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Comparative FT-IR Analysis of Chloroform Fractions of Leaf Extracts of *Anacardium occidentale*, *Psidium guajava* and *Terminalia catappa*

Paul C. Chikezie*, Raphael C. Ekeanyanwu, Adaeze B. Chile-Agada, Franklyn O. Ohiagu

Department of Biochemistry, Imo State University, Owerri, Nigeria

Abstract

The present study identified and characterized phytocomponents from chloroform fractions of leaf extracts of *Anacardium occidentale*, *Psidium guajava* and *Terminalia catappa*. The identification and characterization of phytocomponents from chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa* were carried out using FT-IR system protocol. The weak bands around 2922.2 cm^{-1} and 1714.5 cm^{-1} were indicative of the presence of carboxylic acids, whereas the presence of ethers was represented by relatively medium bands around 1088.4 cm^{-1} and 1047.4 cm^{-1} in chloroform fraction of leaf extract of *A. occidentale*. Likewise, chloroform fraction of leaf extract of *P. guajava* contained ethers (1088.4 cm^{-1} and 1047.4 cm^{-1}), esters and phenolics (1267.3 cm^{-1}). Notable phytocomponents in chloroform fraction of leaf extract of *T. catappa* were ethers (1088.4 cm^{-1} and 1047.4 cm^{-1}) and nitro compounds (1379.1 cm^{-1} and 1323.2 cm^{-1}). A general outlook of the present study revealed peculiarities in phytocomponent profiles of chloroform fractions of the leaf extracts, which depended on the polarity of the fractionation solvent and plant species variations.

Keywords: FT-IR analysis, phytocomponents, *Anacardium occidentale*, *Psidium guajava*, *Terminalia catappa*

1 Introduction

Anacardium occidentale L. (Anacardiaceae), commonly referred to as cashew, originated from Brazil and now well established in the tropical regions of India and East Africa [1, 2]. In Brazil, the cultivation of cashew occupies approximately 700, 000 hectares of land space with a production estimate of 280,000 tons per year [3, 4]. The tree usually grows up to an average height of 12 m with convoluted trunk and spreading branches almost touching the ground. The oval, glabrous and rounded leaves of *A. occidentale* measures about 10-18 by 8-15 cm in dimension with a short petiole [5]. The immature leaves appear pale green or red, whereas the mature leaves are dark green. The cashew tree is primarily cultivated for its edible cashew apple (pseudo fruit) and chestnut, which are of enormous economic and social importance in many countries in Latin America, Asia and Africa [4]. The leaves of *A. occidentale* are rich in phenols, phlobotannins, cyanogenic glycosides, flavonoids, saponins, (E)-β-ocimene, α-copaene [6-8]. The ethnomedicinal and industrial use, as well as biological activities of phytocomponents from the cashew tree, are widely reported [1, 2, 4, 5, 7, 9].

Psidium guajava L. (Myrtaceae) originated from tropical Southern America and now virtually found growing in tropical and subtropical regions of the world, namely, Asia, Egypt, Hawaii, Florida and Palestine [10]. A botanical survey showed that out of the 150 species of *P. guajava*, about 20 species produce edible fruits [11]. The leaves of *P. guajava* are simple with a short petiole, oblong or elliptic leathery blade with conspicuous parallel veins and measures about 3-10 mm long [5, 12]. The *P. guajava* tree is an evergreen shrub with an average height of 3-10 m, which may possess several main stems and many branches that continually flakes [5]. The fruit of *P. guajava* is ovoid or pear-shaped of 4-12 cm long with an average weight of 500 g [5]. The fruits appear green when matured and yellow when fully ripe. Notable phytocomponents of *P. guajava* leaves are the flavonoids, carotenoids, terpenoids, phenolics and triterpenes [13]. Herbal preparations of the leaves are used in folk medicine for the amelioration of several ailments like diarrhea [13], rheumatism, diabetes mellitus, cough, mouth ulcer [14]. Additionally, decoctions of leaf extracts of *P. guajava* are anti-bacterial as well as used for skin and wound healing and the chewing stick is recommended for oral care [15, 16]. The biological activities and ethnomedicinal usefulness of *P. guajava* have been exhaustively reviewed by several authors elsewhere [10, 12, 13, 15-17].

Terminalia catappa L. is an ornamental tree native to Southeast Asia and belongs to Combretaceae family [18, 19]. An agricultural survey showed that the tree is cultivated in subtropical and tropical regions of the world [18]. The *T. catappa* tree, which attains the average height of 15-25 m, is largely deciduous [20] and commonly cultivated to provide shade in housing estates and public spaces. The branches of the trees are arranged in characteristic tiers. The ripe fruit and nut kernel are eaten raw. Anatomical inspections show that the leaves are glossy dark-green and

* Corresponding author; E-mail: p_chikezie@yahoo.com; Phone: +2348038935327

leathery with broad and ovoid dimensions that measure approximately 15-25 cm long and 10-14 cm wide [18]. Nagappa *et al.*, [21] reported the anti-diabetic activity of *T. catappa* fruits. The leaves of *T. catappa* contain phytocomponents such as triterpenic acids, squalene, ethylacetate, phytol, violaxanthin, violeoxanthin, 6, 10, 14-trimethyl-2-pentadecanone, β-cryptoxanthin, lutein epoxide, (E, E)-2, 4-decadienal [22-25]. The summarized ethnomedical usefulness of extracts from various parts of *T. catappa* published in relevant articles in PubMed has been reported [18].

Molecular profiling of phytocomponents provides insights into their ethnomedicinal usefulness and chemical diversities, including toxicity concerns associated with herbal extracts that are of relevance to the toxicologist or pharmacist, clinician and nutritionist [26, 27].

Using Fourier transform-infrared (FT-IR) spectroscopy protocols, the present study identified and characterized phytocomponents from chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa*. The outcome of our studies will provide preliminary insights into the aggregate mixtures of likely phytocomponents from chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa*.

2 Materials and Methods

2.1 Collection of plant specimens

Fresh leaves of *A. occidentale*, *P. guajava* and *T. catappa* were harvested, between the 2nd and 7th April 2019, from botanical gardens of Imo State University, Owerri, Nigeria (Latitude 5° 30.2237'N; Longitude 7° 2.6277'E). A botanist, Professor F.N. Mbagwu of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria identified and authenticated the leaves of the selected plants. The leaves were assigned voucher numbers as follows: *A. occidentale* = IMSUH 009; *P. guajava* = IMSUH 010; *T. catappa* = IMSUH 011. Samples of the leaves were deposited in the herbarium for future reference. Extraction of the leaves was carried out within 24 h of collection of the plant specimens.

2.2 Extraction and preparation of fractionated leaf extracts

Preparation and extraction of the leaves were done according to the methods previously described [26]. Fractionation of the leaf extracts was done according to the methods of Okoye *et al.,* [29] but with slight modifications. Separate volumes of the crude hydro-ethanolic leaf extracts were fractionated using separatory funnels and in successive solvents, such that progressive partitioning was carried out in equal volumes of solvents in the order of increasing polarities; viz. petroleum ether, *n*-hexane, chloroform and ethylacetate. The chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa* were subjected to FT-IR analysis.

2.3 FT-IR analysis

The identification and characterization of phytocomponents from chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa* were carried out using FT-IR system protocol (PerkinElmer Spectrophotometer, USA) according to the methods previously described [30]. Peak values of the extracts were measured within wavelength regions of (4000 – 700) cm^{-1}.

3 Results and Discussion

3.1 FT-IR spectra of leaf extract of A. occidentale

Figure 1 showed the FT-IR spectra of chloroform fraction of leaf extract of *A. occidentale* and summarized in Table 1.

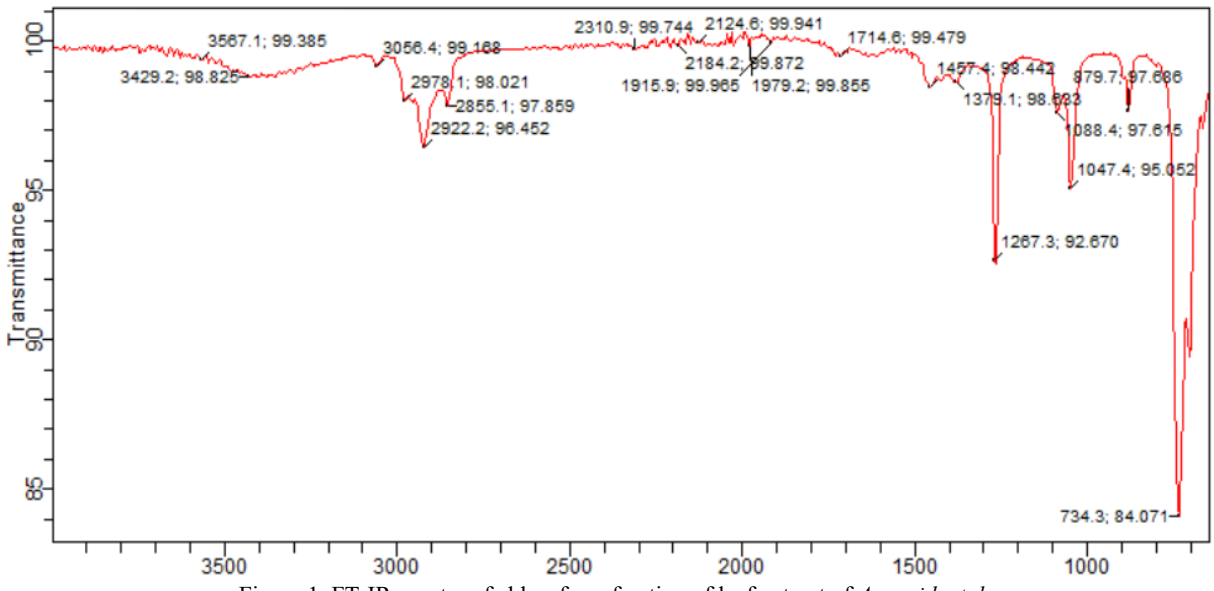

Figure 1. FT-IR spectra of chloroform fraction of leaf extract of *A. occidentale*

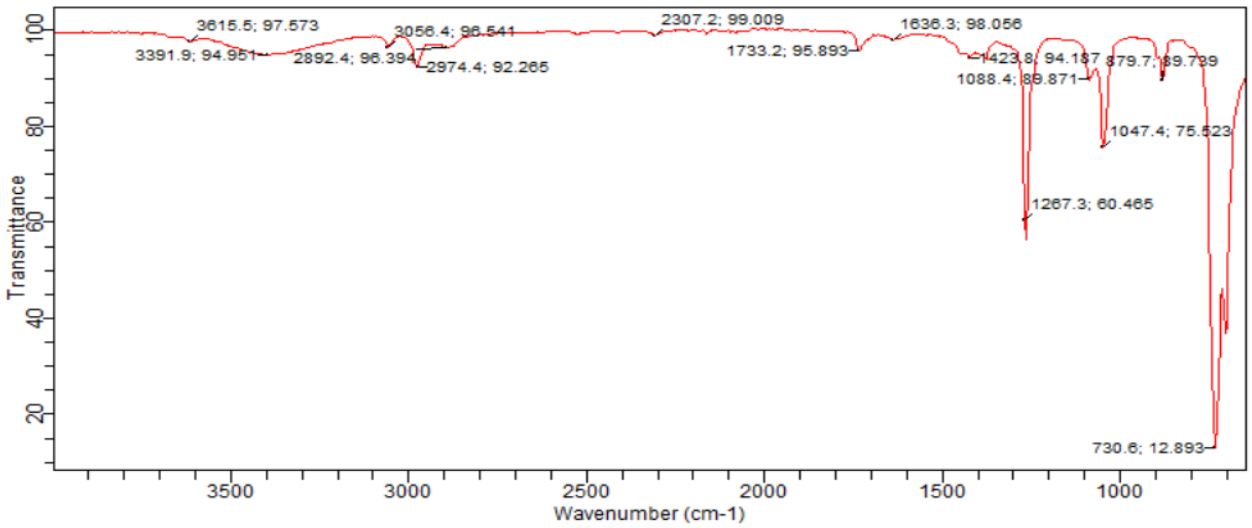

Figure 2. FT-IR spectra of chloroform fraction of leaf extract of *P. guajava*

Table 1. Peak values of FT-IR spectra of chloroform fraction of leaf extract of *A. occidentale*

S. No	Peak/band (cm^{-1})	%T	Functional groups/assignment	Origin
1.	3567.1	99.385	Alcohol O-H stretch	O-H
2.	3429.2	98.825	Alcohol O-H stretch	O-H
3.	3055.4	99.168	Aromatic sp2 C-H stretch	C-H
4.	2978.1	98.021	Aromatic sp2 C-H stretch	C-H
5.	2922.2	95.452	Carboxylic acid O-H stretch	O-H
6.	2855.1	97.859	Methylene C-H stretch	C-H
7.	2184.2	98.872	Alkynes sp C-C stretch	C≡C
8.	2124.6	99.941	Alkynes sp C-C stretch	C≡C
9.	1714.5	98.479	Carboxylic acid C-O stretch	C=O
10.	1457.4	98.144	Aromatic C-C stretch	C=C
11.	1379.1	98.883	Methyl C-H stretch	C-H
12.	1088.4	87.675	Alkoxy C-O stretch	X-O-C
13.	1047.4	85.052	Alkoxy C-O stretch	X-O-C
14.	879.7	87.885	Alkene di-substituted C-C bend	C=C
15.	734.3	84.071	Aryl ortho di-substituted C-C bend	C=C

%T: Percentage Transmittance

The presence of aliphatic alcohol in the fraction was indicative of broad peaks around 3567.1 cm^{-1} and 3429.2 cm^{-1}. The peak values of 3055.4 cm^{-1}, 2978.1 cm^{-1}, 1457.4 cm^{-1} and 734.4 cm^{-1} were evidence of the presence of aromatic compounds in chloroform fraction of leaf extract of *A. occidentale*. The weak bands around 2922.2 cm^{-1} and 1714.5 cm^{-1} were indicative of the presence of carboxylic acids, whereas the presence of alkoxyl groups (ethers) was represented by relatively medium bands around 1088.4 cm^{-1} and 1047.4 cm^{-1}. The presence of aliphatic compounds was indicative of medium bands around 87.885 cm^{-1} as well as the alkynes at 2184.2 cm^{-1} and 2124.6 cm^{-1}. The presence of methyl and methylene groups in chloroform fraction of leaf extract of *A. occidentale* was represented by peak values at 1379.1 cm^{-1} and 2855.1 cm^{-1} respectively.

3.2 FT-IR spectra of leaf extract of P. guajava

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

S. No	Peak/band (cm^{-1})	%T	Functional groups/assignment	Origin
1.	3391.9	94.351	Secondary amines N-H stretch	N-H
2.	3058.4	96.541	Aromatic compounds C-H stretch	-C-H
3.	2974.4	92.265	Carboxylic acid O-H stretch	O-H
4.	2892.4	96.384	Carboxylic acids O-H stretch	O-H
5.	1733.2	95.892	Carboxylic acids C-O bend	C=O
6.	1636.3	98.058	Alkenes sp2 C-C stretch	C=C
7.	1423.8	94.187	Alkanes sp3 C-C bend	C-C
8.	1267.3	60.487	Acyl C-O; phenol C-O stretch	C-OH
9.	1088.4	88.871	Alkoxy C-O stretch	X-O-C
10.	1047.4	75.523	Alkoxy C-O stretch	X-O-C
11.	879.7	89.799	Di-substituted alkenes C-H bend	=C-H
12.	730.8	12.882	Aromatic C-H bend	Ar-C-H

%T: Percentage Transmittance

The functional groups of phytocomponents present in chloroform fraction of leaf extract of *P. guajava* are illustrated in Figure 2 and summarized in Table 2. The relatively weak band around 3391.9 cm^{-1} was indicative of the presence of secondary amines in chloroform fraction of leaf extract of *P. guajava*. Furthermore, the presence of aromatic amines in chloroform fraction of leaf extract of *P. guajava* was indicative by weak bands around 3058.4 cm^{-1} and 730.8 cm^{-1}. Table 2 showed the presence of carboxylic acids, typified by the peak values at 2974.4 cm^{-1}, 2892.4 cm^{-1} and 1733.2 cm^{-1}. The relatively weak bands around 1636.3 cm^{-1}, 1423.8 cm^{-1} and 879.7 cm^{-1}, typified the presence of aliphatic compounds. Table 2 showed that chloroform fraction of leaf extract of *P. guajava* contained ethers

(1088.4 cm^{-1} and 1047.4 cm^{-1}), esters and phenolics (1267.3 cm^{-1}).

weak band around 3056.4 cm^{-1} but strong band around 1449.9 cm^{-1}, 879.7 cm^{-1} and 734.3 cm^{-1}. The peak values at 2974.4 cm^{-1}, 2892.4 cm^{-1}, 1640.0 cm^{-1} and 1420.1 cm^{-1} were

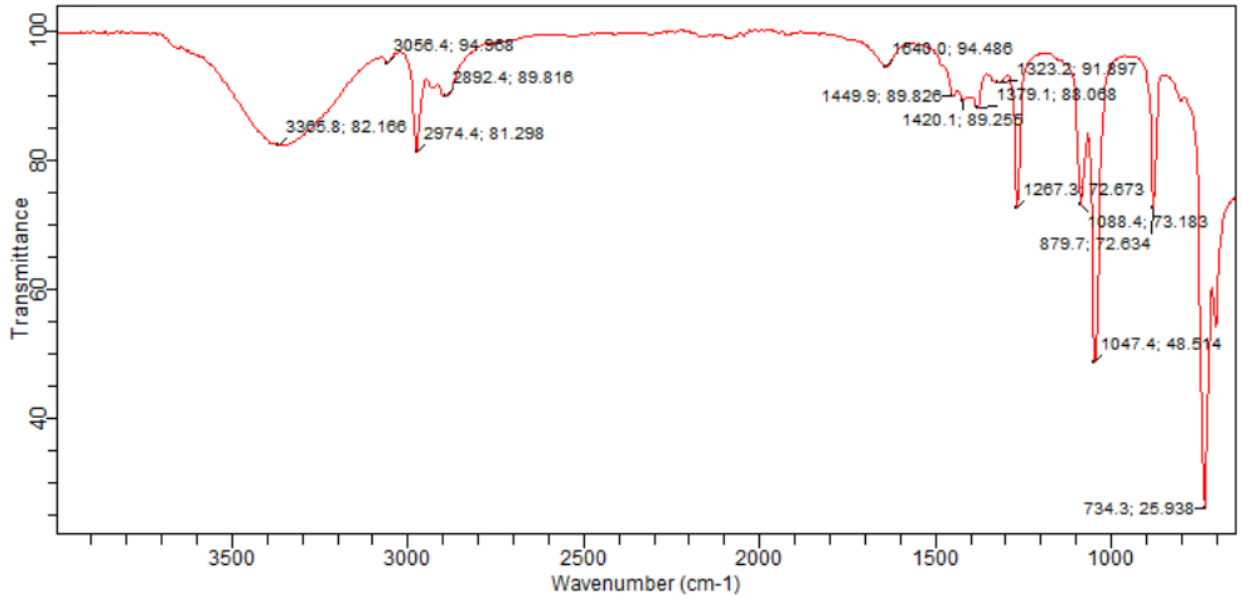

Figure 3. FT-IR spectra of chloroform fraction of leaf extract of *T. catappa*

3.3 FT-IR spectra of leaf extract of T. catappa

Table 3. Peak values of FT-IR spectra of chloroform fraction of leaf extract of *T. catappa*

S/No	Peak/band (cm^{-1})	%T	Functional groups/assignment	Origin
1.	3365.8	82.166	Alcohol O-H stretch	-O-H
2.	3056.4	94.968	Aromatic C-H stretch	Ar-C-H
.	2974.4	81.298	Alkanes C-H stretch	C-H
4.	2892.4	89.816	Alkanes C-H stretch	C-H
5.	1640.0	94.486	Alkenes C-C stretch	C=C
6.	1449.9	89.826	Aromatic compounds C-C stretch	C=C
7.	1420.1	89.255	Alkanes sp3 C-H stretch	C-H
8.	1379.1	88.068	Nitro compounds NO_2 stretch	-N=O
9.	1323.2	91.897	Nitro compounds NO_2 stretch	-N=O
10.	1267.3	72.673	Acyl C-O; Phenol C-O stretch	C-OH
11.	1088.4	73.183	Alkoxyl C-O stretch	X-O-C
12.	1047.4	48.514	Alkoxyl C-O stretch	X-O-C
13.	879.7	72.183	Aromatic sp2 C-H bend	Ar-C-H
14.	734.3	25.938	Aromatic sp2 C-H bend	Ar-C-H

%T: Percentage Transmittance

Figure 3 illustrated the FT-IR spectra of chloroform fraction of leaf extract of *T. catappa* and summarized in Table 3. The presence of alcohol in of chloroform fraction of leaf extract of *T. catappa* was typified by a broad band around 3356.4 cm^{-1}. Aromatic compounds gave relatively indicative of the presence of aliphatic compounds in chloroform fraction of leaf extract of *T. catappa*. Other phytocomponents present in chloroform fraction of leaf extract of *T. catappa* were ethers (1088.4 cm^{-1} and 1047.4 cm^{-1}) and nitro compounds (1379.1 cm^{-1} and 1323.2 cm^{-1}). The presence of phenolics and esters were typified by peak value at 1267.3 cm^{-1}.

Previous reports have established the fact that the phytochemical profile of herbal extracts depends on age, species and genetic constitution as well as growth conditions, geographical location, soil chemistry and seasonal period of the harvest of the plant materials [31-33]. A general profile of the phytocomponents from chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa* indicated the presence of alcohols, esters, aromatic compounds, secondary amines, carboxylic acids, nitro compounds and aliphatic compounds. In agreement with the present findings, previous studies showed that chloroform fractions of plant materials, for the most part, are composed of fatty acids, esters, waxes and terpenoids [27, 34, 35]. The low polarity of chloroform dictated the solubility characteristics of phytocomponents from fractionated leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa*, which to a large extent were hydrophobic molecular species. According to previous reports, the polarities of solvents used for partitioning protocols had a bearing on phytochemical profiles of fractionated herbal extracts [27, 33, 36]. A related study showed that *n*-hexane and dichloromethane fractions of leaf extract of *T. catappa* yielded hydrophobic and volatile phytocomponents such as fatty acids and esters [37] due to relative low polarities of the solvents.

Comparative FT-IR spectra analysis of the present study revealed that the presence of molecular species such as ethers, aromatic compounds, esters and aliphatic

compounds were mutual to chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa*. On the contrary, phenolics and acyl compounds were exclusive to chloroform fractions of leaf extracts of *A. occidentale* and *P. guajava*, whereas nitro compounds appeared to be distinctive to chloroform fraction of leaf extract of *T. catappa.*

4 Conclusion

A general outlook of the present study revealed peculiarities in phytocomponent profiles of chloroform fractions of the leaf extracts, which depended on the polarity of the fractionation solvent and plant species variations. The FT-IR spectra analysis outcome of the present investigation was not exhaustive. Further spectroscopic studies are recommended in order to establish the structural/molecular identities of the phytocomponents from chloroform fractions of leaf extracts of *A. occidentale*, *P. guajava* and *T. catappa*.

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Competing interests

The authors declare that they have no competing interests.

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