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Document heading **doi: 10.21276/apjhs.2017.4.2.10** **Research article**

Standardization and evaluation of anticonvulsant activities of leaf extract and fractions of *Waltheria indica* (Baudilio) (Malvaceae)

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ABSTRACT

This study evaluated the anticonvulsant property of *Waltheria indica* leaf extract and fractions. *Waltheria indica* leaves were harvested, identified with herbarium specimen deposited, air-dried and pulverized. The pulverized leaves were soxhlet extracted using aqueous methanol. The crude extract was dried *in vacuo*. Phytochemical, pharmacognostic standards and toxicity evaluations were carried out. The crude extract was screened for anticonvulsant activity at concentrations of 250mgkg^{-1} and 500mgkg^{-1}; and subsequently subjected to liquid- liquid partitioning with n-hexane, chloroform, n-butanol, methanol and water, to obtain the respective fractions. These fractions were screened for anticonvulsant activity. The most active fraction was subjected to vacuum liquid chromatography (VLC) and the VLC fractions screened using analytical HPLC. The leaf contains saponins (7.2%), alkaloids (10.2%) and flavonoids (6.8%); while proximate analysis showed moisture content of (15.83%), total ash (6%) and acid insoluble ash (5%). The LD_{50} was > 5000mgkg^{-1}. Anticonvulsant evaluations showed that at 250mgkg^{-1} chloroform showed protection of (80%), crude extract (40%), methanol fraction (40%), n-hexane (20%) and n-butanol (20%). At 500mgkg^{-1} chloroform fraction showed protection of (100%), crude extract (60%), methanol (60%), n-butanol fractions (60%) and n-hexane (40%). The HPLC screening identified phyto-compounds with reported anticonvulsant activity (Astragalin, p-Hydroxybenzoic acid, (z)-Oct-2-ene-1, 3, 8-tricarboxylic acid, (z)-2-(7-hydroxyoctyl) pent-2-enedioic acid and Septicine). *Waltheria indica* leaf extract is non-toxic and significantly delayed the onset of convulsion and onset of death due to convulsion and reduced the number of convulsion per minutes. The protections given were dose dependent. This study established anticonvulsant property of *Waltheria indica* leaf, thus supports its ethnomedicinal use.

Keywords: *Waltheria indica*, anticonvulsion, proximate, LD_{50}, HPLC, phyto-constituents.

Introduction

Convulsion has been a global threat. It is a children disease that is characterized by jerking or fitting as a result of abnormal electrical discharge in the brain and lasts for few minutes when it sets in. It can occur at anytime of the day and may cause death before getting medical attention. Much effort has been directed over the past years to evaluate the role of available anticonvulsant drugs in the management of convulsion.

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Unfortunately, valuable comparisons among various studies published are difficult [1]. This is because there are differences in the scheme of evaluation applied. The patient groups are not comparable in terms of age, onset of convulsion and treatment choice [2]. Criteria for evaluation of result are sometimes not indicated and the distribution of factors affecting the treatments is usually different among groups [3].Since 1990s, vigabatrin an inhibitor of gamma- aminobutyric acid A (GABA A) receptor or GABA transminase, has been used and shown to be successful in the resolution of infant convulsion, especially for those associated with tuberous sclerosis and has been a first line therapy, but its potential association with irreversible visual field

study to identify has restricted its use in certain parts of the world [4], however, alternative treatment including *Alum sativum* , *Telferia occidentalis* and *Cassy thafili*, *Waltheria indica* has been used locally. Although these were not yet evaluated, they have been used with variable success. Apart from the fact that, they have not all been evaluated but locally they have given success to the herbalist. In traditional medicine *Waltheria indica* has been used in various treatment of infection; however, the relevant pharmacological bases for these indications are often missing in traditional medicines. Decoction is used in the treatment of convulsion, wounds, skin ulcer, rheumatism and cancer [5]. Maceration and juices were mostly used to treat eye diseases [6]. Infusion of the leaves or roots was used to treat gingivitis and anemia [7]. Chewing of the root is also used by some populations. The root is chewed by Bissa community in Burkina-Faso against sore throat and to treat internal haemorrhage [8]. *Waltheria indica* is used for the treatment of minor ailments (e.g. sore throat, cough, erectile dysfunction) and complicated ailments (like inflammation and asthma). In Hawaii, *Waltheria indica* is considered as one of the ten most recognized medicinal plants. Locally, it is used as aspirin-like anti-inflammatory drugs [9]. In this regard various extracts are used in the management of inflammation and inflammatory conditions. *Waltheria indica* is used in the treatment of diarrhea by traditional healers in Nigeria [10]. In addition, it is traditionally used to treat malaria, dysentery, haemorrhoids, leprosy and epilepsy [11], infertility and bladder ailments [12], erectile dysfunction and impotence [13].The search for alternative drug to use in convulsion, at what duration and the evaluation of the efficacy is the cardinal point and is of great research interest**.** It is a considerable fact that convulsion occurs spontaneously; therefore the absence of anticonvulsant drug can lead to major complications such as brain damage or death. Not every drugs used for convulsion management are allowed to sell locally. This research was carried out with the view to determining the anticonvulsant property of the leaf extract and fractions of *Waltheria indica*; and to ascertain its mechanism of action by determining the constituents responsible for this activity. *Waltheria indica* plant is of the kingdom Plantae, super-division Embroyophyta, division Tracheophyta, sub-division Spermatophytina, class Magnoliopsida, sub-order Rosanae, order Malvales, family Malvaceae, genus Waltheria and species *Waltheria indica.*

Fig 1: Picture of *Waltheria indica* leaves

Methods

Plant collection*:* A fresh *Waltheria indica* plant was collected in June, 2014 in an abandoned farm land in Ebonyi state. Its taxonomic identification was confirmed by Prof. B.A. Ayinde. The leaves were removed and air dried for four weeks and oven dried for two hours before blending with a milling machine.

Extraction: 1100 gram of the powdered sample *Waltheria indica* leaf was soxhlet extracted using methanol. The extract was concentrated using rotary evaporator under reduced pressure. The concentrated sample was dried, stored and the yield determined.

Phytochemistry and proximate analysis of *Waltheria indica* leaf extract: Phytochemical screening and proximate analysis were carried out on the leaf to determine the presence of some phyto-compounds and admixtures in the sample according [14, 15]. In this process, the following were determined: Moisture content, total ash, acid insoluble ash, water soluble ash and water soluble extractive.

Determination of moisture content

An evaporating dish which has been heated to constant weight and stored in desiccator was taken. A 3 g of powdered *Waltheria indica* leaf was accurately weighed into the dish. It was placed in an oven set at 100-105 ^{0}C and was dried for 5 hours and the sample was weighed again. The drying and weighing was continued at one hour intervals till the difference between two successive weighing corresponded to not more than 0.25 %. Constant weight was reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator showed not more than 0.01 g difference. The moisture content was the total weight lost expressed as the percentage of the initial weight of sample.

$$\% \text{ moisture content: } \frac{\text{Differencein average weight - weight of empty evaporating dish}}{\text{weight of the powdered sample}} \times \frac{100}{1}$$

Determination of total ash

The nickel crucible which has been heated, cooled and stored in a desiccator was taken a 2 g of powdered sample of *Waltheria indica* leaf was weighed into it. The powdered sample was spread in an even layer. It was heated gently in the fume cupboard till all the moisture has been driven off and the material has been completely charred. The flame was gradually increased to (450 ^{0}c) until the residue was completely white indicating that it was free from carbon, and then it was cooled and weighed. It was continued with reference to air dried sample. It was carried out in triplicate.

$$\text{Percent total ash: } \frac{\textit{weight} \text{ of sample + crucible - Initial wt of crucible}}{\text{weight of dried sample}} \times \frac{100}{1}$$

Determination of acid insoluble ash

The total ash obtained after burning the sample in a crucible was mixed with 25 ml of dilute hydrochloric acid and allowed to stand for 5 minutes covering the crucible with a watch glass. It was filtered to collect the insoluble matter on an ash-less filter paper. Later, the watch glass and crucible was washed in a hot water and was passed through the filter paper. The washing was continued till the insoluble matter was completely free from acid (i.e. until the filtrate was neutral) and the solid on the tip of the edge of the filter paper was washed. Acid insoluble ash was expressed as weight differences in reference to the initial weight of the sample.

$$\text{Percent acid insoluble ash: } \frac{\textit{weight} \text{ difference}}{\text{weight of dried sample}} \times \frac{100}{1}$$

Determination of water soluble ash

The ashed sample was put in a crucible and was mixed with 25 ml of water. It was boiled for 5 minutes. Then it was filtered into a beaker and put back into a glass crucible. The insoluble matter in a glass crucible and filter paper was washed with a hot water, and ignited in the crucible for 15 minutes at a temperature not more than 450^{0}c. The weight of this residue was subtracted from the weight of the total ash. The content of water soluble ash was calculated in reference to the air dried sample.

$$\text{Percent water soluble ash: } \frac{\textit{Final} \text{ weight of crucible - total ash}}{\text{weight of the dried sample}} \times \frac{100}{1}$$

Determination of water soluble extractives

A 5 g of *Waltheria indic*a leaf powder was accurately weighed into a stoppered conical flask. A 100 ml of water was added into the conical flask and firmly covered and macerated for 24 hours. It was shaken for 6 hours by placing the flask on the mechanical shaker and allowed to shake slowly and after 6 hours, the flask was removed and allowed to stand for further 18 hours. It was filtered rapidly, taking precautions against loss of filtrate. A 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed evaporating dish. It was then dried to constant weight at 105 ^{0}C in the oven and weighed again. The percentage water –soluble

extractive was calculated with reference to the air-dried sample. The experiment was carried out in triplicates.

Percent water soluble extractive: $\frac{\textit{weight} \text{ of evaporating dish + sample - weight of empty dish}}{\text{weight of dried sample}} \times \frac{100}{1}$

Acute toxicity test: A 5 gram of concentrated extract was reconstituted using Tween 80 and used to carry out acute toxicity test. A total of 9 rats were used for each phase, while feeding of the animals were withdrawn 12 hours before the experiment to determine the safety of the drug. Each phase took 24 hours with first phase taking 10 m/kg, 100 m/kg and 1000 m/kg of the extract while phase two took 2000, 3000 and 5000 m/kg of the extract based on their body weight [16].

***In vivo* anticonvulsion assay of the crude extract:** The crude extract was screened for anticonvulsion activity using the method described by Abubakar and Adelaiye, 2008; using 25 rats grouped into 5. The crude extract was administered to the animals orally in three concentrations of 125 mg/kg, 250 mg/kg and 500 mg/kg of their body weight. One hour later 120 mg/kg of pentylenetetrazole (PTZ) was administered intraperitoneally. The time of administration of PTZ, onset of action, duration of convulsion, onset of death were noted and the result obtained was expressed as MEAN + SEM. Also, the percentage death and percentage protection were calculated [17].

***In vivo* anticonvulsion assay of the fractions**

A portion of the crude extract (3/4) was partitioned using n-hexane, chloroform, n-butanol, methanol and water. The respective fractions were concentrated and used to carry out anticonvulsant test using (Abubakar and Adelaive, 2008) method. A total of 60 rats, divided into the groups of five were used. Each fraction was administered to the animals orally in two concentration of 250 mg/kg and 500 mg/kg of their body weight. One hour later 120 mg/kg of PTZ was administered intraperitoneally. The time of administration of PTZ, onset of action, duration of convulsion, onset of death were noted and the result obtained was expressed as MEAN + SEM. Also, the percentage death and percentage protection were calculated.

Vacuum Liquid Chromatography (VLC): VLC was carried out on the most active chloroform fraction using gradient chloroform: ethyl acetate solvent mix according to [18] method. The column was eluted with gradient chloroform/ ethyl acetate solvent system (10:0 to 0:10 ratios).

Bulking of fractions by thin layer chromatography (TLC) of the VLC fractions: The different fractions of VLC were spotted on TLC plates that have been activated in an oven set at 150 degree centigrade for 5 minutes. It was allowed to dry before dipping each into the TLC tank containing n-hexane and ethyl acetate solvent mix (in the ratio of 140:60), pre- saturated with the solvent system vapour. The TLC plate was allowed to develop. The TLC plate was removed; allowed to dry before spraying with concentrated tetraoxosulphate (VI) acid and was viewed in ultra-violet light chamber. The fractions of VLC were bulked according to their similarities as viewed through ultra-violet light chamber. The bulked fractions were subjected to analytical HPLC for effective detection of compound present in each fraction.

Analytical High performance liquid chromatography (HPLC) screening of the VLC fractions

In the HPLC screening, 2 mg each of the VLC fractions was reconstituted with 2 ml of HPLC grade methanol. The mixture was sonicated for 10 minutes and thereafter centrifuged at 3000 rpm for 5 min. 100 µL of each dissolved sample was transferred into HPLC vials containing 500 µL of HPLC grade methanol. HPLC analysis was carried out on the sample using a Dionex P580 HPLC system coupled to a photodiode array detector (UV3440S, Dionex Softron GmbH, Germering, Gremany). Detection was at 235, 254, 280 and 340 nm. The separation column (125 x 4 mm; length x internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nano-pure water adjusted to pH 2 by addition of formic acid and methanol was used as eluent.

Statistical analysis of results: The statistical significance was evaluated using one way analysis of variance (ANOVA). The values were expressed as mean ± standard error of mean (SEM). $P \leq 0.05$ was considered statistically significant.

Results

Percentage Yield: The percentage yield was calculated at the end of the extraction, concentration and drying, using the expression below:

$$\text{Percentage (\%) yield} = \frac{\textit{weight} \text{ of the extract in (g)}}{\text{weight of the powdered sample}} \times \frac{100}{1}$$

The yield was 220g crude extract (from the 1100g of the dried pulverized leaves), showing a percentage yield of 20%

Result of qualitative phytochemical screening: Table 1 below shows the result of qualitative phytochemistry screening of *Waltheria indica* leaf.

Table 1: Result of the phytochemical of *Waltheria indica* leaf

Alkaloids	Saponins	Tannins	Flavonoids	Steroids	Terpenoids	Cardiac glycosides	Proteins
+ +	+ + +	+ +	+ +	+ +	-	+ +	-

Key: + = Trace or mildly present, + + = Moderately present, + + + = Abundantly present, - = Absent

Result of quantitative phytochemical screening: Table 2 below shows the result of the quantitative phytochemical screening of the leaf extract of *Waltheria indica* leaf.

Table 2: Result of quantitative phytochemical screening

Phyto-constituents	Percentage
Alkaloids	**10.2**
Flavonoids	**6.8**
Saponins	**7.2**
Tannins	**0.2**

Acute Toxicity Test: The results of the acute toxicity tests are presented in Tables 3 and 4.

Table 3: Acute toxicity Test: Phase 1

Groups	Wt of animals in (g)	Dosage in mg/kg	Dosage in ml	No of Death
1	32.9(sterile water)	10	0.34	**0/3**
2	33.6(crude extract)	10	0.34	**0/3**
3	37.9(sterile water)	100	0.38	**0/3**
4	**38.8(crude extract)**	**1000**	**0.39**	**0/3**

Table 4: Acute Toxicity Test: Phase 2

Groups	Wt of animals in (g)	Dosage in mg/kg	Dosage in ml	No of Death
1	32.9(normal saline)	10	0.34	**0/1**
2	39.5(crude extract)	2000	0.62	**0/1**
3	40.8(crude extract)	3000	0.80	**0/1**
4	**43.7(crude extract)**	**5000**	**0.88**	**0/1**

Results of proximate analysis of *Waltheria indi*ca leaf: The results of the proximate analysis of *Waltheria indi*ca leaf is presented in Table 5.

Table 5: Proximate analysis of *Waltheria indica* leaf extract

Parameters	Percentage
Total ash	**5.80**
Acid insoluble ash	**5.00**
Water soluble ash	**1.50**
Moisture content	**15.83**
Water soluble extractive value	**0.60**

Anticonvulsant Activity: Result of anticonvulsant activity of *Waltheria indica* leaf extract is presented in Table 6. It showed the anticonvulsant activity of *Waltheria indica* leaf extract on PTZ induced convulsion in rat.

Table 6: Anticonvulsant activity of *Waltheria indica* leaf extract

Group	Pre-treatment	Dose mg kg^{-1}	Time of administration (minutes)	Onset of action (minutes)	Duration of convulsion (minutes)	Onset of death (minutes)	No of Death	No of alive
1	NC	5 ml	9.00±0.01	9.01±0.02	59.01±0.45	0.01±0.01	5	**0**
2	HF	250	9.07±0.01	9.08±0.01	53.41±1.36	0.01±0.03	4	**1**
		500	9.07±0.01	10.09±0.04	51.61±0.92	0.02±0.01	3	**2**
3	CF	250	9.18±0.01	9.22±0.01	39.82±2.81	0.08±1.09	1	**4**
		500	9.32±0.02	9.41±0.03	35.22±2.51	-	-	**5**
4	BF	250	9.58±0.08	9.60±0.09	52.62±0.92	0.02±0.01	4	**1**
		500	10.05±0.01	10.07±0.03	50.82±1.32	0.02+0.02	2	**3**
5	MF	250	10.13±0.02	10.18±0.03	55.21±0.92	0.05±0.01	3	**2**
		500	10.18±0.01	10.22±0.01	49.27±3.19	0.04±0.00	2	**3**
6	AF	250	10.32±0.01	10.33±0.01	55.21±1.39	0.01±0.00	4	**1**
		500	10.42±0.02	10.44±0.04	53.62±0.92	0.02±0.02	3	**2**
7	**Valium**	**5**	**10.50 ±1.10**	**5.0±1.20**	**35±0.10**	**-**	**-**	**5**

Key: NC= Negative control (Tween 80), HF= n-Hexane fraction, CF= Chloroform fraction, BF= Butanol fraction, MF= Methanol fraction, AF= Aqueous fraction and Valium= Positive control. Observation was taken on the time of administration of the drug, the on-set of action, duration of convulsion and on-set of death; all calculate in minutes and expressed as mean ± SEM and analyzed using ANOVA; where values of $p < 0.05$ were considered statistically significant.

Analytical HPLC analysis results

The analytical HPLC results were assessed using UV scan at 235nm. This was because most of the components were detected best at this wavelength compared to wavelengths of 254nm, 280nm and 340nm at which the detection was also evaluated. The HPLC chromatogram of each fraction showed that the chromatogram obtained at 235nm had more compounds detected than those obtained at the other three wavelengths at which this screening was equally carried out.The HPLC machine had an in in-built library of UV scans of compounds with which it compares the UV scans of the component of each fraction eluted; and the comparison expressed as hit. Based on the similarity with data in an inbuilt library (hit values), the close comparison of the UV scans of the component and that of the library; and the literature review of the UV scan; the following compounds were preliminarily identified. Table 7 below shows the summary of preliminarily identified compounds.

Table 7: Summary of preliminarily identified compounds

Fractions	Comparable Peak
VLC F1	A= Astragalin
VLC F2	A= (12-0-deacetyl -12-epi-scalarin), B= (P-hydroxybenzoic acid), C= (protocatechus, ure), D= ((z) –oct-2-ene- 1, 3, 8 tricarboxylic acid), E= (Tensidol B), F= (4-hydroxybezoyl –deltalactame) and G= (Dipiperamide).
VLC F3	A= (Citreonigrin) and B = (z) – 2- (7-hydroxybenzoic) pent -2- enedioic acid).
VLC F4	A= (Septicine) and B= (Scalarolide)

Key: VLC F1 to VLC F4= Vacuum liquid chromatographic fractions 1 to 4.

The analytical HPLC chromatograms of the vacuum liquid chromatographic fractions are presented in Figures 2, 4, 7 and 9 for fractions 1- 4 respectively. The corresponding UV scan or spectrum for each component of each fraction are presented in Figures 3, 5 and 6, 8 and 10 respectively for fractions 1- 4.

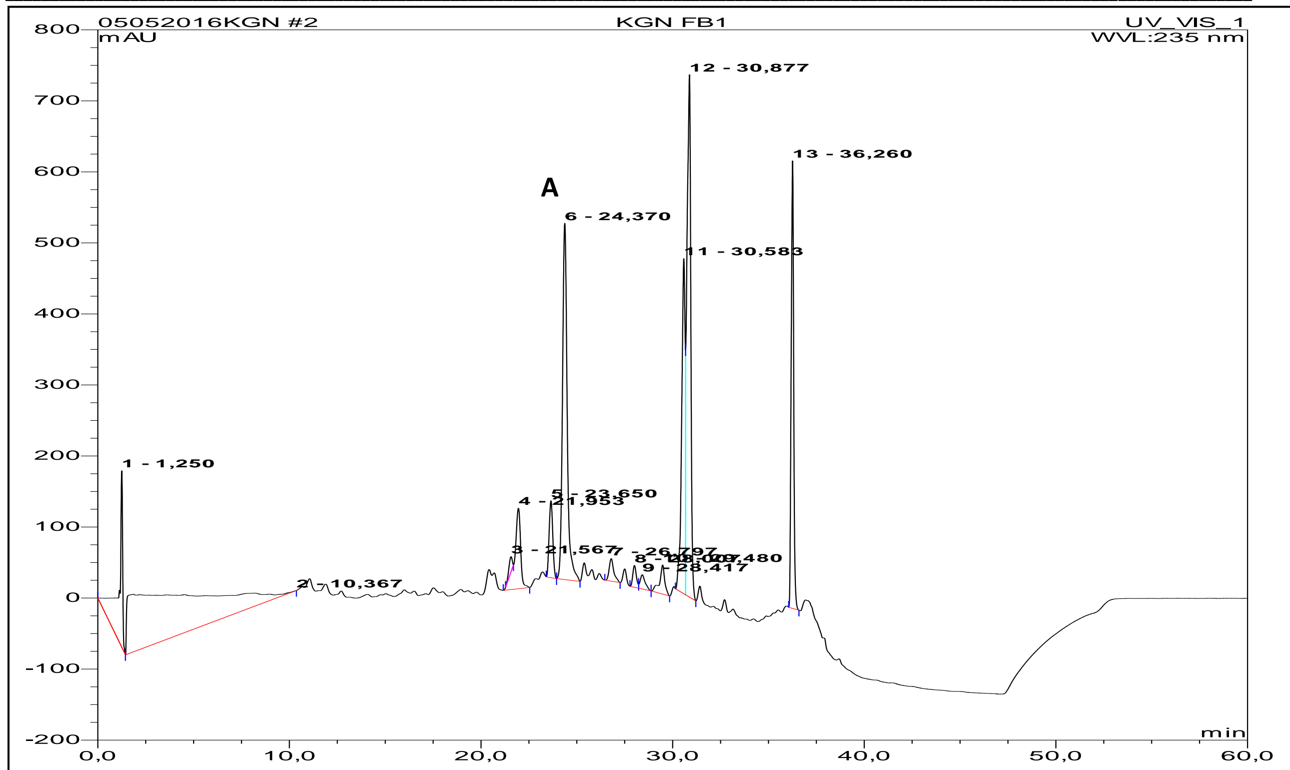

Fig 2: HPLC Chromatogram of VLC F1 showing A= (Astragalin)

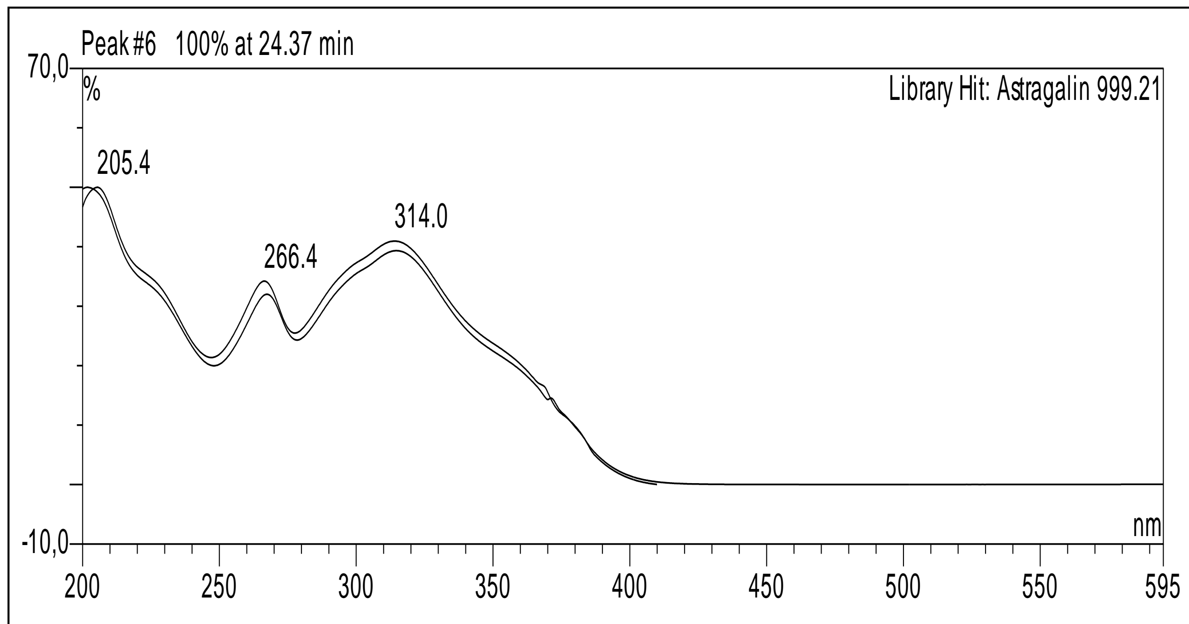

Fig 3: UV spectra of Astragalin detected in VLC F1

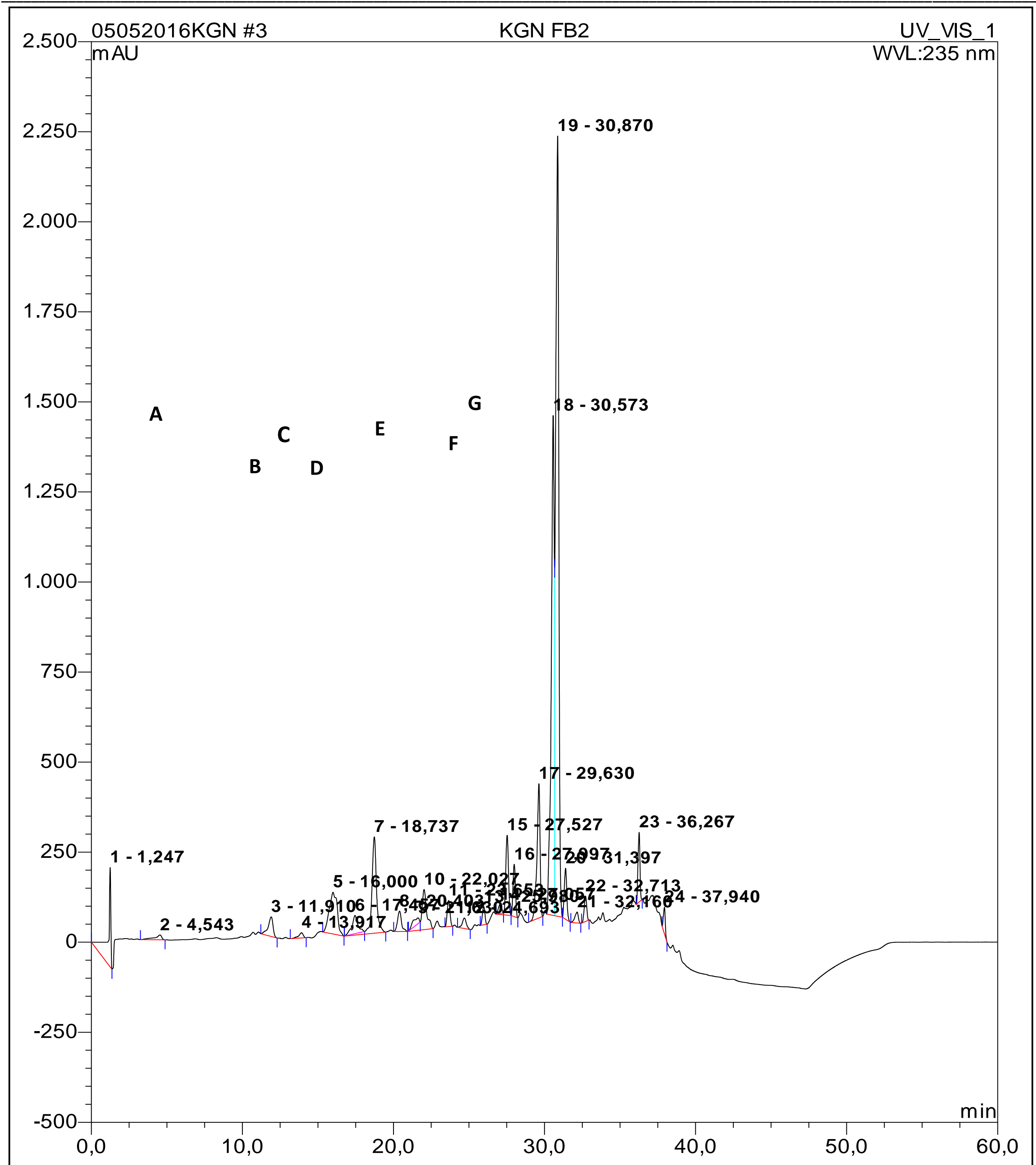

Fig 4: HPLC Chromatogram of VLC F2 showing A (12-0-deacetyl -12-epi-scalarin), B (P-hydroxybenzoic acid), C (protocatechus, ure), D ((z) –Oct-2-ene- 1, 3, 8 tricarboxylic acid), D (Tensidol B), F (4-hydroxybezoyl – deltalactame) and G (Dipiperamide).

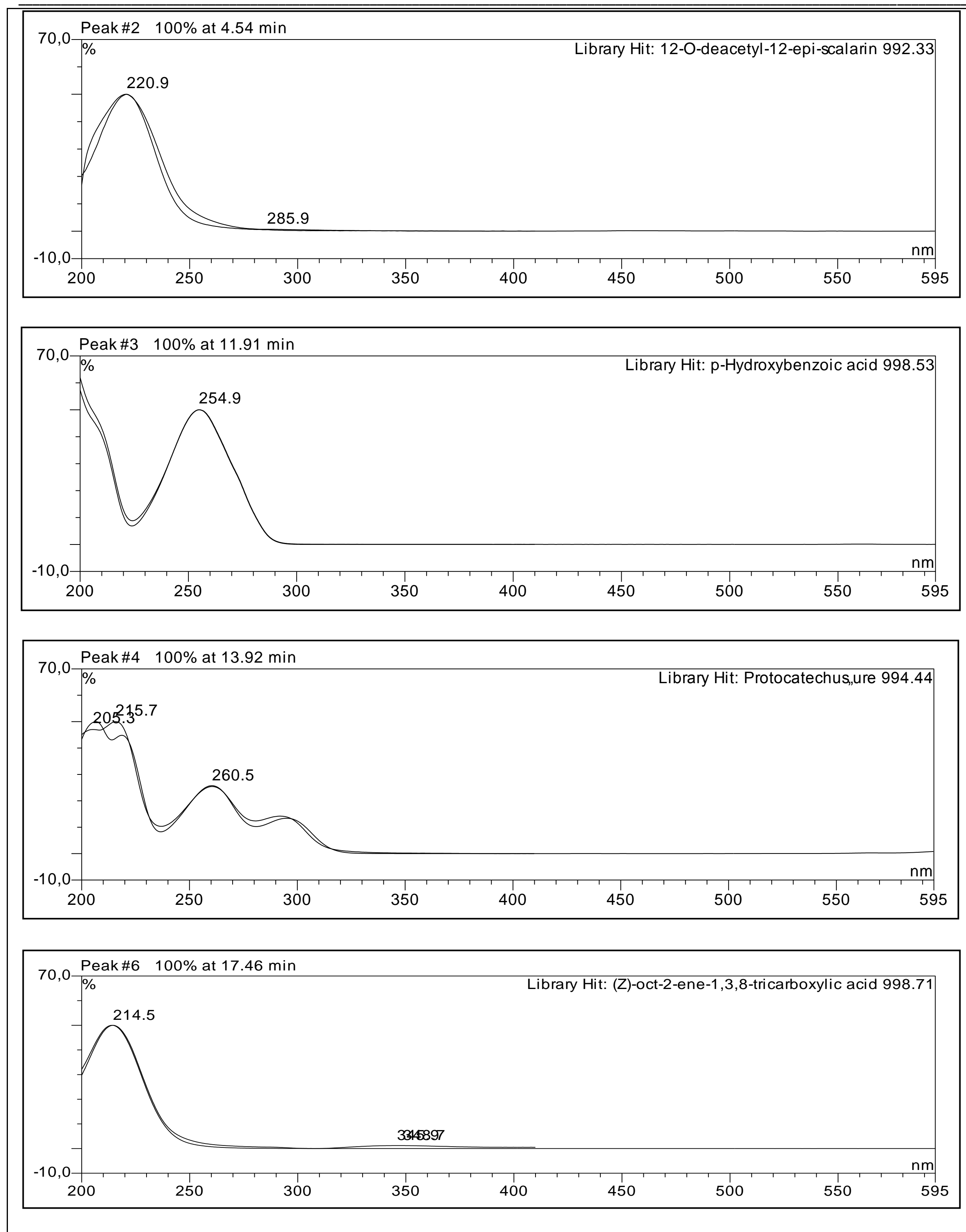

Fig 5: UV scans of detected components of the HPLC chromatogram of VLC F2 (UV spectra of 12-o-deacetyl -12-epi- scalarin, p-hydroxybenzoic acid, protocatechus, ure, (z) – Oct-2-ene- 1, 3, 8 tricarboxylic acid)

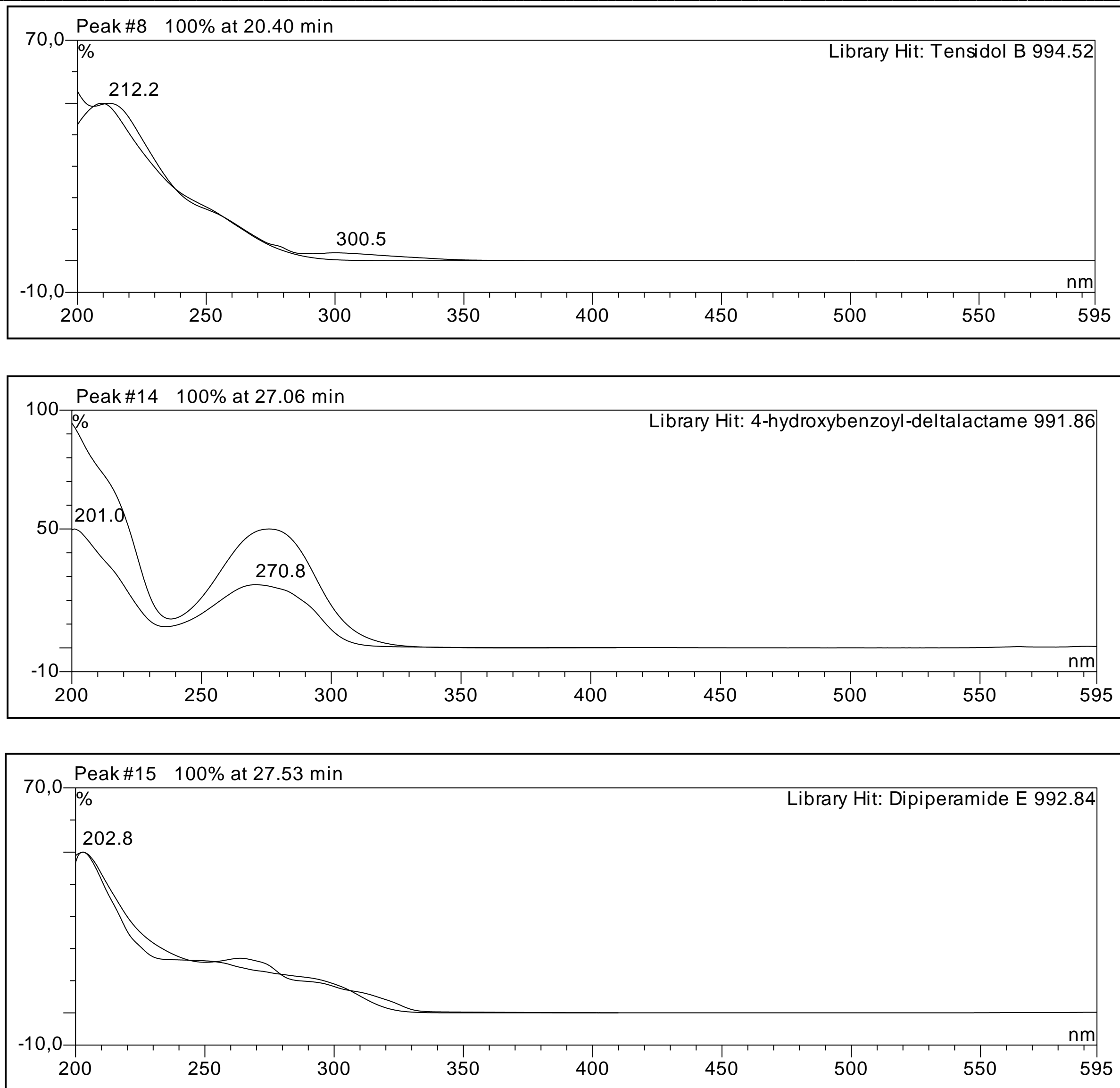

Fig 6: UV scan of detected components of the HPLC chromatogram of VLC F2 (UV spectra of Tensidol B, 4-hydroxybenzoyl–deltalacteme and dipiperamide).

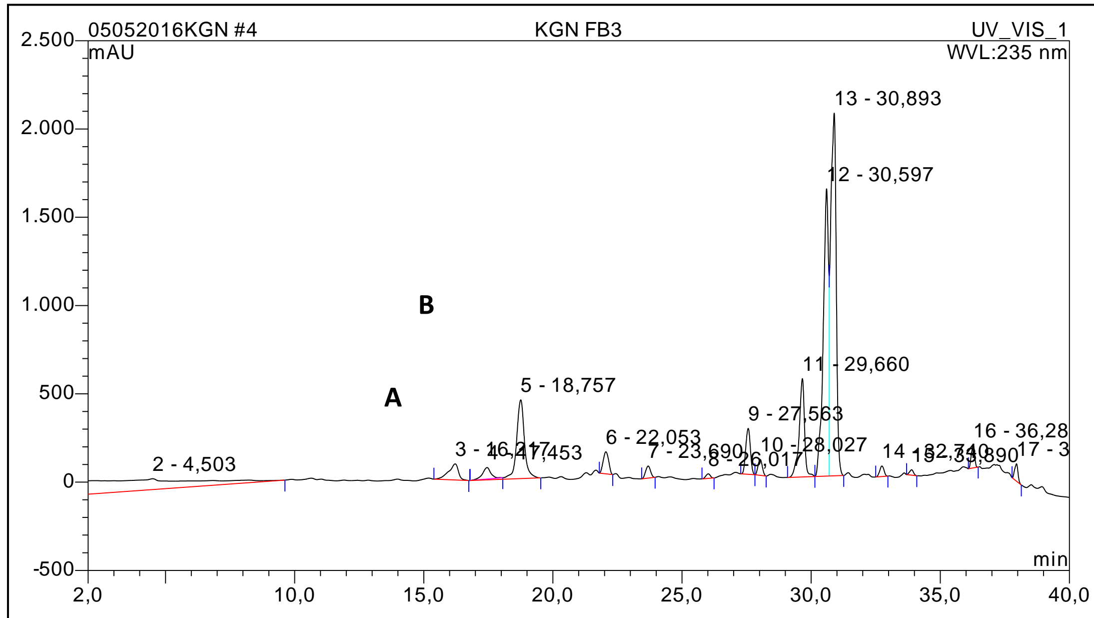

Fig 7: HPLC Chromatogram of VLC F3 showing A= (Citreonigrin) B = (z) – 2- (7-hydroxybenzoic) pent -2-enedioic acid)

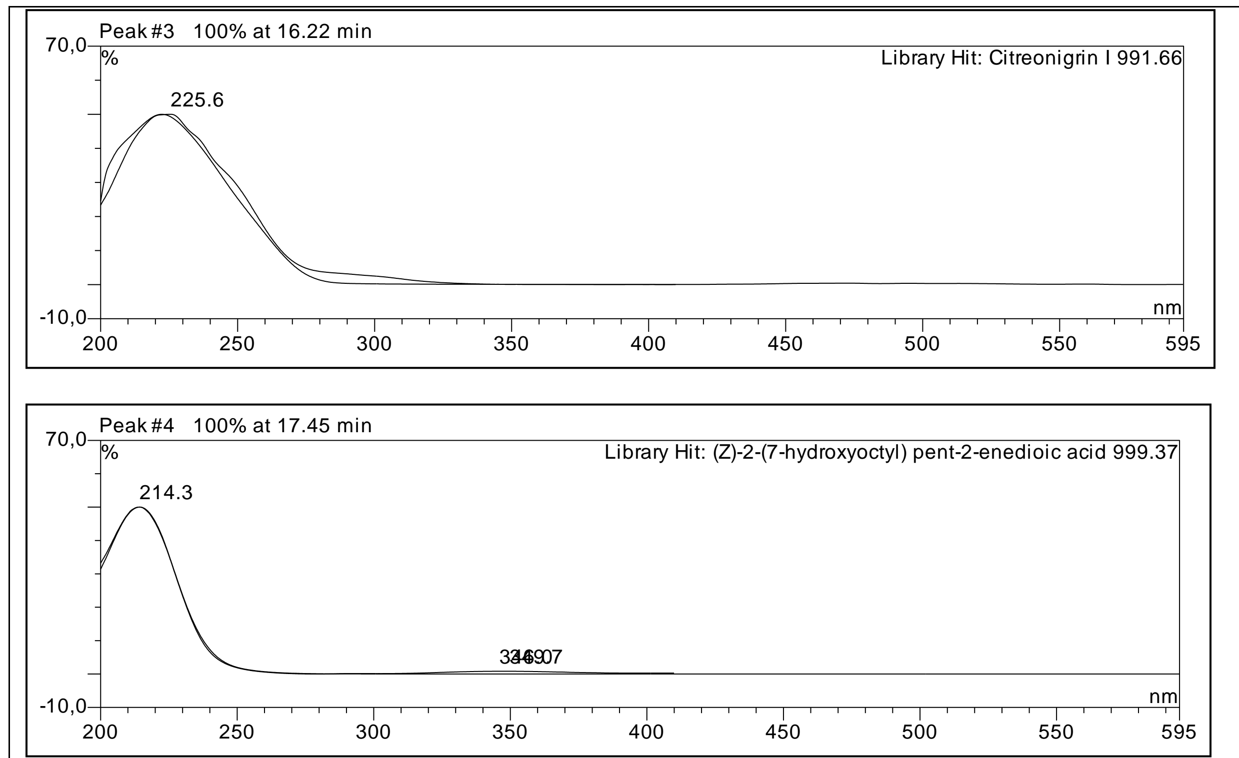

Fig 8: UV spectra of citreonigrin I and (z) -2- (7–hydroxyoctyl) pent -2-enedioic acid detected in VLC F3

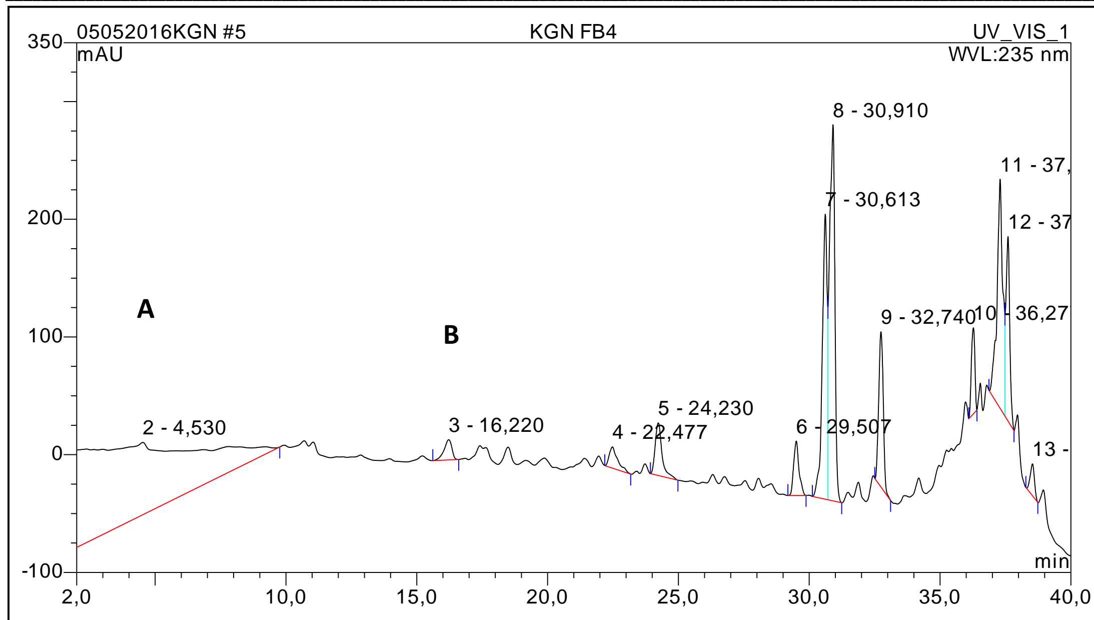

Fig 9: HPL chromatogram of VLC F4 showing A= (Septicine) and B= (Scalarolide)

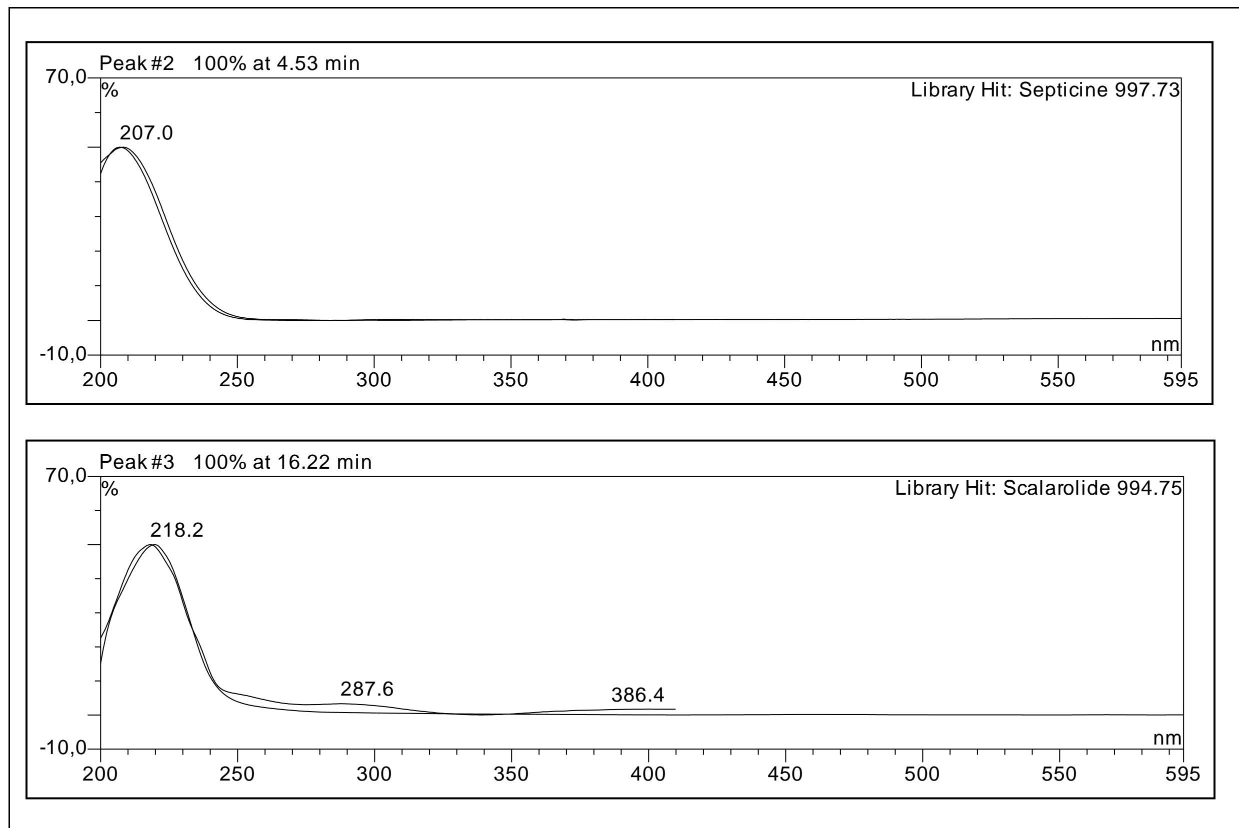

Fig 10: UV spectra of septicine and scalarolide detected in VLC F4

Discussion

The results of qualitative analysis in (Tables 1) showed that *Waltheria indica* leaf extract contained saponins, flavonoids, alkaloids, tannins, steroid and cardiac glycoside. This result was similar to that obtained by [19], [20]. The results of quantitative analysis of the crude extract of *Waltheria indica* leaf (Table 2) showed that *Waltheria indica* leaf extract contains saponins (7.2%), flavonoids (6.8%), alkaloids (10.2%), tannins (0.2%). Alkaloids have been reported and known for their central nervous system depressant activity [21]. Steroids have equally been reported for its use as an anticonvulsant and in the treatment of erectile dysfunction [22].In the median lethal dose test (Tables 3 and 4); the result indicated no death in the two phases of the test. The LD_{50} was thus established to be > 5000 mg/kg and the sample is hence considered relatively non-toxic and safe for use and supports its uses in ethnomedicines.The results of proximate analysis of *Waltheria indica* leaf (Table 5) showed total ash (5.8%), acid insoluble ash (5.0 %), water soluble ash (1.50%), moisture content (15.83%) and water soluble extractive is (0.60%). In this result, moisture content of (15.83 %) could be considered high and could be responsible for its easy microbial infestation [23]. The ash value of (5.8 %) indicates that the plant leaf is of considerable quality [24]. Water soluble ash of (1.5 %) equally supports the good quality of the plant leaf. The methanol crude extract of the leaf of *Waltheria indica* showed central nervous system depressant activity (Table 6). The result of this study showed that *Waltheria indica* leaf extract possessed anticonvulsion property. In general, the chloroform fraction of the leaf extract of *Waltheria indica* plant was found to be most active in the protection from death due to the pentylenetetrazole (PTZ) induced convulsion, as shown in Tables 6. The anticonvulsant activity was dose dependent, as it varied with the doses of the extract and fractions administered in the different groups. The chloroform fraction showed protection of (80 %) at 250 mg kg^{-1} and (100 %) at 500 mg kg^{-1}, followed by methanol fraction with (40 %) protection at 250 mg kg^{-1} and (60 %) at 500 mg kgm,$^{-1}$ butanol fraction gives (20 %) protection at 250 mg kg^{-1} and (60 %) at 500 $mgkg^{-1}$ while n-hexane showed (20 %) protection at 250 mg kg,$^{-1}$ and 40 % at 500 mg kg^{-1} and aqueous extract showed protection of (20 %) at 250 mg kg,$^{-1}$ and 40 % at 500 mg kg.$^{-1}$. Although, all the fractions protected the rat to varying degree in each case; some were more effective than the other. This effectiveness is attributed to the difference in solvent polarity because chloroform is moderately polar with polarity index of 4.1 [25] it mainly extracts steroids and alkaloids, and this played a major role in the management of convulsion as it acts through hypothalamic – pituitary adrenal axis, to stimulate glucocorticoid synthesis. Glucocorticoids are thought to interact with central nervous system steroid receptors that act with central nervous system steroid transcriptional regulator to influence voltage-dependent calcium channels. It may stimulate neuro-steroid synthesis in cells neurons, such as deoxycorticosterone (DOC) and tetrahydrodeoxy– corticosterone (THDOC) both are allosteric modulators of $GABA_A$ receptors [21] .The effectiveness also increases with the increase in the dosage of the drug. This shows that further increase in the dose above 500 mg/kg may completely protect the rat from death or from convulsing at all. The chloroform fraction of the leaf extract at 500 $mgkg^{-1}$ was found to have completely prevented death of animals resulting from PTZ induced convulsion; a finding which is similar to the finding of [17], that aqueous ethanolic extract of aerial portion of *Waltheria indica* plant completely prevented death of animal due to penthylenetetrazole induced convulsion but failed to protect convulsion due to strychnine suggesting differences in their mechanism of action. However, this study disagreed with the assertion that most drug with anticonvulsant property do not counteract penthylenetetrazole convulsion but retards them [26]. The analytical HPLC result revealed the presence of the following compound astragalin, a good antioxidant [27]; 12-0-deacetyl -12-epi-scalarin, an inhibitor [28], sediative [29]; *P*-hydroxybenzoic acid, an antibacterial [30], antimalarial [31]; protocatechus,ure, an antisychotic [32]; (z) – oct -2- ene- 1,3,8 carboxylic acid, an inhibitor [33]; tensidol B, an antischizpherenial, delusion [34]; 4-hydroxybenzoyldeltalactame, an anti-allergic [35], an antibiotic [36]; dipiperamide E, an inhibitor of drug metabolism [37]; Citreonigrin I, a cytotoxic agent [38]; Septicine, an anticonvulsant and muscle relaxant [39] and scalarolide, an antitumor [40]. These preliminarily identified compounds from *Waltheria indica* leaf extract could be acting synergistically in conferring the plant leaf its observed anticonvulsant activity and responsible its various ethnomedicinal uses.

Conclusion and recommendations

In conclusion, the results of qualitative, quantitative phytochemical and physicochemical analyses have shown that *Waltheria indica* leaf extract is a potential crude drug of a considerable quality that could be used

in the management of convulsion and other related cases. The extract of *Waltheria indica* leaf delayed the on-set of convulsion and prolonged on set of death due to pentylenetetrazole (PTZ) induced convulsion. The result of the study equally showed that *Waltheria indica* leaf extract contained relevant phytocompounds which are active against pentylenetetrazole (PTZ) induced convulsion, hence supports the ethnomedicinal use of the plant as an anticonvulsant agent. The findings of the study have established anticonvulsion property of the plant leaf, hence, the data obtained will add to the large body of evidence collected scientifically to show the immense potentials of medicinal plants use in various traditional medicine systems.

Recommendation

In recommendation**,** further study is required in order to establish the mechanism of action of the leaf extract on the central nervous system and for more detailed and conclusive characterization of the leaf extract.

References

1. Akanuma H., Seckijima Y., Tokuda T. A case of severe status epileptics of frontal lose origin suceessfully treated with corticosteroids. Japanese Rinsho Shinkegaku 2010; 38: 461 – 464.
2. Pieribone V.A., Tba J., Soufflet C. Clinical evaluation of ganaxolone in pediatric and adolescent patients with refractory epilepsy. Epilepsia 2007; 48: 1870-1874.
3. Sorel L. A propos of 196 cases of infantile myoclonic encephalopathy with hysarrthmia treatment with purified ACTH. Hazards of synthetic ACTH (French) Rev. Electroencephalogy Neurophysiol Clin 2011; 1: 112-113.
4. Snead O.C. Treatment of infantile spasms. Pediatr. Neurol. 2016; 6: 147-150.
5. Baba-Moussa F., Nacoulma O., Ouatara A., Nguyen H.P., Akpagana K., Bouchet P. Activite antibacterial does extra its aqueux totaux de combretum, micranthum, Lawsonia inermis of Waltheria indica plants. De la Phamacopee ovest Africaine revue de medicines et phamacopee Afrincines 2010; 11: 197 – 203.
6. Blanpin O., Pais M., Quevuviller M.A. Pharmacodynamic study of adovetin Z: Alkaloid of Waltheria Americana L. (Malvaceae). Annals Pharmaceutiques Francaise 2011; 21: 147 – 50
7. Borokini T.I., Omotayo F.O. Phytodemical and ethenobotanical study of some selected Medicinal plants from Nigeria. Journal of Medicinal Plants Research 2012; 6: 1106 – 1118
8. Almagboul A.Z., Bashir A.K., Karim A., Salih M., Farouk A., Khalid S.A. Anti microbial activity of certain Sudanese plants used in Folklric medicine screening for antifungal activities (vi). Fitoterapia 2013; 59:393 – 396.
9. Abbot J.A., Shimazu C. The geographic origin of the plants most commonly used for medicine by Hawailans. Journal of Ethnopharmacology 2012; 14. 213-222.
10. Chhabra S.C., Mahunnah R.L.A., Mshiu E.N. Plants used in traditional medicine in Easthern TaAzania, VI. Angiosperms (Sapotaeae to 2ingberaccae). Journal of Ethnopharmacology 2013; 39: 83-103
11. Bappuji J.L., Ratrian V.S. Traditional uses of some medicinal plants by tribals of Gangaraju Madugula Mandal of Visakhapatnam District, Andhra Pradesh. Ethnobotanical Leaflets 2009; 13: 388-398.
12. Aguilar N.O., Van Valkenburg J.C.C.H. Medicinal and poisonous plants 2. Bachuys Publishers Leiden 2000; pp 585 – 587.
13. Bekkro Y.A., Mamyr B.J.A., Boua B.B., Fezan H., Ehile E.E. Etu deethnobotanique of screening phytochimique de Caesalpinia benthamiana (Baill) Herend at zaruchi (caesalpiniaceae). Science and Nature 2007; 4: 217-225
14. Harbone J.B. Phytochemical Method of London, Chapman and Hall Ltd 2012; pp 49-188.
15. Pulokk and Mukherjee. Quality Control of Herbal Drugs. An Approach to Evaluation of Botanicals Drugs. 2012
16. Lorke D. A new approach to practical toxicity Archives of toxicology. 2012; 53:275-289.
17. Abubakar M.S., Adelaiye A.B., Ayo J.O., Hamidu L.J. Sedative and anticonvulsant effect of ethyl acetate fraction of Waltheria indica in mice. Journal of Pharmacology and Toxicology 2008; 3**:** 261 – 266.
18. Association of Official Analytical Chemist (AOAC). Official Method of Analysis, 16th edn Washington; D.C 1990; 1: 600-792.
19. Jamuna S., Subramaniam P., Krishnamoorthy K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pac. J Trop. Biomed; 2014; 4 (Suppl 1): S359-S367.
20. Olajuyigbe O.O., Babalola A.E., Afolayan A.J. Antibacterial and phytochemical screening of crude ethanolic extracts of Waltheria indica Linn.

African Journal of Microbiology Research 2011; 5(22): 3760-3764.
21. Reddy D.S., Rogawski M.ANeurosteroid replacement therapy for catamenial epilepsy. Neurotherapeutics; 2009; 6: 392-401.
22. Okwu D.E. Evaluation of chemical composition of indigenous species and flavoring agents. J. Pure Applied Science. 2001; 7: 455-459.
23. Mcgee H. On Food and Cooking: The Science and Love of Kitchen. Scriber, New York, 2004 pp: 174.
24. Middleton E.J., Kandaswani C., Theoharides T.C. The effects of plant flavonoid on mammalian cells, implications for inflammation, heart disease and cancer. Journal on Pharmacology, 2000; 52: 673-751.
25. Bake T.A., Benard T.J. Neurological and muscular disorder in children, diagnosis treatment, New York Large Medical book; 18th Edn. 2014
26. Loomis T.A, Hayes A.W. Loomis essential of toxicology 4th edn. California Academic Press 2014; pp 208-245.
27. Kim M.S. Inhibitory effect of astragalin. National University Daegu, Korea 2014; pp 321 – 332.
28. Carmen F., Chiara C.M., Cecile D. Organic Biomol. Chem. London Press 2014; pp 451 – 459.
29. Alasder H., Nelson B. Sediative effect of Bromine component and Brom. Diphether. London Press, 2003; p 346.
30. Narwal S.S. Comparative Immune Modulation by Ferulic acid. Journal of Ethnophamacology 2012; 2: 105 – 107
31. Clarkson C., Maharia V.I., Crouch N.R., Grace O.M., Pillay P., Matsabisa M.G., Bhagrwandin N., Smith P.I., Folb P.I. In Vitro antiplasmodial activities of medicinal plants native to or naturalized in South Africa. Journal of Ethnopharmacology 2016; 92: 171-191.
32. David M. Natural Medicine for Psychiatric Disorder. University Press 2008; p 355.
33. Markyl S.O. Solid Tumor Management. Academic Press 2002; pp 25-30.
34. Sabelli M.C. Sediative Effects of Tensidol. British Press. 2002; pp 106 – 108.
35. Hussan H., Salman M., Abdul J., Alyaa A. Phytochemistry of some plant metabolites. Washington 2012; p 365.
36. Kelvin C. Antimicrobial Sensitivity of Chloramphenicol. University Press 2015; p 258.
37. Natural Research Council. Science and Regulation. American Journal on Ethno-pharmacology, 2000; 5: 266.
38. Chem B.O., Hiton H., Gar T. Anti Oxidative effect of some plant constituents. University Press 2015; pp 95-100.
39. Dam M., Gram L., Philbert A., Hansen B.S. Partial epilepsy and management. UK Press 2007; pp 166-170.
40. Beura D.C (2016). Cancer management and prevention. Academic Press, p 265.

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