]. Carbonic acid, eicosyl vinyl ester, by virtue of its antioxidant activity, was among the phytocomponents from *Cakile maritima* Scop extracts that inhibited the growth of some bacteria, which triggered autoimmune inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis[31]. The presence of phenols, mono- and di-carboxylic acids and their ester derivatives, including nitrogen-containing functional group compounds, were expressly confirmed by FT-IR spectra patterns. Accordingly, the presence of carbonic acid, eicosyl vinyl ester in *P. guajava* validates its use in the treatment of bacterial infections as previously reported by Sanda *et al*.[15], Rahman *et al*.[32], Morais-Braga *et al*.[33] and Diaz-de-Cerio *et al*.[34].

Studies have revealed that 2-methyltetracosane is a free radical scavenging phytocomponent from whole plant methanol extract of *Cenchrus ciliaris*[35]. Fittingly, in concord with previous reports by Sanda *et al*.[15], Diaz-de-Cerio *et al*.[34] and Joseph and Priya[36], the application of *P. guajava* decoction by traditional practitioners in ameliorating pathologic states, induced by oxidative stress, appeared to be connected with the 2-methyltetracosane content of petroleum ether fraction of leaf extract of *P. guajava*.

Further studies aimed at isolating the phytocomponents from petroleum ether fraction of leaf extract of *P. guajava* as well as ascertaining their capacities to ameliorate pathologic conditions associated with oxidative stress are recommended.

CONCLUSION

GC-MS and FT-IR analyses of petroleum ether fraction of leaf extract of *P. guajava* identified five phytocomponents viz., 2-pentadecanol, carbonic acid, eicosyl vinyl ester, bis (2-ethylhexyl) phthalate, 14-pentadecenoic acid and 2-methyltetracosane. The major phytocomponent from petroleum ether fraction of the leaf extract was bis (2-ethylhexyl) phthalate, whereas, the minor phytocomponents include 2-pentadecanol and 14-pentadecenoic acid. Some of these phytocomponents have been reported to exhibit biologic and therapeutic activities.

SIGNIFICANCE STATEMENT

This study discovered that notable phytocomponents from petroleum ether fraction of leaf extract of *P. guajava*, namely, bis (2-ethylhexyl) phthalate, 2-pentadecanol and 14-pentadecenoic acid can be of potential benefit for the treatment of viral, fungal and bacterial infections as well as amelioration of pathologic conditions linked with oxidative stress. This study will help the researcher to uncover the critical area of the use of the identified phytocomponents from petroleum ether fraction of leaf extract of *P. guajava* for the treatment of pathologic conditions that many researchers were not able to explore.

ACKNOWLEDGMENTS

The authors are grateful for the technical assistance offered by Mr. F.C. Emengaha, Chief Academic Technologist, Department of Medical Biochemistry, College of Medicine and Mr. C.O. Kabiri, Senior Laboratory Technologist, Department of Biochemistry, Faculty of Science, Imo State University, Owerri. The efforts of Mr. Franklyn O. Ohiagu are highly appreciated. This work was supported by Imo State University, Owerri and research grant offered by the Tertiary Education Trust Fund (TETFund) Research Based Interventions of Nigerian Universities. Imo State University, Owerri, provided the laboratory space and infrastructures. TETFund provided the financial resources for purchase of laboratory chemicals/reagents and instruments as well as expenses pertaining to transportation and travels. Grant Number: TETFUND/DRSS/UNIV/OWERRI/2015/5RP VOL 1 (7).

REFERENCES

1. Pommer, C.V. and K.R.N. Murakami, 2009. Breeding Guava (*Psidium guajava* L.). In: Breeding Plantation Tree Crops: Tropical Species, Jain, S.M. and P.M. Priyadarshan (Eds.). Springer, New York, USA., ISBN: 978-0-387-71201-7, pp: 83-120.
2. Gutierrez, R.M.P., S. Mitchell and R.V. Solis, 2008. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol., 117: 1-27.
3. Roy, C.K., J.V. Kamath and M. Asad, 2006. Hepatoprotective activity of *Psidium guajava* Linn. leaf extract. Indian J. Exp. Biol., 44: 305-311.
4. Jaiarj, P., P. Khoohaswan, Y. Wongkrajang, P. Peungvicha, P. Suriyawong, M.L.S. Saraya and O. Ruangsomboom, 1999. Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. J. Ethopharmacol., 67: 203-212.
5. Chikezie, P.C., C.O. Ibegbulem and F.N. Mbagwu, 2015. Bioactive principles from medicinal plants. Res. J. Phytochem., 9: 88-115.
6. Varma, N., 2016. Phytoconstituents and their mode of extractions: An overview. Res. J. Chem. Environ. Sci., 4: 8-15.

7. Ezekwe, S.A. and P.C. Chikezie, 2017. GC-MS analysis of aqueous extract of unripe fruit of *Carica papaya*. J. Nutr. Food Sci., Vol. 7, No. 3. 10.4172/2155-9600.1000602.
8. Zahin, M., I. Ahmad and F. Aqil, 2017. Antioxidant and antimutagenic potential of *Psidium guajava* leaf extracts. Drug Chem. Toxicol., 40: 146-153.
9. Su, Z., H. Huang, J. Li, Y. Zhu, R. Huang and S.X. Qiu, 2013. Chemical composition and cytotoxic activities of petroleum ether fruit extract of fruits of *Brucea javanica* (Simarubaceae). Trop. J. Pharm. Res., 12: 735-742.
10. Chikezie, P.C., 2015. Histopathological studies of renal and hepatic tissues of hyperglycemic rats administered with traditional herbal formulations. Int. J. Green Pharm., 9: 184-191.
11. Okoye, T.C., P.A. Akah, C.O. Okoli, A.C. Ezike and F.N. Mbaoji, 2010. Antimicrobial and antispasmodic activity of leaf extract and fractions of *Stachytarpheta cayennensis*. Asian Pac. J. Trop. Med., 3: 189-192.
12. Raskovic, A., N. Pavlovic, M. Kvrgic, J. Sudji, G. Mitic, I. Capo and M. Mikov, 2015. Effects of pharmaceutical formulations containing thyme on carbon tetrachloride-induced liver injury in rats. BMC Complement. Altern. Med., Vol. 15. 10.1186/s12906-015-0966-z.
13. Ighodaro, O.M., O.A. Akinloye, R.N. Ugbaja, S.O. Omotainse and O. Faokunla, 2016. FT-IR analysis of *Sapium ellipticum* (Hochst) pax ethanol leaf extract and its inhibitory effects on pancreatic α-amylase and intestinal α-glucosidase activities *in vitro*. Egypt. J. Basic Applied Sci., 3: 343-349.
14. Nisha, K., M. Darshana, G. Madhu and M.K. Bhupendra, 2011. GC-MS analysis and anti-microbial activity of *Psidium guajava* (leaves) grown in Malva region of India. Int. J. Drug Dev. Res., 3: 237-245.
15. Sanda, K.A., H.A. Grema, Y.A. Geidam and Y.M. Bukar-Kolo, 2011. Pharmacological aspects of *Psidium guajava:* An update. Int. J. Pharmacol., 7: 316-324.
16. Barbalho, S.M., F.M.V. Farinazzi-Machado, R. de Alvares Goulart, A.C.S. Brunnati, A.M.M.B. Ottoboni and C.C.T. Nicolau, 2012. *Psidium guajava* (Guava): A plant of multipurpose medicinal applications. Med. Aromat. Plants, Vol. 1, No. 4. 10.4172/2167-0412.1000104.
17. Biswas, B., K. Rogers, F. McLaughlin, D. Daniels and A. Yadav, 2013. Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. Int. J. Microbiol., Vol. 2013. 10.1155/2013/746165.
18. Naseer, S., S. Hussain, N. Naeem, M. Pervaiz and M. Rahman, 2018. The phytochemistry and medicinal value of *Psidium guajava* (guava). Clin. Phytosci., Vol. 4, No. 1. 10.1186/s40816-018-0093-8.
19. Kabelitz, N., P.M. Santos and H.J. Heipieper, 2003. Effect of aliphatic alcohols on growth and degree of saturation of membrane lipids in *Acinetobacter calcoaceticus*. FEMS Microbiol. Lett., 220: 223-227.
20. Togashi, N., A. Shiraishi, M. Nishizaka, K. Matsuoka, K. Endo, H. Hamashima and Y. Inoue, 2007. Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. Molecules, 12: 139-148.
21. Semwal, P. and S. Painuli, 2019. Antioxidant, antimicrobial and GC-MS profiling of *Saussurea obvallata* (Brahma Kamal) from Uttarakhand Himalaya. Clin. Phytosci., Vol. 5, No. 1. 10.1186/s40816-019-0105-3.
22. Ojinnaka, C.M., K.I. Nwachukwu and M.N. Ezediokpu, 2015. The chemical constituents and bioactivity of the seed (fruit) extracts of *Buchholzia coriacea* Engler (Capparaceae). J. Applied Sci. Environ. Manage., 19: 795-801.
23. Habib, M.R. and M.R. Karim, 2009. Antimicrobial and cytotoxic activity of Di-(2-ethylhexyl) phthalate and anhydrosophoradiol-3-acetate isolated from *Calotropis gigantea* (Linn.) flower. Mycobiology, 37: 31-36.
24. El-Sayed, O.H., M.M.S. Asker, S.M. Shash and S.R. Hamed, 2015. Isolation, structure elucidation and biological activity of Di-(2-ethylhexyl) phthalate produced by *Penicillium janthinellum* 62. Int. J. ChemTech. Res., 8: 58-66.
25. El-Sayed, M.H., 2012. Di-(2-ethylhexyl) phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from the culture filtrate of newly isolated soil Streptomyces (*Streptomyces mirabilis* strain NSQu-25). World Applied Sci. J., 20: 1202-1212.
26. Kanias, T., M. Lanteri, S. Derek, M. Busch and M.T. Gladwin, 2013. Red blood cell storage in pediatric transfer bags is correlated with increased levels of hemolysis and altered osmotic fragility. Blood, 122: 2403-2403.
27. Mineo, H. and M. Moriyoshi, 2019. Carboxylic acids with certain molecular structures decrease osmotic fragility against osmotic pressure in cattle erythrocytes *in vitro*: Appearance of a wedge-like effect similar to RBCs in other animal species. Biochem. Pharmacol., Vol. 8, No. 1.
28. Rowdhwal, S.S.S. and J. Chen, 2018. Toxic effects of di-2-ethylhexyl phthalate: An overview. BioMed Res. Int., Vol. 2018. 10.1155/2018/1750368.
29. Ortiz, A. and E. Sansinenea, 2018. Di-2-ethylhexylphthalate may be a natural product, rather than a pollutant. J. Chem., Vol. 2018. 10.1155/2018/6040814.
30. Sharma, S. and S. Hemalatha, 2017. DNA fingerprinting profile and quality control standardization of folklore medicinal plant *Exacum lawii*. Pharmacogn. J., 9: 775-785.
31. Omer, E., A. Elshamy, A.N. El Gendy, X. Cai, J. Sirdaarta, A. White and I.E. Cock, 2016. *Cakile maritime* Scop. extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. Pharmacog. J., 8: 361-374.
32. Rahman, M.M., S.H. Ahmad, M.T.M. Mohamed and M.Z. Ab Rahman, 2014. Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava* and *Andrographis paniculata*. Scient. World J., Vol. 2014. 10.1155/2014/635240.

33. Morais-Braga, M.F.B., J.N.P. Carneiro, A.J.T. Machado, A.T.L. dos Santos and D.L. Sales *et al*., 2016. *Psidium guajava* L., from ethnobiology to scientific evaluation: Elucidating bioactivity against pathogenic microorganisms. J. Ethnopharmacol., 194: 1140-1152.
34. Diaz-de-Cerio, E., V. Verardo, A.M. Gomez-Caravaca, A. Fernandez-Gutierrez and A. Segura-Carretero, 2017. Health effects of *Psidium guajava* L. leaves: An overview of the last decade. Int. J. Mol. Sci., Vol. 18, No. 4. 10.3390/ijms18040897.
35. Arora, S., G. Kumar and S. Meena, 2017. Screening and evaluation of bioactive components of *Cenchrus ciliaris* L. by GC-MS analysis. Int. Res. J. Pharm., 8: 69-76.
36. Joseph, B. and R.M. Priya, 2011. Phytochemical and biopharmaceutical aspects of *Psidium guajava* (L.) essential oil: A review. Res. J. Med. Plant, 5: 432-442.