Original Research Article

Comparative Profiling of Solvent-mediated Phytochemical Expressions in *Ocimum gratissimum* and *Vernonia amygdalina* Leaf Tissues via FTIR Spectroscopy and Colorimetric Assays

ABSTRACT

Objective: The beneficial role of extraction solvents is often ignored, yet very important in enhancing the therapeutic potential of plant extracts. This study was carried out to comparatively characterize and profile the bioactive phytochemical compounds expressed in different solvent-fractions of *Ocimum gratissimum* and *Vernonia amygdalina* leaf extracts using both colorimetric phytochemical screening assays and Fourier transform infrared (FTIR) spectroscopy.

Methods: Qualitative colorimetric assays were carried out on different solvent-fractions of leaf tissue extracts from both plants to determine the comparative expression profiles of bioactive phytochemical compounds with medicinal importance such as alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, tannins, terpenoids, saponins, and reducing sugars. FTIR spectroscopy was used to characterize, and profile the presence of these compounds based on functional groups such as alcohols (O-H), saturated hydrocarbons (C-H), aliphatic fluoro (C-F), bromo (C-Br) and chloro (C-Cl) compounds, organic sulfates (S=O), esters, ethers, carboxylic acids (C-O), aromatic amines, methane nitriles (C-N), ketones, aldehydes, quinones (C=O), sulphur compounds (C=S), primary and secondary amines (N-H) with bioactive properties in the different solvent-fractions.

Result: Data were generated for methanol, n-hexane, ethyl-acetate, n-butanol and aqueous solvent-fractions of *Ocimum gratissimum* and *Vernonia amygdalina* leaf extracts. We have generated solvent-mediated phytochemical expression profiles for leaf tissue extracts of both plants based on the phytochemistry of their secondary metabolites. The methanolic solvent-fraction expressed the most phytochemicals in both plants.

Conclusion: This study has revitalized the importance of extraction solvents in optimizing phytochemical bioavailability in plant tissues. This may be responsible for variation in medicinal and biological activities reported in prior studies.

Keywords: Phytochemical; Fourier transform infrared spectroscopy; Phytochemistry; *Ocimum gratissimum*; *Vernonia amygdalina;* Plant Extracts.

1. INTRODUCTION

The significance of plants and plant-based products as daily necessities cannot be overemphasized. Reports from the World Health Organization (W.H.O) database suggest that about 80% of the world's population utilize plants and plant-based products for food and health or medicinal purposes every day [1]. Various types of plants have been used traditionally as medicinal alternatives due to known pharmacological and biological activities in animals and humans. Medicinal plants that contain bioactive compounds such as lactones, saponins, glycoalkaloids, alkyl phenol, flavonoids, terpenoids, phlobatannins, glycosides, and tannins have been used over the years for the treatment of various ailments and have been the source of many of the currently available chemotherapeutic drugs [2]. The medicinal properties of these plants emanate from the biochemical activities of the bioactive secondary metabolites (also known as phytochemical compounds) that were originally produced to defend these plants against pathogens, predators, harsh environmental conditions, and for normal homeostatic or physiological

functions [3]. Plant-based products are not only useful for medicinal purposes but can also be used for flavoring, ornamenting, and as preservatives or food additives [4].

Ocimum gratissimum, commonly known as Scent leaf or Clove basil, is an erect perennial wood shrub plant that can grow up to 2.5m high [5], with leaves of about 5-13cm long and 3-9cm wide [6]. It is a herbal plant which belongs to the *Labiatae* family and is found in tropical areas such as India and West Africa [7]. In Nigeria, it is locally called 'Efinrin', 'Ahuji' and 'Daidoya' among the Yoruba, Ibo, and Hausa speaking tribes, respectively [8, 9, 10]. *Ocimum gratissimum* has been shown to have a wide range of medicinal potentials and various parts of the plant have been used traditionally for the treatment of diverse ailments. For example, the leaves are often used for the treatment of stomach upsets, hemorrhoids, diarrhea and mental illness [11, 12] among locals in Nigeria. The flowers and leaves are used in the preparation of teas and infusions due to their richness in essential oils [13]. Decoctions of the roots are used as a sedative for children in Brazil [14]. The whole plant has been used in some parts of India for the treatment of a headache, sunstroke, and influenza [15, 16, 17]. In addition, the plant has many other pharmacological properties and has been used for inhibition or amelioration of convulsion [18], diarrhea [19], and fungal infection [16]. It also has anthelminthic [20] and anti-mutagenic [21] activities.

Vernonia amygdalina, a perennial shrub with a height of 2-5m, belongs to the family Asteraceae and its commonly called 'Bitter leaf' due to its bitter taste [22]. It grows predominantly in tropical Africa and in Nigeria, it's locally called 'Ewuro' in the Yoruba language, 'Onugbu' in the Igbo language, and 'Chusar-Doki' in the Hausa language [23]. Traditionally, the twigs and roots of *Vernonia amygdalina* are used for the treatment of a headache, stomach ache, and gastrointestinal problems in northern Nigeria [5] and the stem barks as chewing sticks for oral hygiene and dental problems in most parts of Nigeria and West African countries [22]. The roots are reportedly used for treating fertility problems, toothache, and malaria [24]. The aqueous extract from the leaves is often recommended by herbalist for the treatment of various ailments some of which includes scabies, diabetes, fever, nausea, loss of appetite, dysentery and sexually transmitted diseases [22, 23, 25]. *Vernonia amygdalina* have been reported to have a lot of other pharmacological properties and has been used for treating antibacterial [26], antimalarial [27], anti-leishmanial [28], anthelminthic [5], and hypoglycemic [29, 30] conditions. In addition, it has hepatoprotective [31], nephroprotective [29], oxytocic [32], phytotoxic [33], antioxidant [34], and analgesic [35] properties.

The medicinal potential of plants and their tissue extracts cannot be completely ascertained without determining the expression profiles of bioactive phytochemical compounds present in them. A wide range of techniques has been used to identify and analyze the psychochemical properties and biochemical composition of these compounds including colorimetric phytochemical assays and FTIR spectroscopy. Colorimetric phytochemical screening assays are based on the ability of certain chemical reagents to react with compounds within an extract to determine the presence of a secondary metabolite or phytochemical in each plant tissue extract by color indication. FTIR spectroscopy is a rapid, essential and inexpensive analytical technique used for the identification of unknown compounds in an extract by characterization of the bioactive functional groups in the compound in comparison to a library of known compounds with similar functional groups [36, 37]. It has been used for fingerprinting herbal extracts or compounds for determining their bioactive constituent and physicochemical/biochemical functions [38, 39].

The present study was undertaken to comparatively characterize the efficiency of different extraction solvents in extracting and expressing the different phytochemicals from *Ocimum gratissimum* and *Vernonia amygdalina* leaf tissues using phytochemical colorimetric assays and Fourier transform infrared (FTIR) spectroscopy. FTIR and colorimetric assay data were then analyzed to generate a metabolomic expression profile to describe the modifying physicochemical effect of each extraction solvent on bioavailable phytochemicals in each plant leaf tissue and their medicinal importance. For this study, we hypothesized that extraction solvents can extrinsically modulate the phytochemical metabolomic

expression profiles of *Ocimum gratissimum* and *Vernonia amygdalina leaf* tissue extracts and that the choice of extraction solvents is responsible for the disparities in the reported medicinal effects of *Ocimum gratissimum* and *Vernonia amygdalina* tissue extracts in prior literature.

2. MATERIALS AND METHODS

2.1 Sourcing, Collection, and Identification of Plants

Fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were handpicked from two local farms located in Abeokuta, Southwest, Nigeria. Identification was done at the Department of Botany, University of Lagos, Nigeria, and further validated and morphologically characterized using the plant catalog on http://theplantlist.org database.

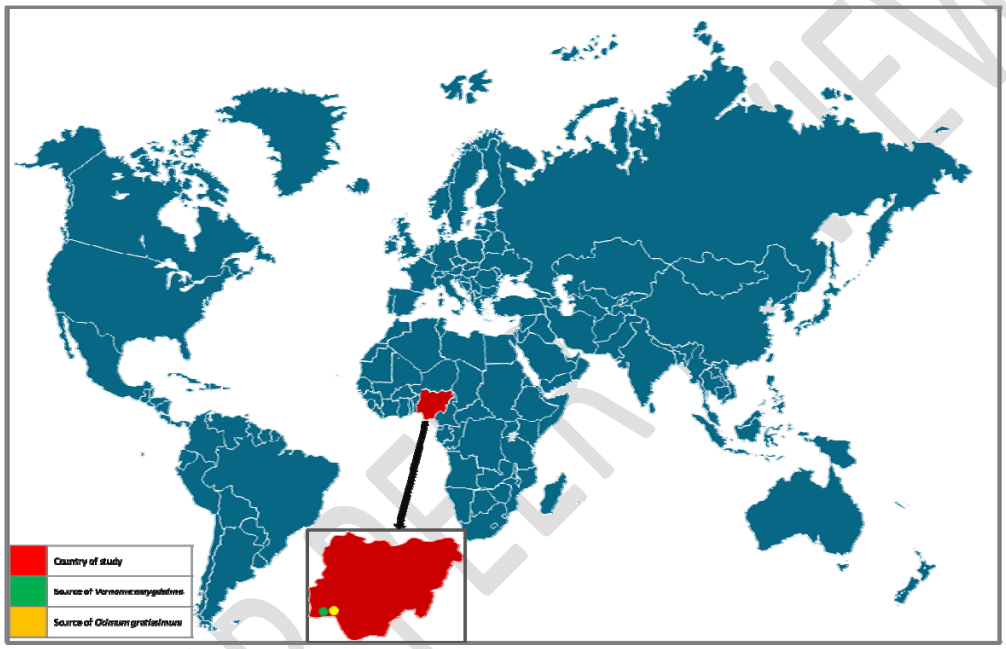

Figure 1: Map showing the geographical location of the country (Nigeria) where the study was carried out and site where *Vernonia amygdalina* and *Ocimum gratissimum* leaves were sourced for this study.

2.2 Chemicals and Reagents

Reagents for colorimetric phytochemical screening and solvent fractionation including all organic and non-organic solvents were purchased (Sigma Aldrich, USA). FTIR grade of Potassium Bromate (Sigma Aldrich, USA) was also purchased for this study.

2.3 Extraction and Fractionation

The *Ocimum gratissimum* and *Vernonia amygdalina* leaves were washed, shade-dried for two weeks and pulverized into a coarse powder with the aid of an electric blender. About 500g of the coarse powder from each leaf extracts were collected and macerated in 80 % methanol at 4:1 ratio (methanol: distilled water) for 21 days. The solubilized crude methanolic leaf extracts of *Ocimum gratissimum* and *Vernonia amygdalina* were thereafter filtered and concentrated using a rotary vacuum evaporator at 40°C. 20g of each dried crude methanolic extracts was suspended in 200 ml of distilled water separately and partitioned in succession with solvents with varying polarities ranging from n-hexane, ethyl acetate, n-butanol and distilled water using a separating funnel.

2.4 Colorimetric Phytochemical Screening Analyses

Phytochemical analysis was done using standard methods [40, 41, 82, 83, and 84] to identify baseline and commonly screened bioactive phytochemical compounds in the leaf tissue extracts of *Ocimum gratissimum* and *Vernonia amygdalina*, such as alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, reducing sugars, saponins, tannins, and terpenoids. Briefly, the following colorimetric screening assays were performed;

Alkaloid assay: Dragendorff's test, Wagner's test, and Mayer's test were done [82, 83, and 84].

Anthraquinone assay: This was assessed using Borntrager's test [83].

Cardiac glycoside assay: Lieberman's, Keller-Kiliani's, Legal's, and Keddes' tests were done [83, 84].

Flavonoid assay: Lead acetate, Ferric chloride, and Sodium hydroxide tests were done [82, 83, and 84].

Phlobatannin assay: Hydrochloric acid (1%) test was done for this purpose [82].

Reducing sugar compounds assay: Benedict's, Fehling's, Barfoed's, Resorcinol, and Phloroglucinol tests were carried out [82, 83, and 84].

Saponin assay: Frothing, Emulsion, TLC, and hemolysis test were done [82, 83, and 84].

Tannin assay: Ferric chloride test was done for this assessment [82, 83].

Terpenoids assay: Salkowski's test was done to determine the presence of terpenoids. [82].

2.5 Spectroscopic Analyses

Dried extracts (2 mg) of *Ocimum gratissimum* and *Vernonia amygdalina* from each of the organic solvent fractions above were mixed with 100 mg of dried potassium bromide (KBr, FTIR grade). The solvent-based mixtures were then compressed into tiny pellets and loaded into the sample holder of a Shimadzu FTIR spectrometer. The extracts were scanned in wavelengths ranging from 4000 – 400cm^{-1} with a resolution of 4cm^{-1}.

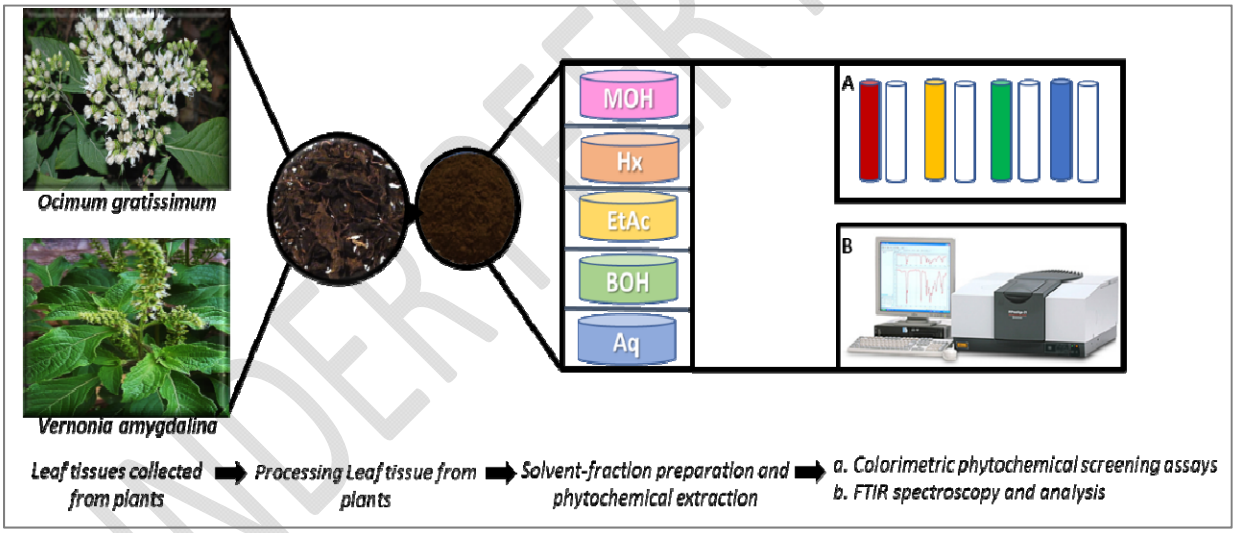

Figure 2: Diagrammatical illustration of an experimental methodology for this study. The two medicinal plants (*Vernonia amygdalina* and *Ocimum gratissimum*) were used as a model for this study. Their leaves were harvested, dried, processed, and differentially fractionated in various organic solvents to determine differences in phytochemical expression profiles via both phytochemical screening assays and FTIR spectroscopy methods. Organic Solvents: MOH: Methanol; Hx: Hexane; EtAc: Ethyl acetate; BOH: Butanol; Aq: Aqueous distilled water.

2.6 Statistical Analyses

All data were expressed as mean ± standard error of means (SEM). Data were also statistically analyzed using Student t-test analysis to test the level of significance of solvent-induced percentage total yield. The differences were considered statistically significant at $P < 0.05$. Graphs were plotted using Microsoft Excel Program and Heatmaps were manually plotted.

3. RESULTS

3.1 Extraction and Fractionation

After extraction, the crude methanolic extracts of the leaves *Ocimum gratissimum* and *Vernonia amygdalina* yielded 97.96g (19.59%) and 88.82g (17.76%). The water or aqueous fractions of both plants yielded the highest percentage of 28.20% for *Ocimum gratissimum* and 25% for *Vernonia amygdalina.*

Screened Compounds	Colorimetric tests	Observations	Inferences	References

While the n-butanol fraction gave the lowest yield of 12.55% and 15.13%, respectively (Table 1).

Table 1: Percentage total yield of different solvent-fractions of *Ocimum gratissimum Vernonia amygdalina* leaf extracts.

Plant leaf extracts	Solvent-fractions	Extract yield (g)	Percentage yield (%)
20g of Crude methanolic extract of *Ocimum gratissimum*	n-Hexane	1.74±0.06	8.86±0.16
	Ethyl Acetate	3.06±0.20	15.28±0.10
	n-Butanol	2.51±0.10	12.55±0.24
	Aqueous	5.63±0.31	28.20±0.77
20g of Crude methanolic extract of *Vernonia Amygdalina*	n-Hexane	1.95±0.04	9.73±0.10
	Ethyl Acetate	3.89±0.70	19.48±0.24
	n-Butanol	3.03 ±0.48	15.13±0.13
	Aqueous	5.17±0.51	25.85±0.31

Data points represent mean ± SEM values of extract yield in grams and percentage yield in percentage.

3.2 Phytochemical Screening Assays

The phytochemical screening of the leaf extracts of *Ocimum gratissimum* and *Vernonia amygdalina* in various solvent-fractions (methanol, hexane, ethyl acetate, butanol and aqueous) was carried out (Table 2). The results of the preliminary phytochemical analysis showed that there were various types of phytochemicals present in the various solvent-fractions of the leaves which are of medicinal value (Figure 3). The methanolic, n-hexane and ethyl-acetate solvent-fractions of *Ocimum gratissimum* have alkaloids, anthraquinones, phlobatannins, and terpenoids in common, but butanol and aqueous solvent fractions have lesser number of phytochemical compounds in them, revealing the presence of cardiac glycosides, flavonoids, and tannins. Saponins and reducing sugars were absent in all the solvent fractions of *Ocimum gratissimum*. While the methanolic, n-hexane and ethyl acetate solvent-fractions of *Vernonia amygdalina* showed the presence of alkaloids, flavonoids, terpenoids, and saponins. The butanol and aqueous fractions have a lesser number of phytochemical constituents compared to others revealing the presence of cardiac glycosides and flavonoids and tannins. Phlobatannins were absent in all solvent-fractions of *Vernonia amygdalina* (Figure 3).

Table 2: Major Phytochemical compounds identified in *Ocimum gratissimum and Vernonia amygdalina* leaf tissue extracts in this study and methods of colorimetric screening assay analysis

Alkaloids	Dragendorff's test	Cloudy orange precipitate	Alkaloid present	[82], [83], [84]
	Wagner's test	Dark brown precipitate	Alkaloid present	
	Mayer's test	Yellow colored precipitate	Alkaloid present	
	Dragendorff's confirmatory test on Thin-Layer Chromatography	Dark color spotted on TLC	Alkaloid confirmed	
Anthraquinones	Borntrager's test	A rose-pink to a red-colored precipitate	Free anthraquinone (glycosides) present	[82], [84]
	Combined anthraquinone test	A pink-red colored precipitate	Anthraquinone derivatives present	
Cardiac glycosides	Legal's test	A deep red color that fades to brownish yellow	Cardenolides present	[82], [83], [84]
	Keddes' test	A violet color that fades to brownish yellow	Lactone ring of Cardenolides present	
	Lieberman's test	A violet colored precipitate	Steroidal nucleus present	
	Keller-Kiliani's test	A brownish ring at the interface and violet ring below.	Steroid ring of glycosides present.	
Flavonoids	Ferric chloride test	A wooly light brown precipitate	Phenolic nucleus present	[83]
	Lead acetate test	Dirty brownish precipitate	Flavonoids present	
	Sodium hydroxide test	Golden yellow precipitate	Flavonoids present	
Phlobatannins	1% HCl + Extract	Reddish precipitate	Phlobatannins present	[82]
Reducing sugar compounds	Fehling test	Deep blue-green color appears	Hexose sugar present	[82], [83], 84]
	Barfoed's test	Red precipitate	Monosaccharides present	
	Resorcinol test	Deep yellow precipitate	Keto- sugar confirmed	
	Phloroglucinol test	Reddish yellow precipitate	Pento- sugar present	
	Benedict's test	Reddish brown precipitate	Reducing sugar present	
Saponins	Frothing test	A foam that persists after 15 minutes	Saponins present	[82], [83], [84]
	Emulsion test	A stable emulsion obtained	Saponins present	
	Thin-Layer Chromatography test	A yellow color precipitate spotted on TLC	Saponin confirmed	
	Hemolysis test	Hemolysis in tubes with extract	Saponins confirmed	
Tannins	1ml Ferric Chloride + Extract	A black or blue green colored precipitate	Tannin present	[82], [83]
Terpenoids	Salkowski's test	A light turbid red-brown color	Terpenoids present	[82]

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	Vernonia amygdalina					*Ocimum gratissimum*				
	MOH	Hex	EtAc	BOH	Aq	MOH	Hex	EtAc	BOH	Aq
Alkaloids										
Anthraquinones										
Cardiac Glycosides										
Flavonoids										
Phlobatannins										
Reducing Sugars										
Saponin										
Tannins										
Terpenoids										

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+++	++	+	–
High	Moderate	Low	None

Figure 3: Heatmap showing solvent-mediated phytochemical expression profiles for *Vernonia amygdalina* and *Ocimum gratissimum* leaf tissue extracts. Solvent-fractions: MOH: Methanol; Hx: Hexane; EtAc: Ethyl acetate; BOH: Butanol; and Aq: Aqueous distilled water.

3.3 FTIR Spectra Analyses

Depending on the peak values in the region of infrared radiation (IR), the FTIR spectra obtained were used to identify and characterize the functional groups of the bioactive compounds present in each solvent-fractions of the plant leaf extracts of *Ocimum gratissimum* and *Vernonia amygdalina* (Figure 4 & 5).

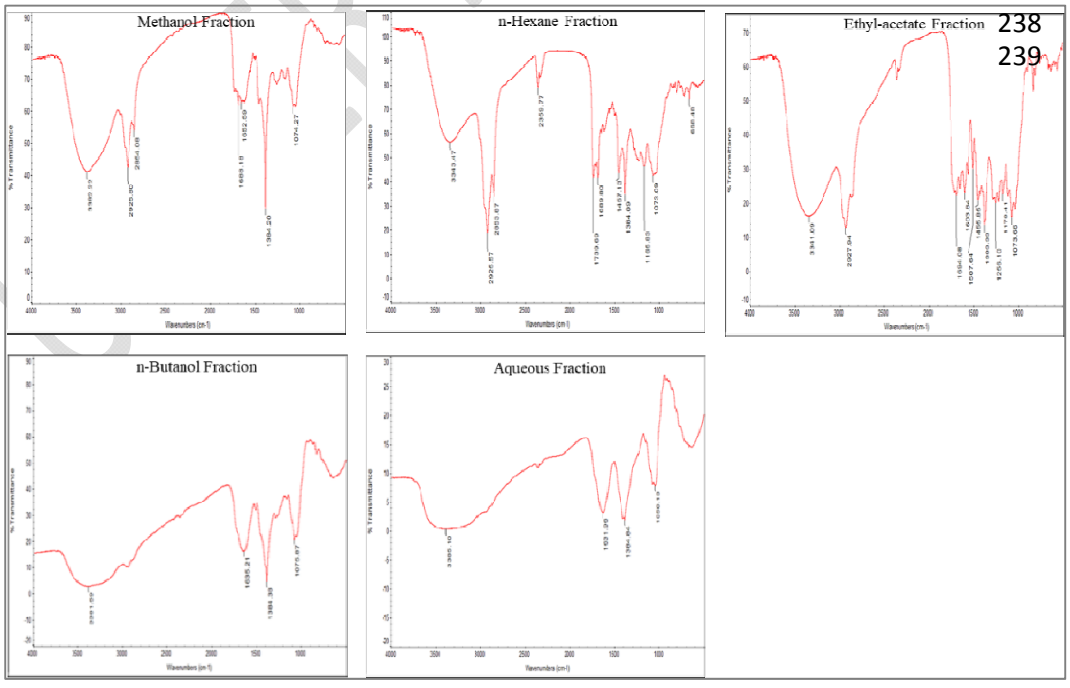


Figure 4: FTIR spectra of

methanolic, n-hexane, ethyl-acetate, n-butanol, and aqueous solvent-fractions of *Ocimum gratissimum* leaf extracts.

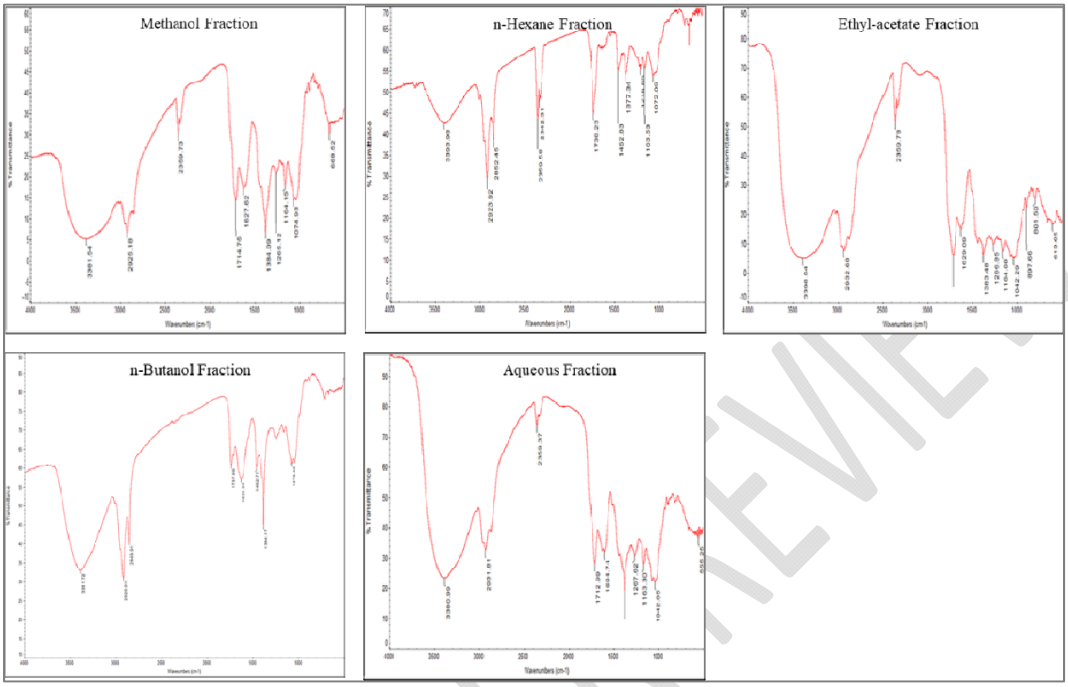


Figure 5: FTIR spectra of methanolic, n-hexane, ethyl-acetate, n-butanol, and aqueous solvent-fractions of *Vernonia amygdalina* leaf tissue extracts.

3.3.1 FTIR Spectral Data Interpretation for *Ocimum gratissimum*

The FTIR analysis for the various solvent-fractions of *Ocimum gratissimum* leaf tissue extracts can be seen in Table 3 and Figure 6. The functional groups identified are summarized in Appendix 1.

3.3.1.1 Spectral Analysis of the Methanolic Extract

The IR spectrum of the crude extract methanolic extract of *Ocimum gratissimum* revealed seven (7) major peaks at the range of 3382.2 – 1074.3 cm^{-1}. The broad peak at 3382.2 cm^{-1} was due to O-H stretching vibrations from a hydroxyl group, which is an important component of many phenolic phytochemical compounds such as flavonoids, phenolic acids and polyphenols [42, 43]. Peaks at 2925.8 cm^{-1} and 2854.1 cm^{-1} are absorptions common to lipophilic phytochemicals [44] and were assigned to C-H asymmetric and symmetric stretching vibrations from hydrocarbons (CH_3 and CH_2 groups) [45, 46]. The peak at 1688.2 cm^{-1} was assigned to C=O stretching vibrations from quinones, which could be due to the presence of anthraquinones [47]. The peak at 1652.6 cm^{-1} can be assigned to C=C stretching vibrations from aromatic ring deformations [48], N-H bending vibrations from aromatic primary amines, and C=O stretching vibrations from amides and carboxylic acids [49], which suggests the presence of amino acids, flavonoids or volatile oils [49, 50, 51]. The peak at 1384.2 cm^{-1} can be assigned to C-H bending from hydrocarbons (CH_3 groups) [49], S=O stretching from organic sulfates [52] and C-O stretch from carboxylic acids likely to be present in the extract [53]. The peak at 1074.3 cm^{-1} reveals the presence of C-F stretch from aliphatic fluoro compounds, C-N stretch from aliphatic primary amines [54] or C-O-C stretch from alkyl-substituted or cyclic ethers [55]. The C-O-C stretching vibrations suggest the presence of monoterpenes (monocyclic or bicyclic) [56] or polysaccharides [54].

3.3.1.2 Spectral Analysis of the n-Hexane Fraction

The peaks at 3343.5 cm^{-1}, 2925.6 cm^{-1}, 2853.9 cm^{-1}, 1689.80 cm^{-1}, 1384.1 cm^{-1} and 1073.1 cm^{-1} were due to the presence of O-H, C-H, C=O, C-H/S=O/C-O, and C-F/C-N/C-O-C functional groups coming from hydroxyl, hydrocarbons (CH_3, CH_2), quinones, organic sulfates, carboxylic acids, aliphatic fluoro compounds, aliphatic primary amines and ethers respectively. The C=O stretching vibration at 1689.80 cm^{-1} suggests the presence of flavonoids [50, 51] or anthraquinones [47] and the C-O-C stretching vibration at 1073.1 cm^{-1} can be attributed to the presence of monoterpenes (monocyclic or bicyclic) [56] or polysaccharides [54] in the leaf extract. The peak at 2359.8 cm^{-1} is likely due to O-H asymmetric stretching vibrations from free OH groups [57], which are an important component of the majority of phenolic phytochemicals such as flavonoids, phenolic acids and polyphenols [42, 43]. The peak at 1739.7 cm^{-1} belongs to C=O stretching vibrations which could come from saturated aliphatic aldehydes or esters [42, 58] and suggests the presence of phospholipids and volatile oils [59, 60]. The peak at 1457.1 cm^{-1} can be assigned to C-H bending vibrations from hydrocarbons (CH_3 groups) and –C=C- stretching vibrations from aromatic rings which could be due to the presence of lipophilic compounds [44, 53] and aromatic compounds respectively [50, 61]. The peak at 1165.8 cm^{-1} belongs to C-N stretching vibrations from aliphatic amines and C-O stretching vibrations from alcohols, carboxylic acids or esters [62]. The C-O stretching vibration suggests the presence of polysaccharides and starch in the extract [63]. The peak at 668.5 cm^{-1} is due to C-Br stretching vibrations from aliphatic bromo compounds, C-H bends vibrations from alkynes or C-S stretching vibrations from disulfides [42].

3.3.1.3 Spectral Analysis of the Ethyl Acetate Fraction

The IR spectrum of the ethyl acetate solvent-fraction revealed ten major peaks within the range of 3341.1 – 1073.7 cm^{-1}. The peaks include 3341.1, 2924.9, 1455.9, 1384.2cm^{-1}, 1178.4 $cm^{-1}$and 1073.7cm^{-1}, which resemble some of the peaks found in the methanolic and n-hexane solvent-fractions. These can be attributed to the presence of O-H, C-H, C-H/S=O/C-O, C-H, C-N/C-O, and C-F/C-N/C-O-C functional groups from hydroxyl groups, hydrocarbons (CH_3, CH_2), organic sulfates, carboxylic acids, saturated aliphatic aldehydes, esters or carboxylic acids, aliphatic fluoro compounds, aliphatic primary amines, and ethers, respectively. The C-O stretching vibration from 1178.4 cm^{-1} suggests the presence of polysaccharides and starch [63]. The C-O-C stretching vibrations of 1073.7cm^{-1} suggests the presence of bicyclic monoterpenes [56] or polysaccharides [54]. The peak at 1694.1 cm^{-1} belongs to C=O stretching vibrations which can be assigned to α, β unsaturated aldehydes, ketones, or carboxylic acids while the peak at 1603.8 cm^{-1} can be assigned to C=O stretching vibrations from carboxylic acid salt or quinones and N-H bending vibrations from primary amines [42, 58]. The C=O stretching vibrations from both peaks (1694.1 cm^{-1} and 1603.8 cm^{-1}) suggest the presence of flavonoids [50, 51] or anthraquinones [47]. The peak at 1507.07 cm^{-1} belongs to N-O stretching vibrations from aromatic nitro- compounds (hetero-aromatic compounds) and C=C from aromatic rings indicating the presence of flavonoids and other aromatic compounds [49]. The peak at 1256.1 cm^{-1} corresponds to C-O stretching vibration from alcohols and esters which suggests the presence of lipids in volatile oils [60] or polyols like hydroxyl flavonoids [61].

3.3.1.4 Spectral Analysis of the Butanol Fraction

The IR spectrum of the butanol solvent-fraction is made up of only four peaks within the range of 3381.6 cm^{-1} – 1075.9 cm^{-1}. The peaks include 3381.6, 1635.2, 1384.4, 1075.9 cm^{-1} corresponding to the presence of O-H, N-H/C=C/C=O, C-H/S=O/C-O and C-F/C-N/C-O-C functional groups from hydroxyl groups, primary amines, aromatic rings, amides, saturated hydrocarbons (alkanes), organic sulfates, carboxylic acids, aliphatic fluoro compounds, aliphatic primary amines, and ethers, respectively. The peaks at 1635.2 cm^{-1} can be attributed to the presence of amino acids, flavonoids or volatile oils while the peak at 1075.9 cm^{-1}, a C-O-C stretching vibration may be due to the presence of alkyl-substituted ethers [55] such as monoterpenes (monocyclic or bicyclic) [56] or polysaccharides [54].

3.3.1.5 Spectral Analysis of the Aqueous Fraction

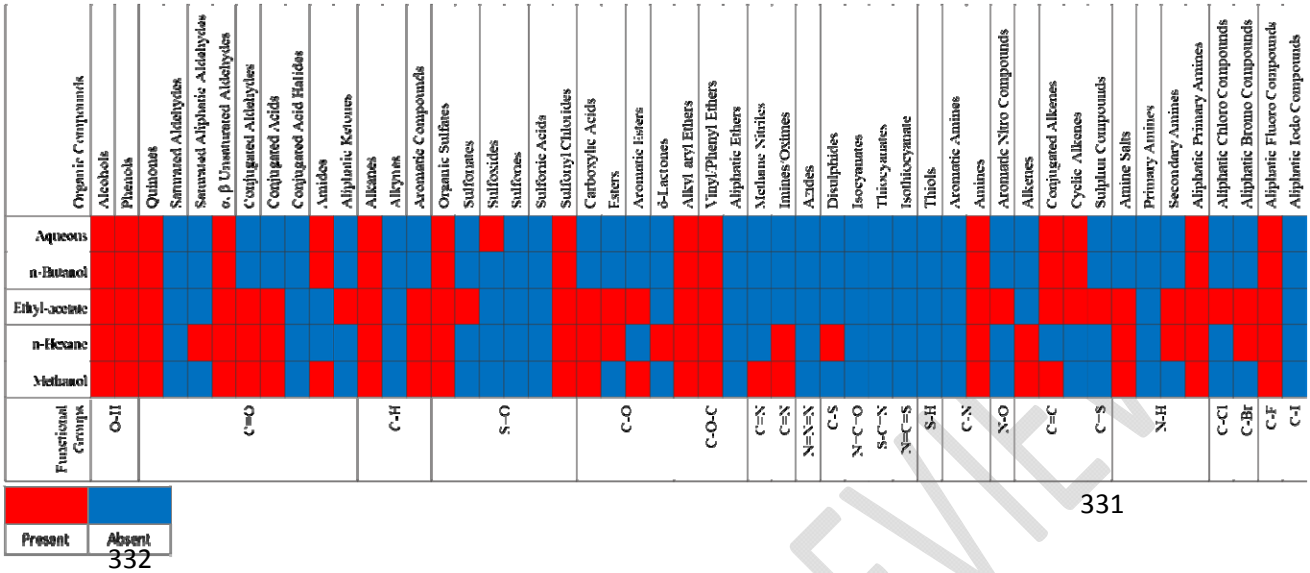
The IR spectrum of the aqueous fraction has four major peaks within the range of 3385.1 cm^{-1} – 1050.1 cm^{-1}, which resembles the peaks observed in the butanol fraction of the leaf extract. The peaks 3385.1,

1631.9, 1384.8 and 1050.1 cm^{-1} belong to O-H, N-H/C=C/C=O, C-H/S=O/C-O and C-F/C-N/C-O-C functional groups from hydroxyl groups, primary amines, aromatic rings, amides, saturated hydrocarbons (alkanes), organic sulfates, carboxylic acids, aliphatic fluoro- compounds, aliphatic primary amines, and ethers, respectively. The peaks at 1631.9 cm^{-1} can be attributed to the presence of amino acids, flavonoids, or volatile oils while the peak 1050.1 cm^{-1}, a C-O-C stretching vibration may be due to the presence of alkyl-substituted ethers [55] such as monoterpenes (monocyclic or bicyclic) [56] or polysaccharides [54].

Table 3: List of probable phytochemicals present in each solvent-fraction of *Ocimum gratissimum*

Solvent fractions	Base group and vibration mode	Possible phytochemical compounds present in leaf extracts
MOH	3382.2 v (O-H), 2925.8 v_{as}(C-H), 2854.1 v_s (C-H), 1688.2 v(C=O), 1652.6 δ(N-H), v(C=C), v(C=O), 1074.3 v_s(C-O-C)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic phytochemicals, anthraquinones, amino acids, volatile oils, sulfur, carboxylic acid, and halogen-containing phytochemicals, monoterpenes (monocyclic or bicyclic), polysaccharides etc.
Hx	3343.5 v (O-H), 2925.6 v_{as}(C-H), 2853.9 v_s(C-H), 1739.7 v_s(C=O), 1688.8 v(C=O), 1457.1δ(C-H), v_{rf}(ar), 1165.8 v(C-O), 1073.1 v_s(C-O-C), 668.5 v(C-Br)	Phenolic compounds (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic compounds, sulphur, carboxylic acids and halogen containing phytochemicals, phospholipids, volatile oils, anthraquinones, polysaccharides, starch, monoterpenes (monocyclic or bicyclic), etc.
EtAc	3341.1 v (O-H), 2924.9 v_{as}(C-H), 1694.1 v_s(C=O), 1603.8 v_s(C=O), 1507.1 v_{rf}(ar), v_{rf}(ha), 1455.9 δ(C-H), v_{rf}(ar), 1256.1 v_s(C-O), 1178.4 v(C-O), 1073.7 v_s(C-O-C)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic phytochemicals, carbonyl containing phytochemicals, sulphur, carboxylic acid and halogen containing phytochemicals, anthraquinones, polyols, polysaccharides, monoterpenes (monocyclic or bicyclic), starch, etc.
BOH	3381.6 v (O-H), 1635.2 δ(N-H), v(C=C), v(C=O), 1075.9 v_s(C-O-C), v_s(C-F)	Phenolic compounds (flavonoids, phenolic acids, tannins, or polyphenols), amino acids, volatile oils, sulfur, carboxylic acid, and halogen-containing phytochemicals, monoterpenes (monocyclic or bicyclic), polysaccharides, etc.
Aq	3385.1 v (O-H), 1631.9 δ(N-H), v(C=C), v(C=O), 1050.1 v_s(C-O-C), v_s(C-F)	Phenolic compounds (flavonoids, phenolic acids, tannins, or polyphenols), amino acids, volatile oils, sulfur, carboxylic acid, and halogen-containing phytochemicals, monoterpenes (monocyclic or bicyclic), polysaccharides, etc.

Footnote: v: Stretching vibration; δ: Bending; v_s: Symmetrical; v_{as}: Asymmetrical; ar: Aromatic; ha: Heteroaromatic; v_{rf}: Ring frame. MOH: Methanol; Hx: Hexane; EtAc: Ethyl acetate; BOH: Butanol; and Aq: Aqueous distilled water.



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333 Figure 6. Secondary metabolite/phytochemical expression profile of different solvent-fractions of
334 *Ocimum gratissimum* leaf tissue extract. Heatmap represents the relative expression profile of secondary
335 metabolites/ phytochemical compounds expressed in leaf tissue of different organic solvent-fractions
336 analyzed with FTIR spectroscopy based on functional group determination.

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338 ### 3.3.2 FTIR Spectral Data Interpretation for *Vernonia amygdalina*

339 The FTIR analysis for the various solvent-fractions of *Vernonia amygdalina* can be seen in Table 4 and
340 Figure 7. The functional groups identified are summarized in Appendix 1.

341 #### 3.3.2.1 Spectral Analysis of the Crude Methanolic Extract

342 The IR spectrum of the methanolic extract of *Vernonia amygdalina* revealed ten (10) major peaks within
343 the range of 3381.6 cm^{-1} – 665 cm^{-1}. The broad peak at 3381.6 cm^{-1} belongs to O-H stretching vibrations
344 from a hydroxyl group, which is an integral component of the majority of phenolic phytochemicals such
345 as flavonoids, phenolic acids and polyphenols [42, 43]. The peak at 2925.2 cm^{-1} belongs to C-H
346 asymmetric/symmetric stretching vibrations from CH_3, CH_2 groups [45, 46], which is common to
347 lipophilic phytochemicals [44]. The peak at 2359.7 cm^{-1} is likely due to the presence of O-H asymmetric
348 stretching vibrations from free OH groups [57], which is the main component of the majority of phenolic
349 phytochemicals [43]. The peak at 1714.8 cm^{-1} is due to C=O stretching vibrations from aldehydes,
350 carboxylic acids, or ketones, which suggests the presence of carbonyl-containing phytochemicals [42, 47].
351 The peak at 1627.6 cm^{-1} can be assigned to N-H bending vibrations of primary amines, C=C stretching
352 vibrations of aromatic ring deformations [48] and C=O stretching vibration of amides or carboxylic acids
353 [49], which suggests the presence of amino acids, flavonoids and volatile oils [49, 50, 51]. The peak at
354 1384.1 cm^{-1} can be assigned to C-H bending from alkanes [49], S=O stretching from organic sulfates [52]
355 and C-O stretch from carboxylic acids [53]. The peak at 1265.4 cm^{-1} corresponds to C-O stretching
356 vibration from alcohols and esters, which may likely be due to the presence of lipids in volatile oils [60]
357 or polyols like hydroxyl flavonoids [61]. The peak at 1164.2 cm^{-1} belongs to C-N stretching vibrations
358 from aliphatic amines and C-O stretching vibrations from alcohols, carboxylic acids or esters [62]
359 predicting the presence of polysaccharides and starch in the extract [63]. The peak at 1074.9 cm^{-1} can be
360 assigned to C-F stretch from aliphatic fluoro compounds, C-N stretch from aliphatic primary amines [54]
361 or C-O-C stretch from alkyl-substituted or cyclic ethers [55]. The C-O-C stretching vibrations suggest the

presence of monoterpenes (monocyclic or bicyclic) [56] or polysaccharides [54]. The peak at 666.5 cm^{-1} can be attributed to C-Br stretching, C-H bending or C-S stretching vibration from aliphatic bromo compounds, and alkynes or disulfides, respectively [42].

3.3.2.2 Spectral Analysis of the N-Hexane Fraction

The IR spectrum of the n-hexane fraction consisted of eleven (11) major peaks between 3393.9 cm^{-1} – 1072.1 cm^{-1}. The peaks 3393.9, 2923.9, 2852.5, 2359.6, 2342.3, 1377.3, 1163.5, and 1072.1 cm^{-1} belongs to O-H, C-H, C-N, C-H/S=O/C-O, C-N/C-O, and C-F/C-N/C-O-C functional groups, which correspond to the presence of hydroxyl groups, saturated hydrocarbons, organic sulfates, carboxylic acids, aliphatic amines, alcohols or carboxylic acids, aliphatic fluoro compounds, aliphatic primary amines, and ethers, respectively. The functional groups suggest the presence of phenolic phytochemicals, lipophilic phytochemicals, polysaccharides, and starch or monoterpenes (monocyclic or bicyclic). The peak at 1738.2 cm^{-1} originated from C=O stretching vibrations of saturated aliphatic aldehydes or esters [42, 58], which suggests the presence of phospholipids and volatile oils [59, 60]. The peak at 1462.8 cm^{-1} can be assigned to C-H bending vibrations (C-H asymmetric and symmetric stretching vibrations from CH_3 and CH_2 groups) and –C=C- stretching vibrations from aromatic rings due to the presence of lipophilic phytochemicals [44, 53] and aromatic compounds [50, 61]. The peak at 1216.9 cm^{-1} corresponds to C-O stretching vibration from alcohols and esters, which suggests the presence of lipids in volatile oils [60] or polyols like hydroxyl flavonoids [61].

3.3.2.3 Spectral Analysis of the Ethyl Acetate Fraction

The IR spectrum revealed eleven (11) major peaks within the range of 3396.5 – 610.1 cm^{-1}. The peaks 3396.5, 2932.7, 2359.8, 1629.1, 1383.5, 1266.9, 1164.0, 1042.3 cm^{-1}, and 610.1 cm^{-1}, which are similar to some of the peaks found in the methanol and n-hexane solvent-fractions belonging to O-H, C-H, C-N, N-H/C=C/C=O, C-H/S=O/C-O, C-N/C-O/C=S, C-N/C-O, C-F/C-N/C-O-C, and C-Br/C-H/C-S bonds indicating the presence of hydroxyl groups, hydrocarbons (CH_3, CH_2), aromatic primary amines, aromatic rings, amides, hydrocarbons, organic sulfates or carboxylic acids, aromatic amines, alcohols, carboxylic acids or sulphur compounds, aliphatic amines, alcohols or carboxylic acids, aliphatic fluoro compounds, aliphatic primary amines or ethers, and alkynes, aliphatic bromo compounds, and disulphides. The peaks suggest the presence of phenolic phytochemicals, lipophilic phytochemicals, amino acids, flavonoids, volatile oils, polyols, polysaccharides, starch, and/or monoterpenes (monocyclic or bicyclic). The peaks at 897.7 and 801.5 cm^{-1} belong to C-H bend from alkenes and N-H wag from primary or secondary amines and/or C-Cl stretching vibrations from aliphatic chloro compounds, respectively [42].

3.3.2.4 Spectral Analysis of the Butanol Fraction

Eight peaks were observed in the IR spectrum of the butanol fraction of the extract 3381.7, 2920.0, 2849.9, 1737.7, 1629.9, 1482.7, 1384.2, and 1078.5 cm^{-1} originating from O-H, C-H, C=O, N-H/C=C/C=O, C-H/S=O/C-O, and C-F/C-N/C-O-C functional groups belonging to hydroxyl groups, hydrocarbons, saturated aliphatic aldehydes, esters, ketones or carboxylic acids, amines, amides, carboxylic acids, organic sulfates, carboxylic acids, aliphatic fluoro compounds, aliphatic primary amines or ethers respectively. The functional groups suggest the presence of phenolic phytochemicals, lipophilic phytochemicals, amino acids, phospholipids, volatile oils, carboxylic, and halogen-containing phytochemicals, monoterpenes (monocyclic or bicyclic) or polysaccharides [51, 54, 56].

3.3.2.5 Spectral Analysis of the Aqueous Fraction

The aqueous fraction of the extract revealed nine peaks (9) on the IR spectrum in which 3380.9, 2931.8, 2359.4, 1712.9, 1267.6, 1163.3 and 1042.9 cm^{-1} belonging to O-H, C-H, C-N, C=O, C-N/C-O/C=S, C-N/C-O, and C-F/C-N/C-O-C functional groups correspond to the presence of hydroxyl groups, saturated hydrocarbons, carboxylic acids or ketones, aromatic primary amines, alcohols, esters or sulphur compounds, aliphatic amines, alcohols or carboxylic acids, and aliphatic fluoro compounds, aliphatic primary amines, and ethers, respectively. The functional groups suggest the presence of phenolic phytochemicals, lipophilic phytochemicals, carbonyl containing phytochemicals, polyols, monoterpenes

410 (monocyclic or bicyclic), and polysaccharides (or starch). The peak at 1604.7 cm^{-1} belongs to C=O
411 stretching vibration from carboxylic acid salts or quinones, or N-H bend from primary amine [58]. The
412 peak at 555.3 cm^{-1} belongs to C-I stretching vibrations from aliphatic iodo compounds [42].

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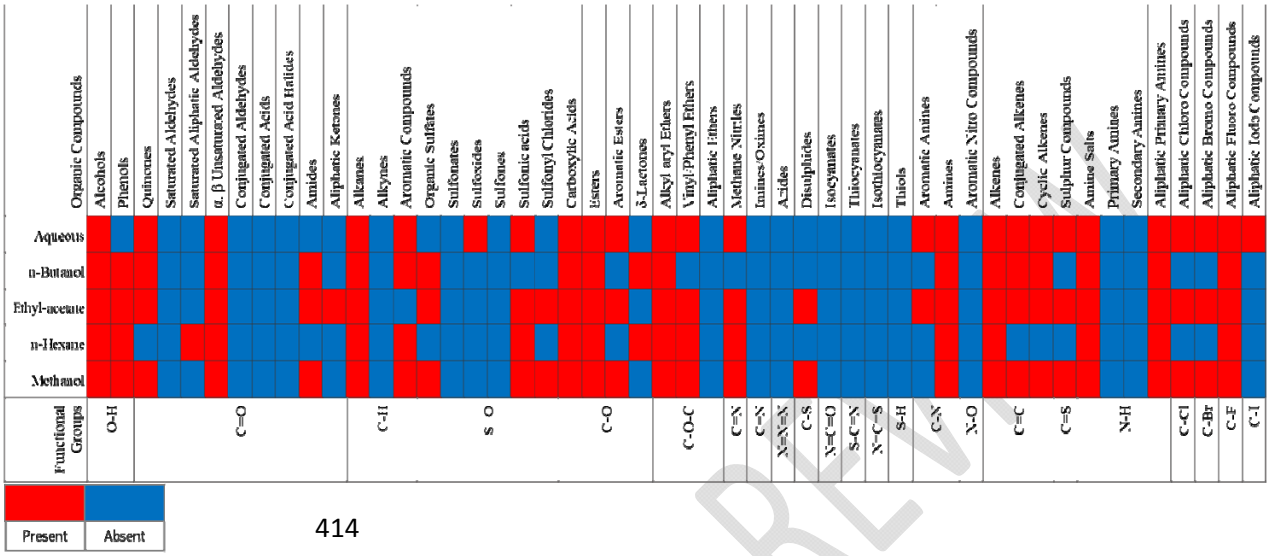

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416 Figure 7. Secondary metabolite/phytochemical expression profile of different solvent-fractions of
417 *Vernonia amygdalina* leaf tissue extract. Heatmap represents the relative expression profile of secondary
418 metabolites/ phytochemical compounds expressed in leaf tissue of different organic solvent-fractions
419 analyzed with FTIR spectroscopy based on functional group determination.

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Table 4: List of probable phytochemicals present in each solvent-fraction of *Vernonia amygdalina*

Solvent-fraction	Base group and vibration mode	Possible phytochemical compounds present in leaf extracts
MOH	3381.6 v (O-H), 2925.5 v_s(C-H), 1714.8 v(C=O), 1627.6 δ(N-H), v(C=C), v(C=O), 1265.4 v_s(C-O), 1384.1 v_s(S=O), 1164.2 v(C-O), 1074.1 v_s(C-O-C), v_s(C-F), v(C-Br), v(C-S)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic compounds, carbonyl containing compounds, sulfur, carboxylic acid and halogen-containing compounds, amino acids, volatile oils, polyols, polysaccharides, starch, monoterpenes (monocyclic or bicyclic), etc.
Hx	3393.9 v (O-H), 2923.9 v_{as}(C-H), 2852.5 v_s(C-H), 1738.2 v_s(C=O), 1462.8 δ(C-H), v_{rf}(ar), 1163.5 v(C-O), 1072.1 v(C-O-C), v(C-F)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic compounds, amino acids, volatile oils, polyols, polysaccharides, starch or monoterpenes (monocyclic or bicyclic), etc.
EtAc	3396.5 v (O-H), 2932.7 $v_{as/s}$(C-H), 1629.1 δ(N-H), v(C=O), v(C=O), 1383.5 v(S=O), 1266.9 v_s(C-O), 1164.0 v(C-O), 1042.3 v_s(C-O-C), v_s(C-F), 897.7, 801.5 v_s(C-Cl), 610.1 v_s(C-Br)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic phytochemicals, amino acids, flavonoids, volatile oils, polyols, polysaccharides, starch or monoterpenes (monocyclic or bicyclic), etc.
BOH	3381.7 v (O-H), 2920.0 $v_{as/s}$(C-H), 2849.9 v_s(C-H), 1737.7 v_s(C=O), 1629.9 δ(N-H), v(C=O), v(C=O), 1482.7 δ(C-H),1384.2 v(S=O), 1078.5 v(C-O-C) v(C-F)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic phytochemicals, amino acids phospholipids, volatile oils, monoterpenes (monocyclic or bicyclic) or polysaccharides, etc.
Aq	3380.9 v(O-H), 2931.8 $v_{as/s}$(C-H), 1712.9 v(C=O), 1267.6 v_s(C-O), 1163.3 v(C-O), 1042.9 v(C-O-C) v(C-F), 555.3 v_s(C-I)	Phenolic phytochemicals (flavonoids, phenolic acids, tannins, or polyphenols), lipophilic phytochemicals, carbonyl containing phytochemicals, amino acids, polyols, monoterpenes (monocyclic or bicyclic), carboxylic and halogen-containing phytochemicals, polysaccharides, or starch, etc.

Footnote: v: Vibration/Stretching; δ: Bending; v_s: Symmetrical; v_{as}: Asymmetrical; ar: Aromatic; ha: Heteroaromatic; v_{rf}: Ring frame. MOH: Methanol; Hx: Hexane; EtAc: Ethyl acetate; BOH: Butanol; and Aq: Aqueous distilled water.

4. DISCUSSION

The presence of secondary metabolites such as phytosterols, alkaloids, saponins, tannins, flavonoids, terpenoids, and phenolic compounds in medicinal plants are responsible for the curative properties of the plants [64]. Importantly, the presence of these so-called phytochemical compounds has been demonstrated to give many plant extracts their medicinal properties such as analgesic, insecticidal, bactericidal, anticancer, antioxidant, antifungal, anthelminthic, antiviral, antidiabetic, antidiarrheal, antiprotozoal, anti-inflammatory, and antimalaria activities [18-21], [23-35], [65]. Colorimetric phytochemical assays were used to qualitatively identify baseline phytochemicals and FTIR spectroscopy to identify the functional groups of secondary metabolites/phytochemicals present in the different solvent-fractions of *Ocimum gratissimum* and *Vernonia amygdalina* plant leaf tissue extracts. Apart from elucidating the structures of biomolecules, functional groups play a significant role in determining the biological activities of bioactive molecules present in extracts [66]. They assist us in understanding the

physicochemical properties of these compounds such as their stereochemistry, absorption or binding interactions, and acid-base properties. Therefore, creating an avenue to understand and determine the absorption, distribution, metabolism, elimination, and toxicological profiles of these bioactive molecules [66].

Alkaloids which are chemical compounds that consist of basic nitrogen atoms and are produced naturally by plants, animals, bacteria, and fungi. Pure alkaloids and their synthetic derivatives act as precursors for making potent drug substances and they are known to have antispasmodic, analgesic and antibacterial activities [65]. Tannins are water soluble polyphenols which are mostly found in both herbaceous and woody plants [67], they have been said to possess various biological activities such as antitumor, antiviral, cardioprotective, anti-inflammatory, and antibacterial properties [68, 69] which can be ascribed to their antiradical and antioxidant activities [70, 71]. Tannins are toxic to micro-organisms and one of the proposed antimicrobial modes of action of tannins is dependent on cutting out substrates needed for microbial growth through the precipitation of microbial proteins [72]. Phlobatannins (also known as Phlobaphenes) are reddish-colored, water-insoluble phenolic substances that are believed to be related to co-occurring condensed tannins [92].

Cardiac glycosides are used for the treatment of congestive heart failure and cardiac arrhythmia as well as for strengthening weakened heart muscles due to its mode of action. It works by inhibiting the Na^+/K^+ pumps causing an increase in the level of Na^+ ions in the myocytes, which results increase the levels of Ca^{2+} available for heart muscle contraction. This enhances the cardiac output and decreases distention of the heart. However, cardiac glycosides must be used carefully because it has been shown to have a fatal toxic effect on the heart due to the closeness of its therapeutic dose and toxic dose [73]. Terpenoids were highly and moderately expressed in the methanolic and hexane solvent-fractions of *Vernonia amygdalina*, but moderately and lowly expressed in methanolic and hexane solvent-fractions of *Ocimum gratissimum*, respectively. Terpenoids are natural lipids found mainly in plants as components of essential oils. They are used commercially as fragrances, flavors in food, cosmetics, and have been shown to have medicinal properties such as anti-ulcer, antimicrobial, antimalarial, anti-inflammatory, and diuretic activities [74].

Anthraquinones were found to be highly and moderately expressed in the methanolic solvent-fractions of *Vernonia amygdalina* and *Ocimum gratissimum* leaf tissue extracts, respectively. Anthraquinones are known to have a broad spectrum of biological activities, which includes anticancer, vasodilatory, anti-inflammatory, phytoestrogen, diuretic, and antimicrobial activities [75]. Anthraquinone and its derivatives are known to have antiviral and viricidal activities [75]. In addition, natural anthraquinones are known to inactivate enveloped viruses [75]. Due to the therapeutic and pharmacological activities of natural and synthetic anthraquinones, they have a wide range of applications in cosmetics, pharmaceutics, dyes, and foods [76]. The sulfur-containing compounds present in the solvent fractions can be used to produce disinfectants and dental creams while the halogen compounds help to produce chlorinated tryptophan within the plant cell walls, which are then metabolized into chlorinated alkaloids [77]. Carboxylic acids present in the extracts show it can be used to produce strong antibacterial agents, curing ulcers and in the treatment of edema and rheumatic joint pains. They can also form aldehydes when combined with phenols [78]. Aromatic amines in the extracts show that it can be used in dye industries as starting materials for making various types of azo dyes, in chemical industries to produce antioxidants, fuel additives and of some chemicals such as motor, transmission, and industrial oils, in cosmetic industries to produce some cosmetics such as varnish and they are also useful for in rubber, textile and plastic industries [76]. Amines are also useful as pharmaceuticals as many drugs contain amine groups such as lidocaine, tetracycline, morphine, and albuterol [79].

Alkynes find their applications in pharmaceuticals to produce contraceptives such as norethynodrel and they also known to have antiviral, antifungal and antitumor activities while alkenes have several applications such as the production of plastics, anesthetic agents, ethylene-oxide flame, illuminants, and fuels [80]. The chlorates in the fractions can be used in the production of disinfectants and bromides for

producing anti-parasitic compounds of bromine origin [81]. Flavonoids are ketone-containing polyphenolic compounds with well-studied medicinal activities. They can be classified as bioflavonoids, isoflavonoids, and neoflavonoids [85]. Flavonoids have been shown to have many biological and medicinal activities including antiviral [86], anticancer [87], antifungal [88], antibacterial [89], anti-inflammatory [90], antioxidant [91], and anti-allergic activities [90] in both *in vitro* and *in vivo* studies. The presence of flavonoids either in low or high quantities in both leaf extracts in this study justifies the prior reported medicinal benefits of *Vernonia amygdalina* and *Ocimum gratissimum*.

Despite the diversity in the solvent-mediated phytochemical expression profiles for both plants, the pattern of expression appears to depend mostly on the intrinsic interaction or solvation of the phytochemical compounds in each solvent-fraction as well as the polarity of the solvents. In this study, the methanol solvent was found to have the best efficacy in inducing optimal extraction (highest phytochemical content) and phytochemical expression profile. These may be due to the ability of these solvents to conserve the stability of chemical structures of desired compounds present in the leaf tissue extracts of both plants. This study has further confirmed the reasons for the disparity in the medicinal activities of different solvent-fractions of plant tissue extracts. The biochemical or physicochemical role of extraction solvent or solvent-fraction and the phytochemistry of the plant tissue must be considered when determining the medicinal benefits of plant tissue extracts.

5. CONCLUSIONS

The present study shows that *Ocimum gratissimum* and *Vernonia amygdalina* leaf tissue extracts are rich sources of various phytochemical compounds such as alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, tannins, terpenoids, saponins, and reducing sugars. However, the expression or bioavailability of these phytochemical compounds may be dependent on the intrinsic physicochemical properties of the solvents used during the process of extraction or solubilization. This is very important as these phytochemical compounds individually have biological and molecular activities that could lead to a cumulative effect, which could be determined by the bioactivity of the most expressed bioactive compound (with the greatest biological effect) within the plant tissue extract. The identified bioactive compounds can serve as drug leads for the design and development of novel plant-based drugs. The functional groups present in the extracts includes O-H, C-H, C=C, C-N, N-H, C=O, C-O, S=O, C=S, C-O-C, C-F, C-Br, and C-O-C groups, which indicate the presence of alcohols, alkanes, alkenes, nitriles, amines, quinones, amides, aldehydes, ketones, esters, carboxylic acids, organic sulfates, sulphur compounds, ether, chloro-, and bromo-compounds, respectively that may be responsible for the various medicinal properties of *Ocimum gratissimum* and *Vernonia amygdalina* leaf tissue extracts. The solvent-mediated phytochemical expression pattern in this study is consistent and followed a profiling order: "Methanol > Hexane > Ethyl acetate > Butanol > Aqueous distilled water" for both plants. In this study, the FTIR spectroscopy was able to give an overview of all probable compounds present in the leaf tissue solvent-fractions of both plants while the colorimetric assay provided us information about the expression patterns. Since the latter is qualitative, Care must be taken in interpreting data from the latter. This colorimetric method needs to be upgraded or followed up with other analytic methods. Further studies using more quantitative analytical techniques such as NMR, mass spectroscopy (MS), and UV-spectroscopy will be done to give more insight into the role of solvent-mediated expression profiles of phytochemical compounds in medicinal properties of plant-based products.

COMPETING INTEREST

The authors declare that no competing interests exist.

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