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Comparative Proximate and Phytochemical Analyses of the Leaf Extracts of two Species of Vernonia

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Abstract Comparative proximate and phytochemical analyses of two variants of bitter leaf; *Vernonia amygdalina* and *Vernonia colorata* were studied using AAS, UV, IR and TLC. Extraction was done with ethanol using the powdered leaves of the two variants, distilled off, and labelled as: ethanol extract of *Vernonia amygdalina* (EEVA) and ethanol extracts of *Vernonia colorata* (EEVC). The same procedure was repeated with acetone and water to obtain: acetone extracts of *Vernonia amygadalina* (AEVA); acetone extracts of *Vernonia colorata* (AEVC); aqueous extracts of *Vernonia amygdalina* (QsEVA) and aqueous extracts of *Vernonia colorata* (QEVC). Phytochemical screening of the extracts showed the presence of saponins, tannins, cardiac glycosides flavonoids and steroids. TLC result using n-hexane/ethyl acetate (3:1) and chloroform/methanol (4:1). Solvent system on crude ethanol extracts of the plant showed the presence of 2 and 3 spots respectively. Proximate analysis showed: 79.66% moisture, 16.6% protein, 2.75% fats, 8.25% crude fibre, 0.17% ash and 11.33% carbohydrate for *V. amygdalina*, while 79.02% moisture, 11.30% protein, 1.50% fats, 8.40% crude fibre, 0.12% ash and 10.85% carbohydrate for *V. colorata*. Elemental analyses of the samples showed; Zn 221mg/kg, Cu 504.05mg/kg, Fe, 1785.45mg/kg, Mn, 258.85mg/kg, for *V. amygdalina* and Zn, 275.95mg/kg, Cu, 336.45mg/kg, Fe, 1645.5mg/kg, Mn, 236.65mg/kg was obtained for *V. colorata.* The concentration of Fe was observed to be highest. While Pb and Cd were below detection limit of 0.05mg/kg. Infrared spectra analyses showed characteristic absorption bands: C-C1 (609.53cm-^{1}), C-N or C-O 1031.4cm^{-1} and 1039.67cm^{-1} bond stretching, C-H (2850-2926.11 cm^{-1}), OH (3150.77cm-^{1}) for AEVA and AEVC, while C-C1 (607.6cm^{-1}), C-H (2891.39cm^{-1}), C-O (1248.95cm^{-1}) for EEVA and EEVC. QEVA and QEVC showed C=C (2133.34-2136.23cm^{-1}); CH, CH_2 and CH_3 groups 1407.12, 1375 and 1408.8cm^{-1}) for the two variants of *Vernonia* extracts. The extracts were UV-detected from 200-900nm and all showed two distinct peaks around 408 and 665nm. The results of the proximate, TLC and spectra analyses proved that aromatic and aliphatic compounds could be attributed to specific biological activities. Steroidal saponins and tannins are responsible for bitter principles. Comparing the nutrients and chemical constituents with Recommended Dietary Allowance (RDA) values of leafy vegetables studied in Nigeria, the result revealed that the leaves contain an appreciable amount of nutrients, phytochemicals and low level of toxicants.

Keywords Vernonia, proximate analyses, phytochemical screening, extracts

1. Introduction

Plants usefulness to the animal kingdom dates back as the existence of the earth itself. Plants provide food, fuel and shelter hence each of these plants has been synthesizing a large variety of chemical substances. These substances in addition to their basic metabolites include phenolic compounds, terpenes, steroids, alkaloids, glycosides and a host of others referred to as secondary metabolites [1]. Leaves are chemical laboratories of plants where photosynthesis occurs. Photosynthesis involves the whole set of chemical processes by which the plant produces complex chemical substances from inorganic substances of the air and soil. The cells of the leaves contain chlorophyll, a substance that absorbs sunlight, energy, turning it into chemical energy. It has been asserted that leaves are the most widely used parts of medicinal herbs producing most of the plant's active components, especially alkaloids, essential oils, glycosides and tannins. Some of the most useful leaves including, aloe, hazelnut, Mexican damiana, foxglove, bearberry, witch hael, laurel, mistletoe, chestnut tree, olive tree, grape vine and bramble [1]

2. Materials and Methods

Sample Collection and Preparation

Bitter leaf samples were obtained from a cultivated garden along the bank of River Benue, just before the left arm of the bridge in Makurdiin six batches. Vernonia amygdalina and Vernonia colorata were obtained, bearing in mind that they are prevalent herbs cultivated at the river bank before the rainy season, specifically in February 2010 and May 2010 and were identified by a taxonomist, Mr. P.O Ekweonu, of University of Agriculture, Makurdi. The leaf samples were rinsed, air-dried for two weeks and pounded in a wooden mortar and pestle and stored in polyethene bags in the laboratory.

Extraction

60 g powdered leaf material was extracted with 250mL of 95% ethanol in a 500mL round-bottomed flask, using Soxhlet apparatus for 7h until the refluxing ethanol became clear. The ethanol was distilled off and the extract was kept in a dessicator. Th eprocedure was repeated with acetone, and water.

Phytochemical Analysis

The extracts were evaluated for the presence of alkaloids, tannins, glycosides, saponins, steroids, and flavonoids using standard methods [2, 3].

Mineral analysis

2.0g of each sample of Vernonia species was weighed and digested with l0mL nitric acidand after complete digestion, the volume was made up to l00mL with deionized water in a volumetric flask, filtered and stored in a polypropylene container. The samples were analyzed using computer controlled atomic absorption spectrometer (AAS 969 model) atShedda Science and Technology Complex Gwagwalada, Abuja.

Preparation of Reagents

The following solvents and materials were obtained from the Chemistry Laboratory of the Benue State University Makurdi. n-hexane, 95% ethanol, acetone, petroleum spirit (40-60°C). Chloroform, ethyl acetate, methanol, sodium nitroprusside, sodium hydroxide, 3,5-dinitrobenzoic acid, potassium hydroxide, ferric chloride, hydrochloric acid, tetraoxosulphate (VI) acid, boric acid, sodium thiosulphate, lead acetate, iodine crystals, copper (II)tetraoxosulphate (VI), methylene blue, methyl red, Thin Layer Chromatographic (TLC) plates coated with silica gel, developing chamber and drown-out capillary tubes. Mayer's reagent, a solution of 1 g mercuric chloride and 3.65g of potassium iodide dissolved in a minimum amount of distilled-water and transferred quantitatively and made up to 100cm3 volumetric flasks, was prepared.

Preparation of Solutions used for Analyses were done as reported in [7]

Extraction using cold Maceration Method

60g of second batch of powdered leaf of *Vernonia* species was infused in 250mL ethanol for 24 h tie in a white filter cloth. The filtrate was concentrated by distilling off the ethanol using a steam bath. The residue of the ethanol extract in the cold was further extracted using water as solvent. The aqueous extracts yielded a pale brown concentrateand proved difficult to distil off the water due to excessive frothing and quite prone to decomposition. The aqueous extracts from the ethanolextract residue, was labeled as QEVA and QEVC.

Phytochemical Analysis

The extracts; EEVA, EEVC, AEVA, AEVC, QEVA, QEVC EEVA and EEVC were subjected to analyses for the presence of cardiac glycosides, tannins flavonoids, alkaloids, steroids and saponins using standard methods [17, 19].

Report of Thin Layer Chromatography (TLC) of Leaf Extracts of *Vernonia* Species (Two Variants of *Vernonia* Species)

n-Hexane, chloroform, ethylacetate, and methanol were employed as developing solvents in a thin layer chromatography assay of the extracts. The solvent system used was n-hexane: ethylacetate in the ratio of 3:1; and chloroform: Methanol in the ratio of 4:1.

EEVA and EEVC of the two *Vernonia* variants show clear tailing from the base spot and two other spots that moved on the plate in all the solvents.

AEVA and AEVC gave two spots, a base spot and a spot that moved on the plate in hexane and ethylacetate, while only a base spot was observed in chloroform and methanol solvent system. QEVA and QEVC showed two *spots* - a base spot and a spot with low R_f value in all the solvent systems. The retention factor, R_f was determined using a meter rule.

$$R_f = \frac{distance\ \ moved\ \ by\ \ the\ substance}{distance\ \ moved\ \ by\ \ solvent\ \ front}$$

Proximate Analysis

The proximate compositions of the two variants of bitter leaf powder were determined using standard methods [4, 5, 6]. The samples were analyzed for moisture, ash, crude protein, crude fat and carbohydrate determined by difference.

Determination of Moisture

Moisture content was determined by the oven method as described in previous literature. 2 g of the prepared sample were weighed in triplicate into an aluminum metal dish and placed in an oven at 150°C for three hours. The samples were then removed and placed in a desiccator and allowed to cool for 15 minutes before weighing. This was repeated until constant weights were recorded. The loss in weight from the original weight (before weighing) was reported as the moisture content.

Determination of Protein

2g of the prepared sample were first digested in Kjeldahl digesting system under a fume chamber. To the digested samples were added few drops of Tashiro indicator (0.2g of methyl red and 0.19g of methylene blue dissolved in 100mL of ethanol), allowed to cool and then distilled in boric acid after being appropriately diluted first with water and after with sodium thiosulphate and sodium hydroxide solution. The samples were then titrated against 0.1M HCl solution. A blank titration was similarly carried out, using the formula;

$$\textbf{Parentage nitrogen} = \frac{titre - blank \times 0.01 \times 5\ Normality \times 100}{weight\ of\ sample}$$

Percentage protein = percentage nitrogen × 6.25

Determination of Fat

2g of the samples were weighed into extraction thimbles and fixed into extraction flask of known weights. Extraction was carried out exhaustively for 16 hours using petroleum spirit (boiling point 40-60^{o}C) on a heating mantle. At the completion of the extraction the petroleum spirit was evaporated. The remaining fat in the flask dried at 60^{o}C for 30 minutes in the oven, cooled for 15 minutes and weighed. The percentage fat content was calculated as follows:

% fat content $=\frac{W_2-W_2}{W_2-W_2} \times 100$

Where, W_1 = weight of extraction flask, W_2 = weight of extraction flask and sample, W_3 = weight of extraction flask and fat

Determination of Crude Fibre

Fibre content was determined following the procedure outlined [7, 8]. 2g of the prepared samples were extracted using diethyl ether. This was digested and filtered through the California Buchner system. The resulting residue was dried at 1300^{o}C for two hours, cooled in a desiccator and weighed. The dried, cooled and weighed residue was then transferred into a muffle furnace and ignited at 600^{o}C for 30 minutes, cooled and re-weighed.

The % crude fibre in the sample = $\frac{w_1 \times w_2}{w_0}$x 100

Where: W_1 = loss in weight on ignition W_2 = loss in weight of blank, W_0 = weight of sample

Determination of Ash Content

5g of the sample was weighed in triplicates into ash dishes that had been previously weighed. The dishes were placed in the furnace and ignited at 550^{o}C for five hours, cooled and weighed. The resulting ash was calculated as follows:

Percentage ash content = $\frac{w_2-w_0}{w_1-w_0} \times 100$

Determination of Carbohydrates

Carbohydrate was calculated by difference [9, 10], as follows:

100 - (% moisture + % crude protein + % crude fibre + % fat + % ash)

3. Results and Discussion

Result of Phytochemical Analysis

The result of phytochemical analysis of the two variants of the leaf extracts of *Vernonia amygdalina* and *Vernonia colorata* are present in Tables 1 and 2.

Table 1: Phytochemical Screening of the Leaf Extract of *Vernonia ainygdalina*

Sample (Extracts)	Cardiac glycosides	Tannins	Flavonoids	Saponins	Steroids	Alkaloids
EEVA	+	+	+	+	+	-
AEVA	+	-	+	+	+	-
QEVA	+	+	+	+	+	-

Key: + = present - = absent EEVA = Ethanolic Extract of *Vernonia ainygdalina* AEVA = Acetone Extract of *Vernonia ainygdalina* QEVA = Aqueous Extract of *Vernonia ainygdalina*

Table 2: Phytochemical Screening of Leaf Extract of *Vernonia colorata*

Sample (Extracts)	Cardiac Glycosides	Tannins	Flavonoids	Saponins	Steroids	Alkaloids
EEVC	+	+	+	+	+	-
AEVC	+	-	+	+	+	
QEVC	+	+	+	+	+	-

Key: + = present - = absentEEVC = Ethanolic Extract of *Vernonia colorata,* AEVC = Acetone Extract of *Vernonia colorata,* QEVC = Aqueous Extract of *Vernonia colorata*

Table 3: Proximate Analysis of two Variants of *Vernonia*

Parameter	***V. amygdalina***	***V. colorata***
Moisture content (%).	79.66	79.02
Protein (%)	16.6	11.3
Fat (%)	2.75	1.50
Crude fibre (%)	8.25	8.40
Ash (%)	0.17	0.12
Carbohydrate (%)	11.33	10.85

Table 4: Metal analyses of the two variants of *Vernonia*

Mg/kg	**Zn**	**Cu**	**Pb**	**Fe**	**Mn**	**Cd**
A = *Vernonia amygdalina*	221	504.05	N.D	1785.45	258.85	N.D
B = *Vernonia colorata*	275.95	336.95	N.D	1645.5	236.65	N.D

Detection limits of the equipment (0.05mg/kg) N.D. = Not Detected

Table 5: IR Characteristic Absorption Bands observed for powdered leaf material of *Vernonia amygdalina*

Bond	**Compound type**	**Wave number (cm^{-1})**
C-Cl	Alkyl chloride	1390.72-1719.6(s)
C-N	Amine	1050-1350
C=C	Alkynes	3435.34-3964.81(s)
C-H	Alkanes	
C=O	Aldehydes, ketones	
C-O	Alcohols, ethers	
O-H	Hydroxyl	

Table 6: IR Characteristic Absorption Bands Observed for Powdered Leaf Material of *Vernonia colorata.*

Bond	**Compound type**	**Wave Number (cm^{-1})**
C-C1	Alkyl chloride	602.77
C-N	Amine	1032.4-1360(s)
C=C	Alkynes	2210-2260(m)
C-H	Alkanes	2850-2923.81
C=0	Aldehydes, ketones	719.6
C-0	Alcohols, ethers	1031.4-1039.67
O-H	Hydroxyl	3150.77

Table 7: Finger print region of the two variants of *Vernonia* extracts determined

Extract	Wave Numbr (cm^{-1})
AEVA	607.6 – 1390.72
AEVC	602.7 – 1390.72
EEVA	609.53 – 1398.44
EEVC	651.96 – 1430.26
QEVA	498.62 – 1407.12
QEVC	492.83 – 1408.08

Table 8: UV-Visible Spectra of two variants of *Vernonia* extracts

Extracts	**λ_{max} (nm)**	**Absorption**	**ε(log10)**
AEVA	408.50	0.902	2.647
AEVC	408.50	2.434	2.612
EEVA	409.00	0.953	2.612
EEVC	408.50	1.814	2.612
QEVA	443.50	0.461	2.647
QEVC	443.00	0.442	2.636

Discussion

Phytochemical Screening

Legal test

For the two samples ethanolic extracts, EEVA and EEVC, change in colour from their initial green to brown indicates the probable presence of cardenolides in these extracts. AEVA and AEVC similarly showed possible presence of cardenolides on displaying a change in colour from slight green to brown colour, while the aqueous extracts changed from pale brown to red colour signifying the presence of cardenolides in QEVA and QEVC as shown in table 1 and 2 above.

Lieberman- Burchard test

The EEVA and EEVC showed a visible change of its initial green colour to blue indicating the probable presence of a steroid nucleus in the structure of the compounds in the extracts. AEVA and AEVC showed the likely presence of a steroid nucleus in the extracts as the slight green of the extracts changed to deep green. Similarly, QEVA and QEVC, the pale brown colour changed to deep brown. The results were taken for possible presence of a steroid nucleus in all extracts.

Tannins

AEVA and AEVC gave negative result for the presence of tannins, while EEVA, EEVC, QEVA and QEVC gave positive results for the presence of tannins for the variants of *Vernonia.* These inferences were made as a result of change of colour from green to dark green forethanol extract and from initial pale - brown to deep red with the aqueous extracts. No remarkable colour change or reaction was observed on treatment with 10% KOH for the acetone extracts.Alternatively, 5% $FeCl_3$ also did not indicate any noticeable reaction in AEVA and AEVC as shown above in tables 1 and 2.

Flavonoids

The six (6) extracts all indicated the presence of flavonoids. These inferences were based on the changes in colour observed as the ethanolic extract - EEVA and EEVC gave a deep green colour from the initial green on adding 1.0 mL of 10% NaOH. Similarly, AEVA and AEVC changed from pale- green to deep green colour, indicating problem presence of flavonoids while QEVA and QEVC showed a deep- red colour from the initial pale- brown substance.

Saponins

The six (6) extracts all gave positive indication for the presence of saponins when 5 drops of olive oil were added and agitated - persistent foaming was taken as presence ofsaponins(Table 1 and 2).

Steroids

All the 6 extracts showed the presence of steroids on adding 5 drops of concentrated H_2SO_4 EEVA and EEVC changed from initial green to reddish brown colour at the interface, similarly AEVA and AEVC went from pale-green to deep-red colour and the QEVA and QEVC showed a change of colour from pale- brown to red colouration and was taken as probable presence of steroids. Alkaloids were absent in all the samples (Table 1 and 2).

Proximate Analysis

The percent moisture content, protein, fats, crude fibre, ash and carbohydrate vary slightly between the two leaves variants of vernonia studied. The results of proximate composition of *V. amygdalina* and *V. colorata* leaves (Table 3) show high moisture content (79.66 and 79.02%, wet weight). This is closely related to reported range (81.4-90.3%) in some of Nigerian green leafy vegetables. Ash content, which is an index of mineral contents in biota is low, (0.17 and 0.12%) when compared with *A. hybridus* (13.8% DW), *T. triangulare* (20.05% DW) [11, 12]. *Ipomea batatas*, (11.10%), *Moringa oleifera* (15.09% DW) and *Hibiscus esculentus*

(8.00% DW) all showed higher content of ash [13, 14]. The crude protein of *V. amygdalina* (16.60%) V. *colorata* (11.30%) varies markedly between the variants, but *V. coloratais* comparable to *Momordica balsamia* L. (11.29% DW), *Lesianthera africana* (13.1-14.9%), *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland [41, 42], and lower than /. batatas (24.85% DW), *Amaranthus candatus* (20.5% DW), *Piper guineeses* (29.78 DW) and *T. triangulare* (31.00% DW) [15].

However, *V. amygdalina* compares favourably with *Gnetum Africana* (17.50% DW) and Leptadenta liastata (19.1% DW) [16]. Plant food that provides more than 12% of its calorific value from protein is considered good source of protein [16]. Reports also indicate that adults, children, pregnant and lactating mothers require 34-56, 13-19 and 17 and 71 g of protein daily respectively [17]. These Vernonia species can afford a reasonable alternative requirement for protein. However, the presence of tannins is known to inhibit he bioavailability of proteins and minerals [17]. Both variants are poor sources of lipids (2.75 and 1.50%) this is very low compared to reported values of (8.3-27.0% DW) in some vegetables consumed in West Africa [17] [17] *A. hyhriflus* (4.65%), *Calchorus Africana* (4.20%) and, 1.85-8.71% DW of some edible green leafy vegetable of Southern India and Nigeria. [17].

The carbohydrate content of (11.33 and 10.85%) is lower than 20, 23.7, 39.05 and 52.18% reported for *Senna obtusfol-ia, Amaranthus incurvatus, M. balsamina* leaves respectively. [18].

The crude fiber content of (8.25 and 8.40% is higher than *batatas* (7.20%) *T. triangulare* (6.20%), *P. gitineese* (6.40%), *Corchorus olitofus* (7.0%) *Vernonia amygdalina* (6.5%) [18]. This may be due to soil fertility.

The Recommended Dietary Allowance (RDA) of fibre for children, adults, pregnant and lactating mother are 19-25, 21-38, 28 and 29g respectively. This shows that the plants arecapable of contributing 34-45, 23-41, 3l and 30% of their respective daily requirement when l00g dried leaves are consumed and as such could be valuable sources of dietary fibre in human nutrition.

Similarly, RDA values for children, adult, pregnant and lactating mothers are 130g, 130, 175 and 210g respectively. [19]. It implies that 40, 40, 30 and 25 of their respective daily requirements can be met when about l00g dried leaves are consumed.

AAS

Table 4 shows the trace elements composition of the two species of *vernonia*, and from the table it can be seen that *V. amygdalina* contained higher concentration of iron Fe, of the entire determined mineral and higher than *V. colorata* leaves. Iron is essential for the building of red corpuscles, essential formation of hemoglobin, the oxygen-carrying pigment in red blood cells. Iron is useful against anaemia, tuberculosis and disorder in growth. Iron is an energizer, even though reports indicate that sources are just adequate in amount needed [20].

Copper contained in the two variant was observed to be next to the iron content (Table 4). The concentration of copper in *V. amygdalina* was more than *V. colorata*leaves. Copper is often seen with iron naturally [13]. It helps in absorbing iron and it is important in cellular defense and protection of the mucous membranes, anti anaemic and essential for formation of iron and hemoglobin.

Manganese which was observed to be about the third in appreciable amount (Table 4). Manganese supports growth and development and is essential in combating anemia.

Zinc content obtained proved to be least in concentration. The level zinc determined was higher in *V. colorata* that in *V. amygdalina* (Table 4), as is the case with all other minerals determined, probably due to soil fertility rate or mineral absorption level of plants as samples were obtained around the same environment and treated.

The presence of trace zinc amount in the leaves of two variants indicates that it is good foliage for reproduction organs, fertility and healthy functioning of the heart [18]. Zinc is important in many enzyme functions and keeping the skin fresh. Report suggests zinc as a necessary component of diet for HIV/AIDs infected persons [14]. Lead, Pb and Cadmium, Cd were below detection limits of the equipment used (0.05mg/kg) in each of the Vernonia species studied. Lead is a major environmental pollutant and its toxicity continues to be a major public health problem in many segments of the population [15]. of the non-essential heavy metals, most attention is currently paid to lead, mercury and cadmium because they pose the greatest risk to-human health

from environmental restriction on the use of these as new research reveals that they may be causing neurological damage of the unborn babies and young children [16]. Though lead poisoning is cumulative, it causes physiological and neutrological disorders such as stillbirth, abdominal pains and anaemia. Neuro-toxic effects of lead include reduction in nerve conduction and inflammation of the brain.

Infrared Spectra Analysis

Spectroscopic results revealed the presence of some functional groups in the isolated extracts of the two variants of *vernonia*. Characteristic absorption bands which was first determined for powered leaf material has been shown (Table 5 and 6). The infrared spectra of *V. amygdalina* and *V. colorata* fractions: EEVA, EEVC, AEVA, AEVC, QEVA and QEVC were analysed by a Nicolet (thermoelectronic corporation, USA), Fourier transform infrared spectrometer, (Nicolet IR 8400s FT-IR). It showed absorption peaks at 3435.34 cm^{-1} for hydroxyl groups, 1039.67 cm^{-1} for C-O or C-N, bonding stretching, 1654.01 cm^{-1} for C-C double bond and 1719.84 and 1248.95 cm^{-1} were taken for C-O groups in a non-cyclic ester or Glactones respectively. The peaks at 2925.15 and 2926.11, 2891.39, 1390.72, 1398.44, 1407.12, 1390.72 and 1408.08 cm^{-1} are absorptions bonds due to aliphatic CH, CH_2, and CH_3 groups for AEVA and AEVC, QEVA and QEVC. EEVC provided 23 different peaks and this was taken as evidence for different components that could be separated and purified. These data are similar to previous result of IR data carried out for 10 steroid glycosides isolated from *V. amygdalina* using High-Speed Countercurrent Chromatography (HSC-7) [17]. In summary the IR spectra revealed the presence of some functional groups from the extracts of the two variants of *vernonia*. Based on characteristic absorption bands observed, AEVA and AEVC possessed C-C1, C-N, C-H, C-O,
C-O, O-H, EEVA and EEVC showed the following bonds; C-C1, C-O, C=O, O-H or C=C group of atoms. EEVC showed characteristic frequency range of several broad over lapping absorptions (2500-3500 cm^{-1}). QEVA and QEVC showed in addition distinct features with 3-4 absorption in this region and very considerable intensity overlaps with C-C bending vibration. This is a prove for aromatic residue, as it is with AEVA and AEVC extracts, that showed an out-of-plane C-H bending, and indicates strong absorption at 675-870 cm^{-1} regions.

UV Spectra

The peak at the region with frequency 670.21 and 671.25 cm^{-1} corresponding to the range as shown in literature (600-800 cm^{-1}) for C- Cl depicting an auxochrome, they intensify the colour of compounds with Cl having the strongest effect according the decreasing order of effect: I, Br, Cl and are generally ortho-para directing in structure. The ultraviolet region, especially the wavelengths from 200-400 nm is very helpful as a guide to the presence of 'group's, particularly the unsaturated ones [20].

The O-H stretching is synonymous to all types of organic acids with frequency range of 3000-2500 cm^{-1}.

Most bonding vibrations have lower frequencies and usually appear in the finger print region below 1500 cm^{-1}, with exception of the N- H bending vibration which occurs in the 1600 cm^{-1} region.

This closely related frequency ranges could be used to confirm the structures of the compounds isolated and indicate the fact that the samples are of the same variants.

This is further investigated via UV- visible spectra scan analysis which produced closely related result when the λ_{max} of six extracts isolated were determined, and these fractions, UV-detected from 200-900 nm and all showed two distinct peaks around 408 and 665nm.

Table 8 is the result of conjugated chromophores that show characteristic absorptions in the useful UV-VIS region of individual highest absorption for the extracts. The ultraviolet absorptions of the conjugated chromophores certainly represent that of aliphatic compounds $-(CH=CH)_6-$ double bond with n=8, and approximate intensity that agrees with the ε_{max} at that region, as this does not represent an unsaturated carbonyl functional group at the λ_{max} value. UV peaks may be used as predictive tools to determine *V.amygdalina* and *V. colorata* extracts activities.

Conclusion

From the results, it is concluded that cardiac glycosides, tannins, flavonoids and saponins, are present in the *V. amygdalina* and *V. colorata*. This is based on spots seen on TLC plates and results of phytochemical analysis. Alkaloids could not test positive for the two samples IR-spectroscopy proved that basic medicinal constituents can be got from the very bitter species, i.e. *V. amygdalina*. Both *V. amygdalina* and *V. colorata* had high amount of iron implying that they can serve as blood tonic.

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