



IR and UV Analysis of VLC Fractions Derived from Ethylacetate Extracts from the Roots of *Maranthes Polyandra*

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ABSTRACT

The research work was aimed at examining the medicinal value of the root of the plant, *Maranthes polyandra* as well as isolation and analysis of secondary metabolites using available spectroscopic techniques. The roots of *M. polyandra* were collected, dried, crushed and extracted with ethyl acetate via cold extraction method. The crude extract was fractionated using Vacuum Liquid Chromatography (TLC grade silica gel as the stationary phase and solvents of increasing polarity as mobile phase) and monitored using Thin Layer Chromatography. Fractions with similar TLC R_f values were pooled together and assigned Pool Number 1 to 14. White crystals were observed for pool number 4 – 11 and relabeled A – H. The crystals isolated were subjected to melting point, IR and UV spectroscopic analysis. Preliminary phytochemical screening of the ethyl acetate root extracts revealed the presence of alkaloids, cardiac glycosides, saponins, sterols, terpenoids, and tannins which are responsible for the therapeutic effects of *M. polyandra* in African traditional medicine. IR analysis showed characteristic absorption bands at 3440-3200 cm⁻¹, 1705-1760 cm⁻¹, 1680-1600 cm⁻¹, and 1300-1000 cm⁻¹ which are indicative of the presence of hydroxyl (-OH), carbonyl (C=O), alkene/aromatic (C=C) and ester (C-O) functional groups respectively. Other absorption bands include: 3000-2850 cm⁻¹, 3100-3000 cm⁻¹, 1470-1370 cm⁻¹ and 900-690 cm⁻¹ which depicts Sp³ C-H_{str} (asymmetric and symmetric), Sp² C-H_{str}, C-H_{bend} of alkanes and C-H_{bend} of aromatics respectively. UV analysis showed major absorption bands at longer wavelengths (> 300 nm) and shorter wavelengths (< 250 nm). This can be attributed to n → π* and π → π* transitions respectively which is characteristic of carbonyl functional groups. The m.pt of the white crystals isolated ranged between 141 – 173 °C.

1. INTRODUCTION

Since ancient times, in search for cure for diseases, man looked for drugs in nature. Due to insufficient

information, either about the illnesses or concerning which plant and how it could be utilized as a cure,

everything was based on trial and error. With time, the reasons for the usage of specific medicinal plants for the treatment of certain diseases were discovered; thus, medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts [1].

Plants contain active compounds also referred to as natural products, deposited in specific parts of plants such as leaves, bark, seeds, root, etc. which are of potential use in medicine and other applications. Natural products are chemical compounds that are formed by living systems - found in nature. Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. These biologically active compounds; such as alkaloids, steroids, tannins, glycosides, phenols, flavonoids etc. forms the constituents of medicinal and poisonous plants [2].

The therapeutic effects of plant materials typically result from the combination of these active compounds. Many of these compounds are secondary metabolites and they often have an ecological role in regulating the interactions between plants, micro-organisms, insects and animals. They can be defensive substances, anti-feedants, attractants and pheromones. In some cases, these natural products deposited in plants have been isolated and characterized, and their mechanisms of action are understood (e.g., ephedrine alkaloids in some species of Ephedrine) [3].

Maranthes polyandra as reported by Yakandawala *et al.*, belongs to the family: *Chrysobalanaceae*. The family is composed of seventeen genera and about 525 species [4]. Asase *et al.*, described *Maranthes polyandra* as a tree up to about 12m tall, sometimes low and bushy, with finely-fissured, smooth bark. The leaves were described as shortly petiolate, broadly ovate or ovate-elliptic, glabrous, shining and green or brownish-green [5]. Some other species of the genus *Maranthes* include *Maranthes aubrevillei*,

Maranthes chrysophylla, *Maranthes corymbosa*, *Maranthes floribunda*, *Maranthes gabunensis*, *Maranthes glabra*, *Maranthes goetzeniana*, *Maranthes kerstingii*, *Maranthes panamensis*, *Maranthes robusta*, and *Maranthes sanagensis* [6].

Maranthes polyandra which is synonymous to *Parinari polyandra* is known as “ibyua kuna” among the TIV people of Benue State, Nigeria and “idofun” (Ilorin) or “iyeri osun” (Ibadan) by the Yoruba of Western Nigeria [7].



Figure 1; The leaves and flower of *Maranthes polyandra* [8]



Figure 2; The leaves and fruits of *Maranthes polyandra* [8]

Otun *et al.*, reported the isolation of 14-methyl pentadecanoate, hexadecanoic acid, 9-octadecenoic acid methyl ester, 9-octadecenal, Cis, cis-linoleic acid, glycidol stearate, and oleic acid from the young and old stem bark hexane extract of *Parinari polyandra* [9].

Plants are complex matrices having a range of secondary metabolites of different functional groups and polarities. As a result, a range of techniques may be used for the extraction of plant materials. Although water is used as extractant in many traditional medicines, organic solvents of varying polarities are generally used in modern methods of extraction to exploit the various solubilities of plant constituents. Obviously, wrong choice of solvent and method will cause the entire processes to fail or the desired compounds from the material may not be released from the matrix completely [10]. Some extraction procedures usually applied for the extraction of natural products from plants include; maceration, percolation, soxhlet extraction and steam distillation.

Secondary metabolites are sensitive to extreme temperature, and can be easily denatured by heat. Hence crude extracts of plant materials are advisably concentrated using a temperature controllable distillation apparatus equipped with a vacuum pump such as the rotary evaporator. Concentrated form of secondary metabolites is usually isolated by any of the suitable chromatographic techniques. The common chromatographic techniques discussed are: column chromatography, gas chromatography (GC), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC).

Pure isolates are often subjected to structural investigation so as to determine their structures using combined spectral techniques of modern methods of spectroscopic studies. Spectroscopic method has already established itself as an authentic as well as one of the most significant techniques not only in solving structural problems but also in analytical and preparative works [11]. These methods include: UV-Visible, IR, Mass spectrometry, NMR etc.

In this paper, we present the phytochemical screening of the root extract of *Maranthes polyandra* as well as the isolation and spectroscopic analysis of the ethyl acetate root extract of *M. polyandra* using available spectroscopic techniques.

2. MATERIALS AND METHODS

2.1. Plant material and sample preparation

Roots of *M. polyandra* were collected from herbal practitioners at Oje market in Ibadan, Oyo State. Identification was done at the Department of Forestry, University of Ibadan, Ibadan. They were rinsed with tap water followed by distilled water to remove the dirt on the surface and then cut into smaller pieces. They were then air-dried until a constant mass was obtained.

2.2. Extraction

Cold extraction method was used. Five hundred gram (500 g) of the pulverized root sample was soaked in 3 Liters of ethyl acetate and left standing for 10 days. The ethyl acetate extract was then filtered and concentrated using rotary evaporator at a constant temperature of 40°C and the crude extract kept in desiccators.

2.3. Chemicals and reagents

All chemicals used were of analytical grade. n-hexane, ethyl acetate, methanol, chloroform, acetic anhydride, glacial acetic acid, concentrated sulphuric acid, 1% hydrochloric acid solution, dilute sodium hydroxide solution, 5% ferric chloride solution, distilled water, Dragendorff's reagents, iodine and silica gel (TLC and column grade). All chemicals and reagents were used without further purification.

2.4. Phytochemical Screening

The crude ethyl acetate extract of the roots were screened for the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids using simple qualitative method of analysis as reported by [12]. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

2.4.1. Test for Alkaloids

Two mL of the extract was acidified with 1% hydrochloric acid and then treated with few drops of Dragendorff's reagents in a test tube. The formation of orange-brown precipitate indicates the presence of

alkaloids.

2.4.2. Test for Cardiac Glycosides

The crude extract was dissolved in 3 mL glacial acetic acid containing traces of ferric chloride. One mL of concentrated sulphuric acid was added down the side of the test tube. Purple ring colour at the interface indicates the presence of cardiac glycosides.

2.4.3. Test for Flavonoids

Two mL of the crude extract was dissolved in dilute sodium hydroxide. A yellow solution that turns colourless on addition of concentrated hydrochloric acid indicates the presence of flavonoids.

2.4.4. Test for Saponins

Two mL of the crude extract was dissolved in 5 mL of distilled water. Two mL of the resulting solution was taken into a test tube and shaken vigorously for few minutes. Frothing which persists on warming was taken as an evidence for the presence of saponins.

2.4.5. Test for Steroids

One mL of crude extract was treated with drops of chloroform, acetic anhydride and conc. H₂SO₄, and observed for the formation of dark pink or red colour.

2.4.6. Test for Tannins

Extract (0.5g) was dissolved in 10 mL of distilled water, and then filtered. Few drops of 5% ferric chloride solution were then added to the filtrate. Formation of a blue-black precipitate indicates hydrolysable tannins and green precipitate indicates the presence of condensed tannin.

2.4.7. Test for Terpenoids

Two mL of chloroform was added to 0.5g of the crude extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced indicates the presence of terpenoids.

2.5. Chromatographic Analysis of Extract

The fractionation of the ethyl acetate root extract was carried out using vacuum liquid chromatographic technique and monitored using thin layer chromatographic (TLC) techniques.

2.5.1. Vacuum Liquid Chromatography

About 6.53 g of the ethyl acetate extract was pre-adsorbed on silica gel and then loaded on a VLC column packed with 196 g of TLC grade silica gel as stationary phase.

The column was eluted stepwise under vacuum with solvents (n-hexane, chloroform and methanol) of increasing polarity. Twenty-eight (28) fractions were collected at 50 mL each. The samples were then concentrated and allowed to stand at room temperature. Fractions with similar TLC R_f values were pooled together and assigned Pool Number 1 to 14. White crystals were observed for pool number 4 - 11 and relabeled A - H.

2.5.2. Thin Layer Chromatography

Thin layer chromatographic analysis of the different fractions obtained from VLC was carried out using silica gel pre-coated thin layer chromatographic plates as stationary phase and different solvent ratios of hexane-ethyl acetate as mobile phase. Spots on TLC plates were detected using iodine tank and the retention factors of the spots were calculated and recorded.

2.6. Analysis of Isolated Compounds

Basic information on the structure of the isolated compounds were obtained from the analysis of spectral data and physical properties such as melting point. The functional groups present in the isolated compounds were investigated using the spectra data obtained from a combination of experimental techniques; FT-IR spectrum and UV spectrum. Melting point of the isolated compounds was also determined.

2.6.1. Ultra-violet (UV) Spectrum

The crystals obtained on fractionation were

dissolved in a suitable solvent, poured into a quartz curvet and then inserted into the spectrometer. The UV spectrum was recorded between 190 – 400 nm. The Ultra-violet spectrum (λ in nm) was run on Spectro UV-VIS Double Beam PC Scanning Spectrometer, UVD – 2960 at the Department of Chemistry, University of Ibadan, Ibadan.

2.6.2 Infra-red (IR) Spectrum

The isolated compounds were dissolved in a suitable solvent and a drop of the dissolved compound was then placed between a pair of polish sodium chloride plates and the solvent allowed to evaporate. The sodium chloride plate was then inserted into a sample holder in the spectrometer and the IR spectrum recorded between 4000-400 cm^{-1} . Infra-red spectrum (ν in cm^{-1}) was run on an FT-IR PerkinElmer Spectrum Two (LABOMED, INC.) at the Department of Chemistry, University of Ibadan, Ibadan.

2.6.3. Melting Point Determination

The melting points of the crystals obtained were determined using the Barnstead Electrothermal (1900) Melting Point apparatus at the Department of Chemistry, University of Ibadan, Ibadan. The crystals were introduced into a capillary tube in which one end was sealed and then inserted into the melting point apparatus and the temperature allowed to increase gradually at the rate of 1°C per min. The temperature at which the crystal starts melting and the temperature at which it completely melted were noted and recorded.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1. Percentage yield

The percentage yield of the crude extract was obtained using the formula below:

$$\% \text{ yield} = \frac{a}{b} \times 100$$

Where; a = weight of crude extract (6.98 g);

b = weight of dry plant root (500 g).

Therefore;

$$\% \text{ yield} = \frac{6.98 \text{ g}}{500 \text{ g}} \times 100 = 1.40\%$$

3.1.2. Phytochemical screening of crude extract

Phytochemical screening carried out on the ethyl acetate extract of the root of *Maranthes polyandra* as shown in table 1, indicates the absence of flavonoids.

Table 1: Phytochemical screening of root extracts of *M. polyandra*.

Test	Inference
Saponins	+
Alkaloids	+
Tannins	+
Terpenoids	+
Flavonoids	-
Cardiac glycosides	+
Steroids	+

Keys: + = Present - = Absent

3.1.3 Vacuum Liquid Chromatography

The ethyl acetate extract was fractionated via gradient elution solvent system using VLC. The column was eluted stepwise under vacuum with solvents of increasing polarity as shown in table 2. Twenty-eight (28) fractions were collected at 50 mL each, concentrated and weighed.

The % column recovery was calculated as shown below:

Total weight = 5.47 g

% column recovery =

$$\frac{\text{total weight}}{\text{weight of extract in column}} \times 100$$

Hence:

$$\% \text{ column recovery} = \frac{5.47 \text{ g}}{6.53 \text{ g}} \times 100 = 83.77\%$$

Table 2: VLC fractionation of ethyl acetate root extract of *Maranthes polyandra*

Column Eluents	Single or Pooled Fractions	Pool No.	Weight (g)
Hex (100%)	1-2	1	0.16
Hex:Chl (80:20)	3-4	2	0.35
Hex:Chl (60:40)	5-6	3	0.60
Hex:Chl (40:60)	7	4	0.50
Hex:Chl (40:60)	8	5	0.31
Hex:Chl (20:80)	9	6	0.32
Hex:Chl (20:80)	10	7	0.41
Chl (100%)	11	8	0.24
Chl:Meth (95:5)	12-13	9	0.41
Chl:Meth (95:5)	14	10	0.80
Chl:Meth (95:5)	15 – 16	11	0.45
Chl:Meth (95:5)	17 – 20	12	0.32
Chl:Meth (90:10)	21 – 24	13	0.27
Chl:Meth (90:10)	25	14	0.13
Chl:Meth (90:10)	26 – 28	15	0.20

Keys: Hex = Hexane, Chl = Chloroform, Meth = Methanol

3.1.4. Thin Layer Chromatographic Analysis

The fractions obtained from VLC were subjected to TLC analysis using pre-coated TLC plates. The solvent systems as well as the retention factor of each spot were recorded. Fractions with pool numbers; 4, 5, 6, 7, 8, 9, 10, and 11 were then labelled A, B, C, D, E, F, G and H respectively as shown in table 3 and submitted for IR and UV spectroscopic analysis.

Table 3: TLC Analysis of Isolates

Isolates	Description	Solid/Mobile phase	R _f (cm)
A	W. crystal	Sg/Hex-Ethyl A, 7:1	0.35
B	W. crystal	Sg/Hex-Ethyl A, 7:1	0.38
C	W. crystal	Sg/Hex-Ethyl A, 7:1	0.50
D	W. crystal	Sg/Hex-Ethyl A, 7:1	0.55
E	W. crystal	Sg/Hex-Ethyl A, 6:2	0.30
F	W. crystal	Sg/Hex-Ethyl A, 6:2	0.50
G	W. crystal	Sg/Hex-Ethyl A, 4:4	0.43
H	W. crystal	Sg/Hex-Ethyl A, 4:4	0.35

keys: W = white, Sg = Silica gel, A = Acetate

Table 4: UV Analysis of Isolated Compounds

Isolates	Absorption band (λ_{\max}) nm
A	312, 240, 223, 218
B	331,327, 243, 218
C	300, 243, 203
D	300, 233, 208
E	301
F	371, 360, 300
G	300, 238, 218
H	300

Table 5: IR Analysis of Isolates

Iso.	Observed Peaks (cm ⁻¹)	Int.	Types of vibration
A	3340.07	b	O-H _{str} (Hb)
	2928.64, 2854.60	s	Sp ³ C-H _{str} (alkanes)
	1735.20	s	C=O _{str} (carbonyl)
	1642.68	w	C=C _{str} (a/a)
	1465.37, 1377.77	m	C-H _{bend} (alkanes)
	1245.91, 1174.64, 1051.83	m	C-O _{str} (esters)
	884.60, 800.08, 722.64	w-m	C-H _{bend} (aromatics)
	B	3444.55	b
2928.29, 2854.46		s	Sp ³ C-H _{str} (alkanes)
1735.13		s	C=O _{str} (carbonyl)
1643.41		w	C=C _{str} (a/a)
1465.91, 1377.94		m	C-H _{bend} (alkanes)
1245.88, 1174.57, 1053.00		m	C-O _{str} (esters)
884.85, 840.85, 722.62		w-m	C-H _{bend} (aromatics)
C		3444.02	b
	2926.72, 2854.69	s	Sp ³ C-H _{str} (alkanes)
	1734.53	s	C=O _{str} (carbonyl)
	1643.41	w	C=C _{str} (a/a)
	1456.57, 1377.10	m	C-H _{bend} (alkanes)
	1245.90, 1174.59, 1055.78	m	C-O _{str} (esters)
	722.81	m	C-H _{bend} (aromatics)
	D	3443.12	b
2926.62, 2854.67		s	Sp ³ C-H _{str} (alkanes)
1734.32		s	C=O _{str} (carbonyl)
1643.00		w	C=C _{str} (a/a)
1456.18, 1377.06		m	C-H _{bend} (alkanes)
1246.04, 1174.66, 1056.64		m	C-O _{str} (esters)
722.95		m	C-H _{bend} (aromatics)
E		3467.80	b
	3008.05	m	Sp ² C-H _{str} (alkenes)
	2925.63, 2854.43	s	Sp ³ C-H _{str} (alkanes)
	1746.89	s	C=O _{str} (carbonyl)
	1646.50	w	C=C _{str} (a/a)
	1464.55, 1376.67	m	C-H _{bend} (alkanes)
	1241.26, 1162.69, 1056.65	m	C-O _{str} (esters)
	722.38	m	C-H _{bend} (aromatics)
F	3465.22	b	O-H _{str} (Hb)
	2984.61, 2932.64, 2851.40	m	Sp ³ C-H _{str} (alkanes)
	1757.26	s	C=O _{str} (carbonyl)
	1448.54, 1374.01	m	C-H _{bend} (alkanes)

	1245.91, 1097.88, 1049.73	s	C-O _{str} (esters)
G	3502.90	b	O-H _{str} (Hb)
	2995.07, 2925.52, 2854.36	m	Sp ³ C-H _{str} (alkanes)
	1757.80	s	C-O _{str} (carbonyl)
	1461.14, 1375.71	m	C-H _{bend} (alkanes)
	1245.95, 1161.96, 1056.90	m	C-O _{str} (esters)
H	3395.62	b	O-H _{str} (Hb)
	3008.46	m	Sp ² C-H _{str} (alkenes)
	2926.21, 2854.60	s	Sp ³ C-H _{str} (alkanes)
	1711.64	s	C-O _{str} (carbonyl)
	1623.43	w	C=C _{str} (a/a)
	1464.85, 1377.66	m	C-H _{bend} (alkanes)
	1241.57, 1165.31, 1116.53	m	C-O _{str} (esters)
	843.78, 723.12	w-m	C-H _{bend} (aromatics)

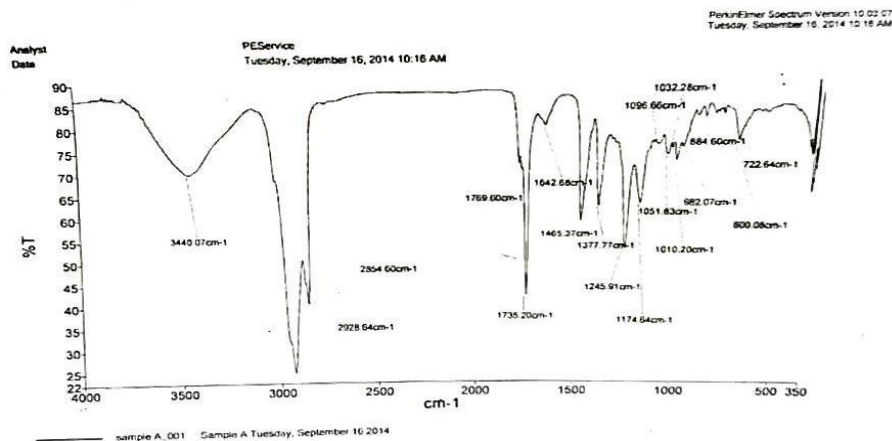
keys: Iso = Isolates, Int. = Intensity, Hb = Hydrogen bonded, a/a = alkenes/aromatics, Str = Stretch, b = broad, m = medium, s = strong, w = weak

3.1.5 Melting Point Analysis of Isolated Compounds

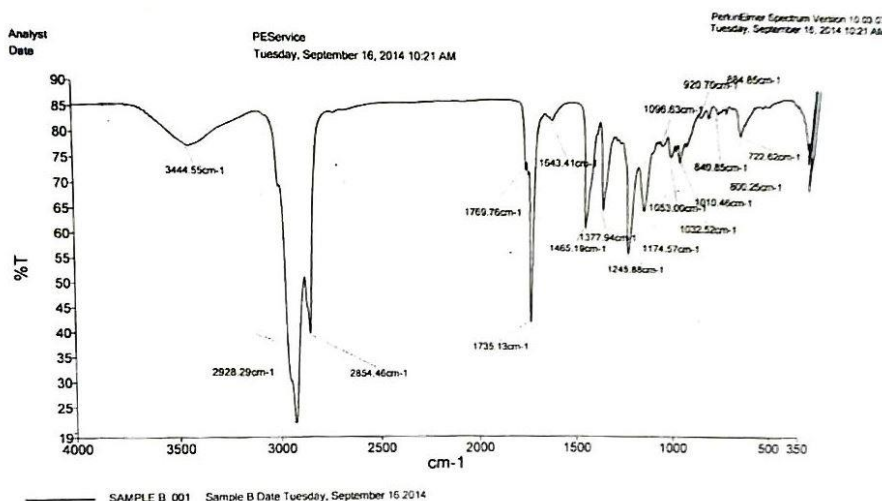
Table 6: Melting Point Analysis of Isolates

Isolates	Melting point (°C)	Degree of purity
A	148-155	Not pure
B	150-158	Not pure
C	160-173	Not pure
D	161-162	Pure
E	158-169	Not pure
F	141-145	Not pure
G	144-146	Pure
H	165-170	Not pure

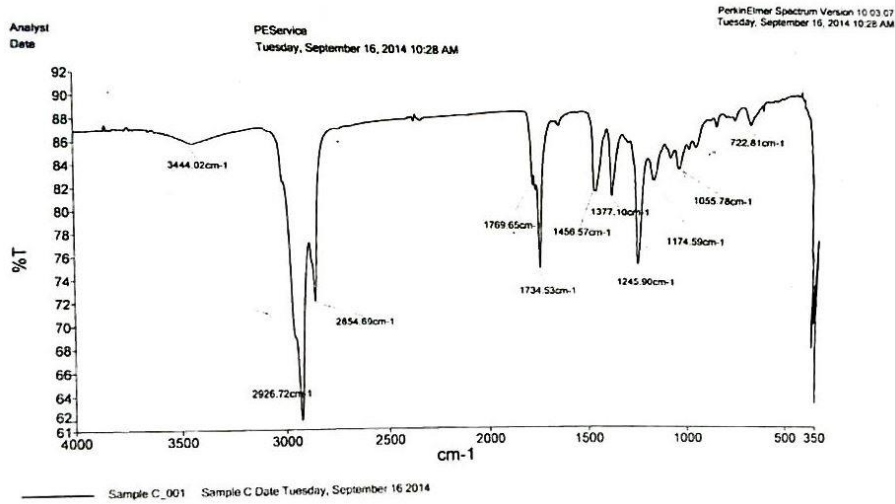
IR Spectrums Isolates



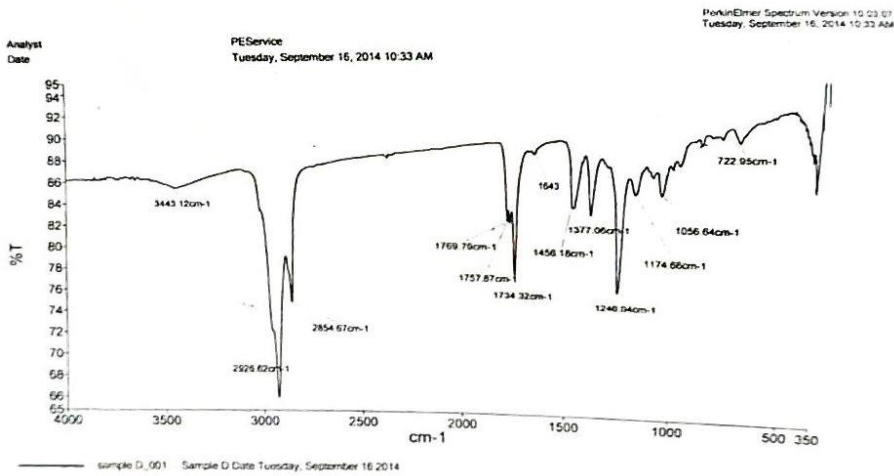
IR spectrum of isolate A



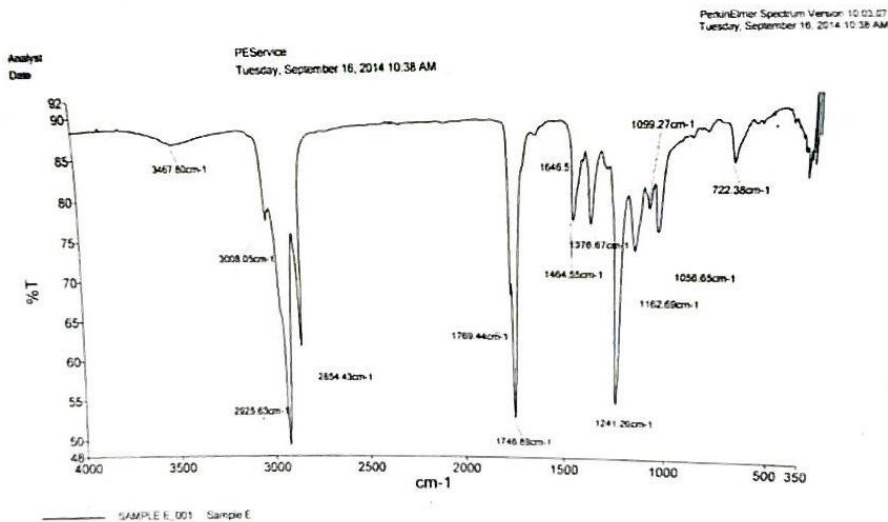
IR spectrum of isolate B



IR spectrum of isolate C

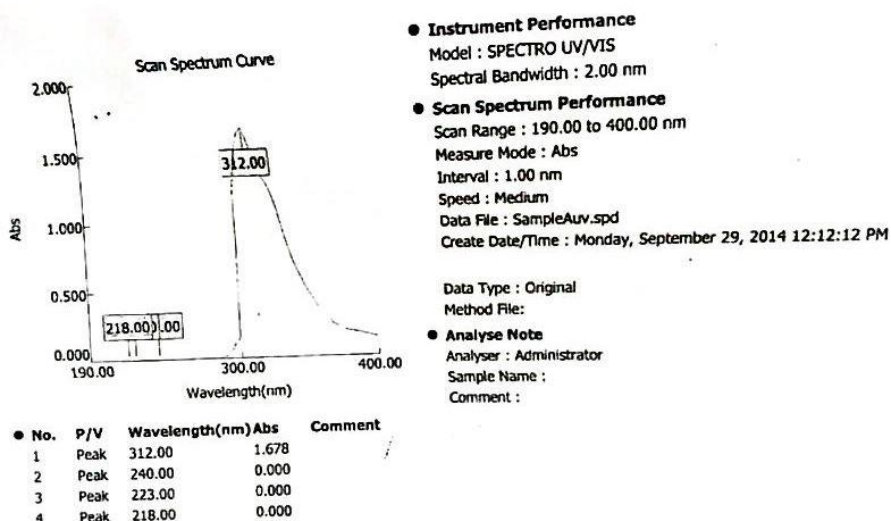


IR spectrum of isolate D

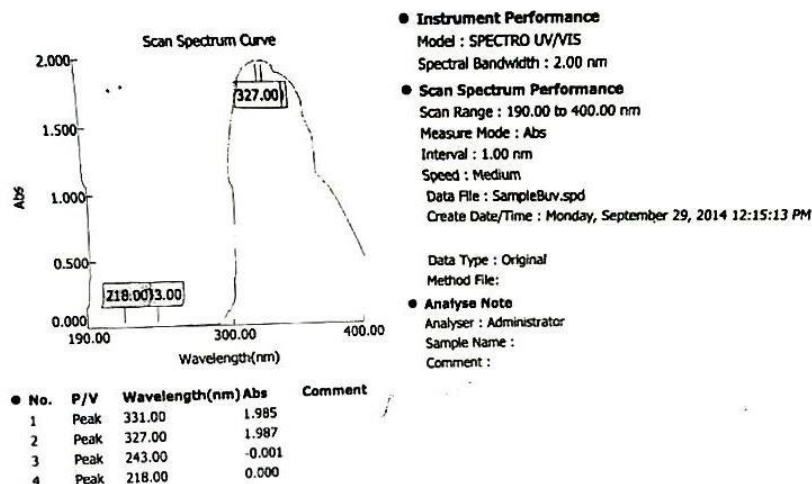


IR spectrum of isolate E

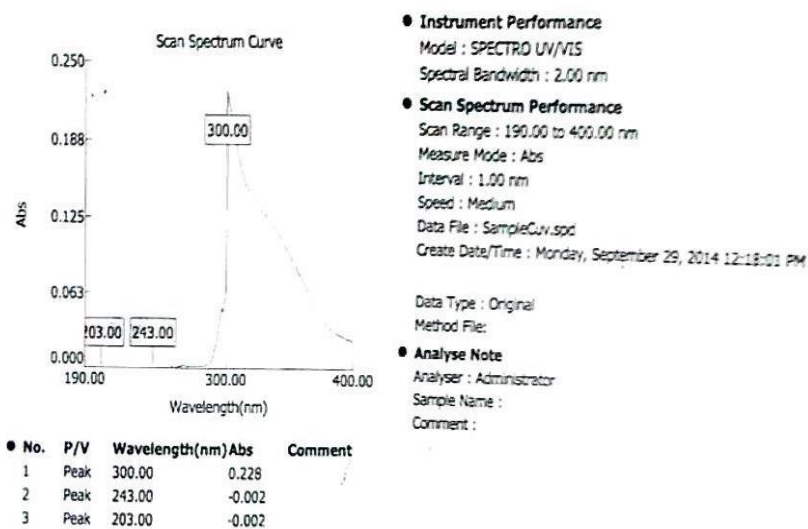
UV Spectrums of Isolates



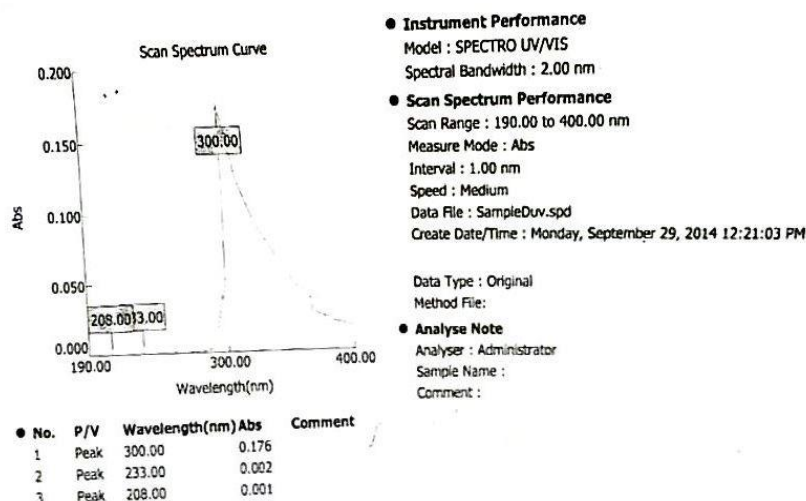
UV spectrum of isolate A



UV spectrum of isolate B



UV spectrum of isolate C



UV spectrum of isolate D

3.2. Discussion

The result of qualitative phytochemical screening of the ethyl acetate root extract of *Maranthes polyandra* (table 1) indicates the presence of alkaloids, cardiac glycosides, saponins, steroids, terpenoids and tannins. Flavonoid was seen to be absent which is contrary to the report of Mundi and Alhassan [13]. This difference could be as a result of geographical or topological factors. The curative properties of plants are perhaps due to the presence of these various secondary metabolites. These classes of compounds were known to have curative activity against several pathogens and therefore could suggest the traditional use of this plant for the treatment of various illnesses.

Alkaloids have been proven to have pharmacological properties such as hypotensive, anticonvulsant, antiprotozoal, antimicrobial and antimalarial activities. Plants that possess Tannin, cardiac glycoside and alkaloid are the most effective for managing hypertension and also providing protection for the heart [14].

Tannin has been found to have astringent properties that hasten the healing of wounds and inflamed mucous membranes. Terpenoid have also been shown to decrease blood sugar level in animal

studies. Steroids show analgesic properties as well as anti-inflammatory and analgesic properties.

Saponin stimulates the release of insulin and blocks the formation of glucose in the blood stream which affirms the potential use of this plant in the treatment of diabetes and other diseases [14].

The UV spectrum of the isolates (table 4) showed major absorption bands at longer wavelengths (> 300 nm) and shorter wavelengths (< 250 nm). These can be attributed to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions respectively. These involve the promotion of electrons from occupied molecular orbitals (π , n) to unoccupied molecular orbitals (π^*). These transitions generally occur in chromophores that contain an O, N or S atom such as C=O, COOR, COOH, CONH₂, R-SH etc. (Pavia et al., 2001). However, the absence of N-H and S-H bands as well as the presence of O-H and C-O bands in the IR spectrum of the isolated compounds shows that their UV absorption bands are due to C=O, COOR or COOH functional groups.

The IR analysis of the isolates (table 5) showed a very broad absorption band between 3440 and 3200 cm⁻¹ which is indicative of the presence of H-bonded hydroxyl (O-H_{str} of an alcohol) functional group. The compounds also showed peaks between 3000 and 2850 cm⁻¹ which indicates the presence of an sp³ C-

H_{str} (asymmetric and symmetric) and 1470 to 1370 cm^{-1} indicative of $C-H_{bend}$ of an alkane as well as 3100 to 3000 cm^{-1} and 1680 to 1600 cm^{-1} depicting the presence of an $sp^2 C-H_{str}$ and $C=C_{str}$ of alkenes/aromatics respectively.

The strong absorption observed in the spectra of these compounds between 1705 to 1760 cm^{-1} indicates the presence of a carbonyl ($C=O_{str}$) functional group while those observed between 1300 to 1000 cm^{-1} are due to $C-O_{str}$. The weak to medium absorption observed in the spectra between 900 to 690 cm^{-1} is characteristic of $C-H_{bend}$ of aromatics. The melting point of the isolates gives an idea on the degree of purity of that particular isolate. A sharp melting point indicates that the isolate is pure whereas an impure isolate will melt over a long temperature range. Hence, from the result obtained (table 6) compound D and G are pure whereas the rest are impure and was subjected to further purification process before characterization.

4. CONCLUSION

The result of this study shows the presence of some phytochemicals such as Tannins, alkaloids, saponins, cardiac glycosides, sterols and terpenoids in the root of *Maranthes polyandra* and these are responsible for its therapeutic effects and supports its potential use in traditional medicine. The isolates shows the presence of hydroxyl (-OH), carbonyl ($C=O$), alkene/aromatic ($C=C$) and ester ($C-O$) functional groups as well as C-H group usually found in saturated hydrocarbons. Information obtained from UV showed $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions characteristic of carbonyl functional groups.

RECOMMENDATION

It is recommended that further spectroscopic analysis such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and x-rays analysis be carried out for complete elucidation of the structure of these isolates.

Conflict of Interest Declaration

The author(s) declare NO Conflict of Interest.

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