Molecular Structure Profiles of Major Chemical Components of *Vernonia amygdalina* and *Tephrosia vogelii* Leaf Extracts

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Abstract

Plants contain various chemical components some of which play vital roles in the bodies of plants and animals. It is essential that the chemical composition of these plants be well elucidated in o rder to come up with their mode of action. The objective of this study was to determine the major chemical constituent of Vernonia amygdalina and Tephrosia vogelii leaf extracts and elucidate the molecular structure profiles of the chemical components. Mature leaves were collected from Kenya Agriculture Research institute (KARI) Naivasha. Collection was done during two peak seasons, dry season February and wet season April. Chemical analysis involved determination of chemical extractive content of the plant leaves; the crude extract of the plants was obtained by Soxhlet extraction using the different solvents comprising of hexane, ethyl acetate, methanol, toluene/ethanol (2/1 v/v) mixture and water, followed by characterisation of the plant extracts by Nuclear Magnetic Resonance (^{1}H ^{13}C NMR), Fourier-Infra red Analysis (FTIR) and Gas Chromatography Mass Spectrometry (GC-MS). Assays for Chemical Constituents and compound identification were based on National Institute of Standards and Technology (NIST) library. The major secondary metabolites detected in all plants were glycosides, whereas, tannins and rotenoids were detected in Tephrosia only. A higher amount of these compounds were observed during the dry season compared to the wet season. This could be attributed to the increase in maturation of leaves during the dry season. Sesquiterpene lactones were on the other hand detected only in Vernonia during both the wet and dry season at the same amounts.

Keywords: *Vernonia amygdalina, Tephrosia vogelii, rotenoids, sesquiterpene lactones, glycosides and tannins*

Introduction

Vernonia amygdalina is a shrub or small tree of 2-5m with petiolate leaf of about 6mm diameter and elliptic shape. The leaves are green with a characteristic oduor and taste (Beentje , 1994). No seeds are produced and the tree has therefore to be distributed through cuttings (Bentje , 1994). It grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant, (Bonsi *et al.,* 1995). It is a popular African vegetable (Abosi *et al* 2003); the leaves are used for human consumption where the bitterness can be abated by boiling or by soaking in several changes of clean water. All parts of the plant are pharmacologically useful (Adaramoye *et al.,* 2008). Both aqueous and alcoholic extract of the stem, bark, root and leaves are reported to be extensively used as a purgative, antimalarial and in the treatment of eczema (Afloyan *et al.,* 2006). It is easily recognized and used for self medication by parasitized chimpanzees (Huffman, 1996). Pharmacological studies have also shown that the leaf extract has both hypoglycaemic and hypolipidaemic properties in experimental animals and could be used in managing diabetes mellitus (Akah, 1992). The common and documented medicinal uses of *Vernonia amygdalina* include the treatment of schistosomiasis, amoebic dysentery and gastrointestinal problems (Huffman *et al.,* 1996). In Nigerian herb homes, extracts of the plant are used as tonic, in the control of ticks and treatment of coughs, feverish condition, constipation and hypertension (Adaramoye *et al.,* 2008).

Tephrosia vogelii is a soft, woody branching herb or small tree with dense foliage, 0.5- 4m tall with velutinous to sericeous indumentum, Stems and branches tomentose with long and short white or rusty-brown hairs. Leaves arranged spirally, imparipinnate; stipules 10-22x 3-3.5 mm early caduceus;

rachis 5-25 cm long including petiolule; leaflets in 5-14 pairs, narrowly elliptical to elliptical-oblanceolate, up to 7x2 cm, base acute to obtuse apex rounded to emarginated, venation most distinct on lower surface, silky tomentose. Inflorescence a terminal or axillary pseudo -raceme, 8-26 cm long, rusty tomentose; basal bracts leaf like, penduncle stout, as long as pseudo -raceme; flower 18-26 mm long, fragrant when fresh, white-purple or blue; pedicel up to 23 mm long. Bracteoles sometimes present on calyx. Pod linear, slightly turgid, 5.5-14x 0.8-1.5 cm. Brown-green, wooly to sericeous, 6-18 seeded. Seed are ellipsoid to reniform, 5-7 x3-5 mm, and dark to black. (Beentje, 1994).

Tephrosia vogelii is found in widely varying habitats, including savannah -like vegetation, grassland, forest margins and shrub land, waste land and fallow fields. *vogelii* has been cultivated for insecticide, fish and arrow poison obtainable from the leaves. The poison stupefies fish, which are then easily caught (Dale, 1961). Dry, crushed leaves are used as insecticides against lice, fleas, and ticks, and as a mollusticide. Used as an abortifacient, emetic, bactericide, purgative and cure for skin disease, schistosomiasis, ringworm and parasitic infections. Leaf decoctions are used in treatment of scabies and yaws; a weak infusion of leaves is taken as an anthelmintic. Root decoctions are used to treat constipatio n (Dzenda *et al.*, 2007).

Materials and Method

Mature *Tephrosia vogelii* and *Vernonia amygdalina* leaves were collected from Kenya Agriculture Research Institute (KARI) Naivasha. KARI Naivasha is in Naivasha district of Rift Valley province. It lies between Latitude 0 degree 45‘ South and longitude 36 degree East. It receives a mean annual rainfall 750mm. The long rains occur in March- June and the short rains in October-November). Collection was done during two peak seasons, dry season February 2008 and wet season April 2008.

To obtain the crude extract Soxhlet extraction was carried out at University of Eldoret Chemistry laboratories. Phytochemical analysis of the extracts was carried out at Kenya Agricultural Institute (KARI) Naivasha branch and Lermab in Nancy University France.

Pesticide-free mature leaves from each plant were collected from branches which had produced the final raceme Between 6 and 10 compound leaves below the terminal florescence were picked separately and transported while fresh to University of Eldoret Chemistry laboratories . In the laboratory, the leaves were dried to a constant weight in an electric oven and reduced to fine powder in a hammer mill. The fine powder was passed through a 115-mesh sieve and dried further to constant weights before extraction using a soxhlet extractor. Soxhlet extraction was followed by characterisation of the plant extracts by ^{1}H ^{13}C NMR (Nuclear Magnetic Resonance) and FTIR (Fourier-Transformed Infra red Analysis), GC-MS (Gas Chromatography Mass Spectrometry) and Screening for Chemical Constituents.

Soxhlet extraction was done using the different solvents comprising of hexane, ethyl acetate, methanol, toluene/ethanol (2/1 v/v) mixture and water. 10gm of sample powder was extracted with 180ml of the solvent for 15 hours at the rate of 10 to 12 cycles per hour. To quantify the amount of extractives in the leaves, series extraction was carried out using the different solvents starting from the least polar solvent to water on the same batch of powder success ively in the order hexane, ethyl acetate methanol, toluene/ethanol (2/1 v/v) mixture, then water. After each extraction, the solvent was evaporated under reduced pressure in a rotavapor and the residue dried over P_2O_5 under vacuum before weighing.

Two methods based either on direct determination of extractives after solvent evaporation (direct methods, DM) or on the difference between dry weight of powder before and after extraction (indirect method, IM) were used to evaluate extractive contents.

The percentage of extractives was determined according to the formula.

$$\%\ \mathrm{DM} = \frac{m_e}{m_s} \times 100$$

$$\%\ \mathrm{IM} = \frac{(m_s - m_d)}{m_s} \times 100$$

Where m_e is the weighed mass of extracts after solvent evaporation m_s is the dry mass of the powder before extraction, and (m_d) is the dry mass of extracted powder.

Characterisation of V. amygdalina and T .vogelii Extracts

^{1}H ^{13}C NMR and Infra red Analysis

About 1mg of the dry extract was dissolved fully in methanol-d_4, chloroform d_6 or DMSO-d_6 in a special NMR test tube. The tube was then inserted into an automatic operated ^{1}H and ^{13}C NMR testing machine. The spectra so produced were recorded in $CDCL_3$, methanol-d_4, chloroform d_6 or DMSO-d_6 as

required on a Bruker DRX 400 spectrometer. Chemical shifts were expressed in parts per million (ppm) and calculated relative to TMS. For infra red analysis about 1mg of finely ground and dry extract samples were dispersed in a matrix of KBr and pressed to form disks before introducing into an automatic operated Perkin Elmer IR machine. Infra red analysis (FTIR) spectra were recorded as KBr disks on a Perkin Elmer FTIR spectrometer SPECTRUM 2000 between wave number ranges of 4000-5000cm^{-1}.

GC-MS Analysis

Test samples were analyzed for trimethyl derivatives using the following procedure. In a screw-capped vial, a sample of approximately 1 mg of dry leaf powder was dissolved in 0.5 ml of anhydrous acetonitrile (Across Organics) and 0.4 ml of *N,O*-bis-trimethylsilyl (trifluoroacetamide) containing 1% trimethylchlorosilane (BSTFA / 1% TMCS) (Across Organics) was added. The solution was sonicated for about 1 min and heated at 60°C for 60 min. After evaporation of the solvent in stream of dry nitrogen, the residue was diluted in 1 ml of anhydrous acetonitrile. GC-MS analysis was performed on a Clarus ® 500 GC gas chromatograph (Perkin Elmer Inc., USA) coupled to a Clarus ® 500 MS quadrupole mass spectrometer (Perkin Elmer Inc., USA). Gas chromatography was carried out on a 5 % diphenyl / 95 % dimethyl polysiloxane fused-silica capillary column (Elite-5ms, 60 m x 0.25 mm, 0.25 mm film thickness, Perkin Elmer Inc, USA). The gas chromatograph was equipped with an electronically controlled split / splitless injection port. The injection (injection volume of 1 µl) was performed at 250°C in the split mode (split flow of 20 ml/min). Helium was used as carrier gas, with a constant flow of 1.2 ml/min. The oven temperature program was as follows: 200°C constant for 4 min, 200°C to 330°C at a rate of 5°C/min and then constant for 330°C. Ionization was achieved under the electron impact mode (ionization energy of 70 eV). The source and transfer line temperatures were 250°C and 330°C, respectively. Detection was carried out in scan mode: m/z 35 to m/z 700 a.m.u. The detector was switched off in the initial 10 min (solvent delay).

Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Sofowora (1993). 2ml of extract was added to 2mls of ferric chloride solution ($FeCl_3$), a deep bluish green solution was formed with presence of phenols. The colour intensity gave an estimate of compounds present.

3g of the powdered sample was boiled in 50ml distilled water for 3minutes on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled wat er in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. A blue or green colour indicated the presence of tannins.

25ml of dilute sulphuric acid was added to 5ml of extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5ml of fehling solution A and B was added. A brick red precipitate of reducing sugars indicated presence of glycosides.

Results and Discussion

Quantification of Extractives

Table 1. Percentage Extracts from *Vernonia amygdalina* and *Tephrosia vogelii* Leaves by Soxhlet

Solvent	*Vernonia amygdalina*		*Tephrosia vogelii*	
	Direct method	Indirect method	Direct method	Indirect method
Hexane	0.7	0.9	0.7	0.7
Ethyl Acetate	0.4	0.5	0.4	0.4
Methanol	1.9	2.0	1.6	1.8
Toluene/ethanol	1.5	1.8	1.4	1.8
Water	5.6	5.8	4.4	4.8

The results show that in all cases, indirect measurements (IM) gave higher extracts content than direct measurements (DM). This may have been due to loss of some extractive components during the removal of solvent in the Rotavapor or manipulation of the sample. In both cases , the direct and indirect methods of water extraction yielded higher values.

Table 2. Series Extractions with Different Solvents of Increasing Polarity

Solvent	*Vernonia amygdalina*	*Tephrosia vogelii*
Hexane	0.6	0.4
Ethyl acetate	0.9	0.8
Methanol	2.1	1.9
Toluene/Ethanol	2.5	2.1
Water	3.2	3.0
Total Extractive	**9.3**	**8.2**

The quantity of extractives increases with polarity of the solvent used and these results are in agreement with observations performed on other tropical plant species. Water extraction leads to the highest quantity of extractives in the two plant species ranging between 3.0 and 3.2%. High quantities of extractives as described in the literature on other plant species contribute to fungal an d toxicity and in general their utilization for various uses as biocides (Afolayan *et al.*, 2006*).*

Identification and Characterisation of Vernonia amygdalina and Tephrosia vogelii Extractives

Vernonia amygdalina Leaf Extractives

Methanol and ethyl acetate gave similar results; ^{1}H NMR analysis of *Vernonia amygdalina* crude leave extractives by methanol and ethyl acetate (figure 1) indicated the presence of methylene protons between 0.8ppm and 2.0ppm and typical fatty acids and simple sugars between 3.0 ppm a nd 4.0 ppm. ^{1}H NMR for methanol and ethyl acetate extract indicated allylic protons between 2.0 and 2.5ppm and broad vinyl protons at 5.4ppm typical of fatty acids.

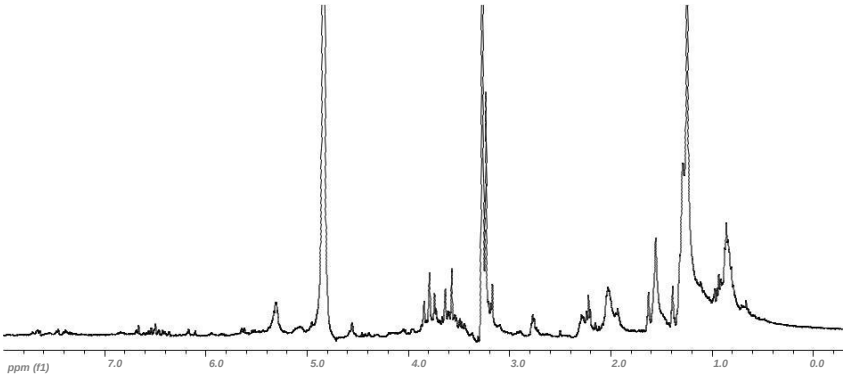


Figure 1. ^{1}HNMR Spectra of Methanol and ethyl acetate *Vernonia amygdalina* Crude Extractives

The FTIR spectrum (Figure 2) indicated a characteristic OH group absorption at 3400cm^{-1} and carbonyl C-H vibrations at 1715 and absorptions at between 2850 and 2920 reported to be present in fatty acids. All spectra indicated characteristic hydroxyl group absorption at 3350 cm^{-1} and aromatic C=C skeletal vibrations at 1620, and 1456 cm^{-1}typical of flavan like compounds.

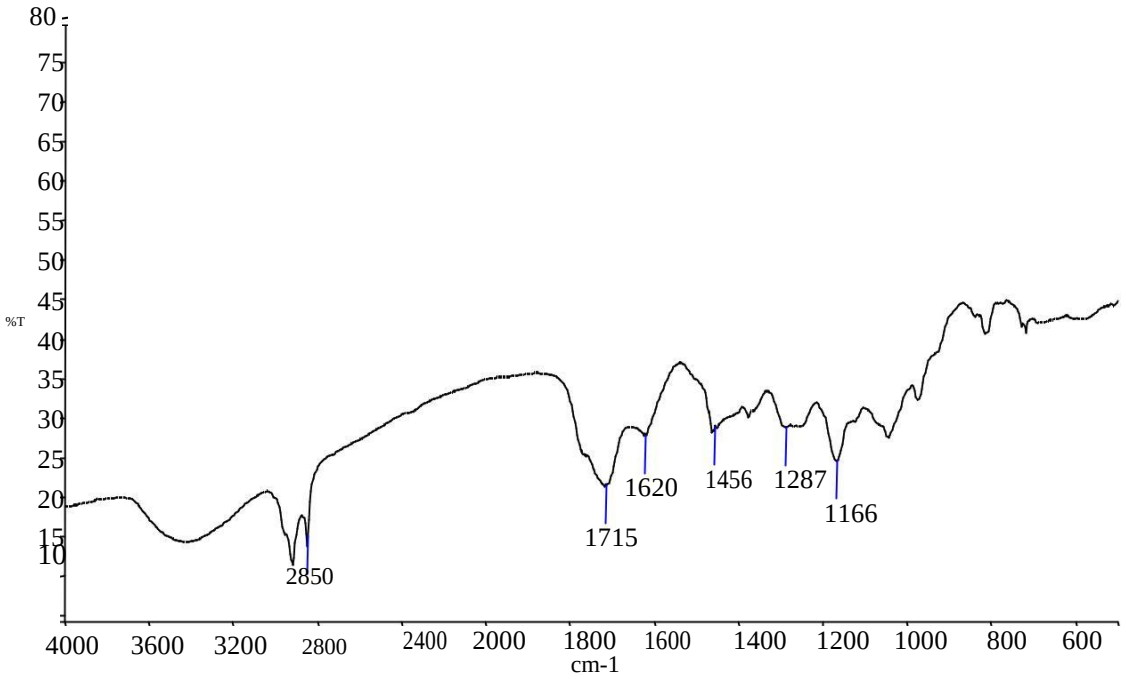


Figure 2. FTIR spectra of methanol and ethyl acetate extractives of *Vernonia amygdalina*

GC-MS analyses of the TMS derivatives of methanol and ethyl acetate extractives from *Vernonia amygdalina* leaves are presented in Figure 3& 4.

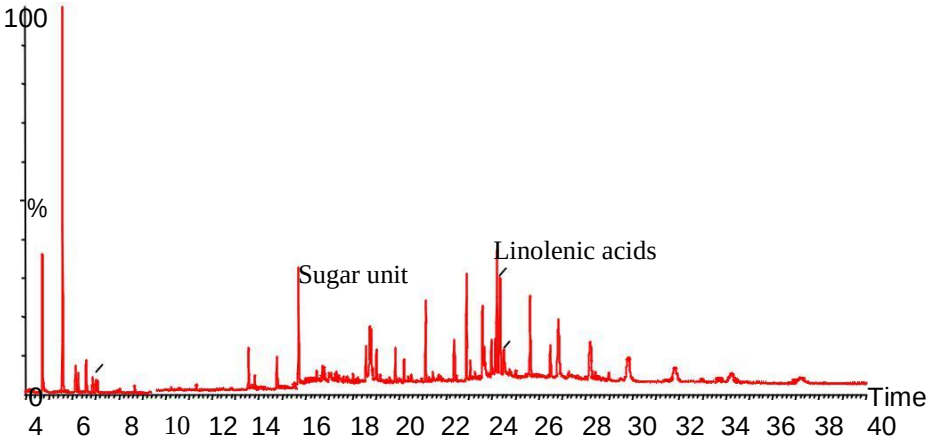


Figure 3. GC- MS chromatograms of methanol extractives of *Vernonia amygdalina*

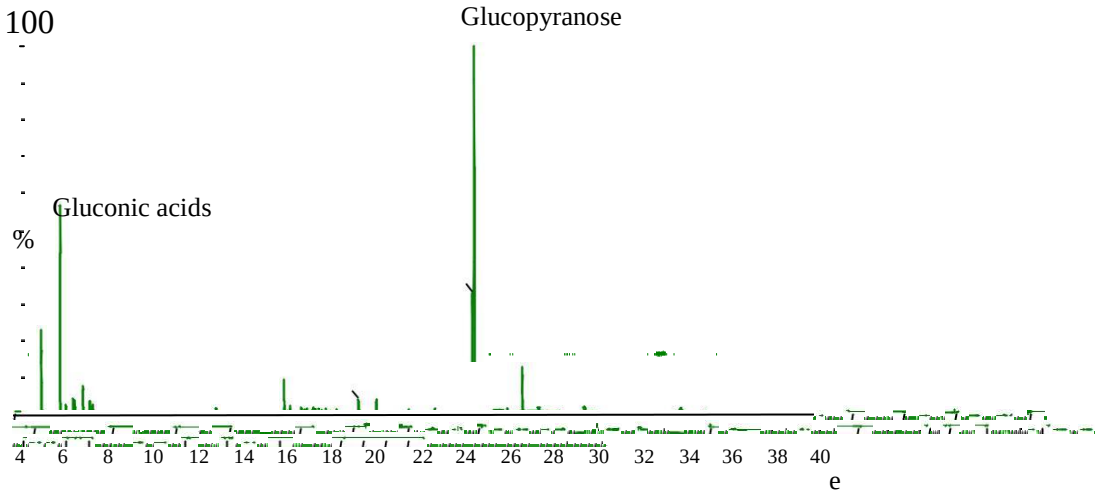
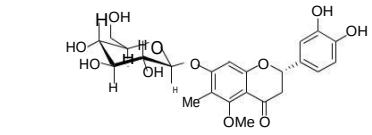
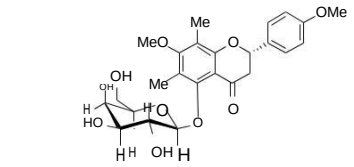
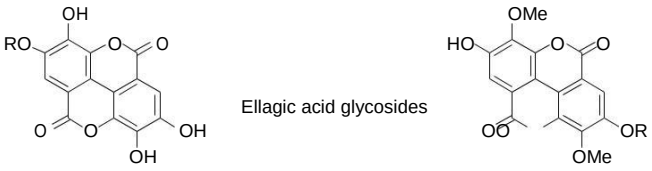


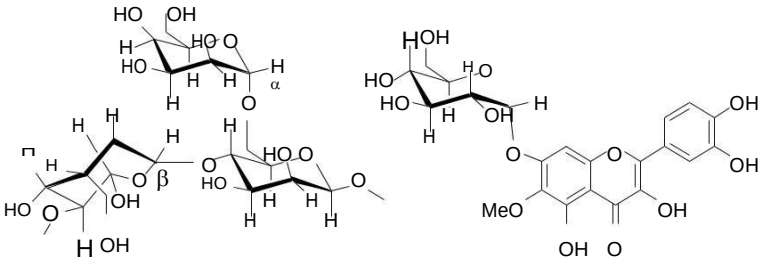
Figure 4. GC- MS chromatograms of ethyl acetate extractives of *Vernoni aamygdalina*

The molecular structures of *Vernonia amygdalina* elucidated from this study are as shown in Figure 5

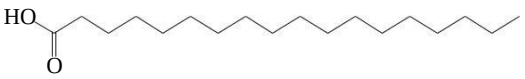

Glucopyranoside


Galactopyranoside .




Galactomannan sugar unit

Patulitrin sugar unit


Octadecanoic acids


MW = 98
Phosphoric acids

Figure 5. Probable Structures of the Compounds of *Vernonia amygdalina* Leaf Extracts *Tephrosia vogelii* leaf extractives

^{1}H NMR analysis of *vogelii* crude leaf extractives by methanol and ethyl acetate (Figure 6) indicated the presence of methylene protons between 0.8ppm and 2.0ppm and typical fatty acids and simple sugars between 3.0 ppm and 4.0 ppm. ^{1}H NMR for methanol and ethyl acetate extract indicated allylic protons between 2.0 and 2.5ppm and broad vinyl protons at 5.4ppm typical of fatty acids.

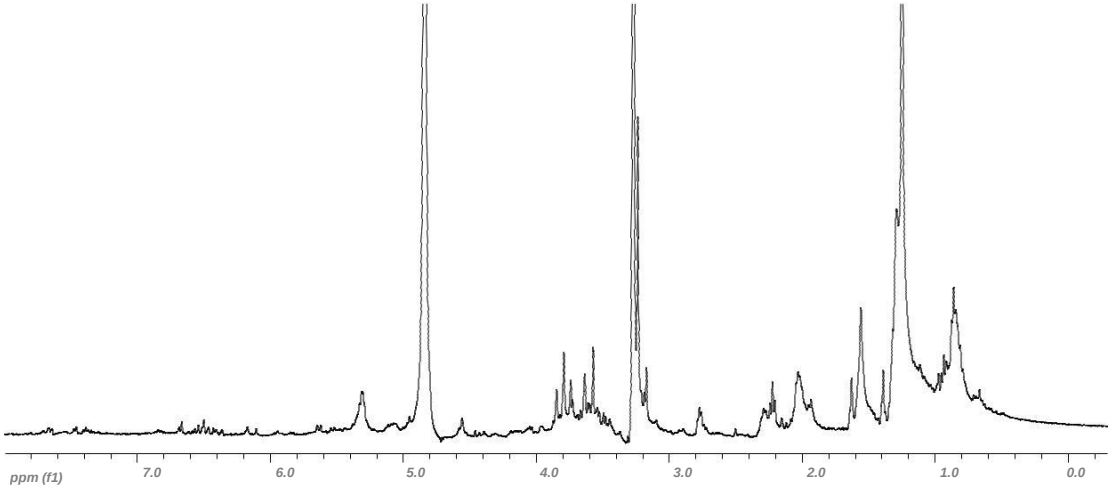


Figure 6. ^{1}HNMR spectra of methanol and ethyl acetate *Tephrosia vogelii* crude extractives

The FTIR spectrum (Figure 7 & 8) indicated a characteristic OH group absorption at 3400cm^{-1} and carbonyl C-H vibrations at 1715 and absorptions at between 2850 and 2920 rep orted to be present in fatty acids. All spectra indicated characteristic hydroxyl group absorption at 3350 cm^{-1} and aromatic C=C skeletal vibrations at 1602, 1506 and 1456 cm^{-1}typical of flavan compounds.

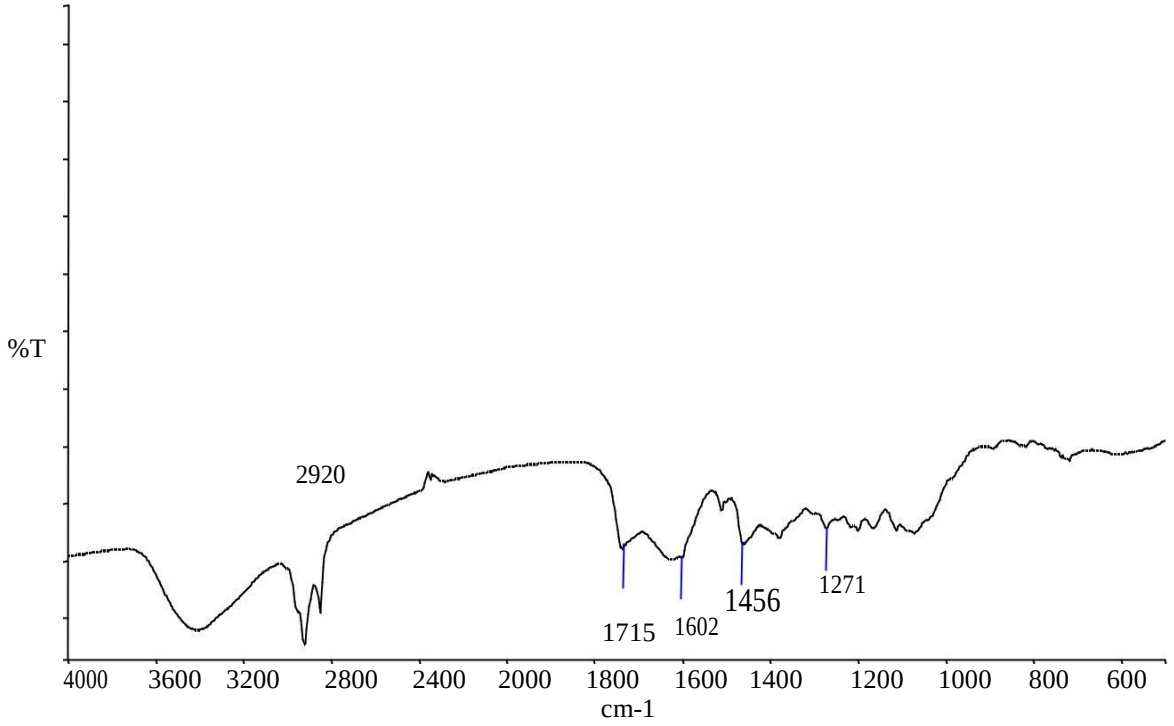


Figure 7. FTIR spectra of methanol extractives of *Tephrosia vogelii*

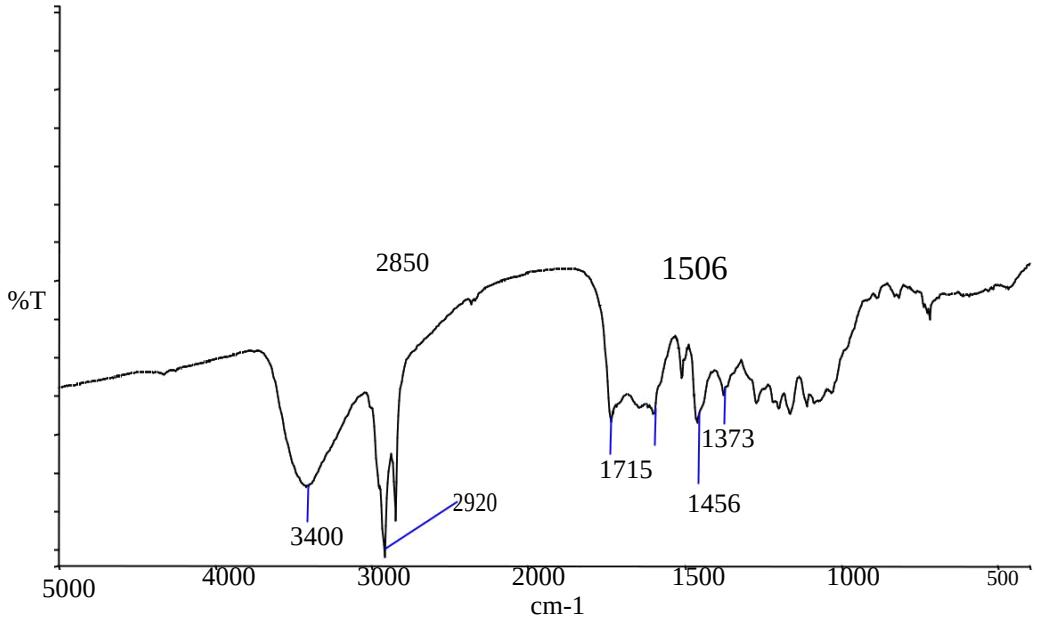


Figure 8. FTIR spectra of ethyl acetate extractives of *Tephrosia vogelii*

GC-MS analyses of the TMS derivatives of different crude extractives from leaves are presented in Figure 9 & 10.

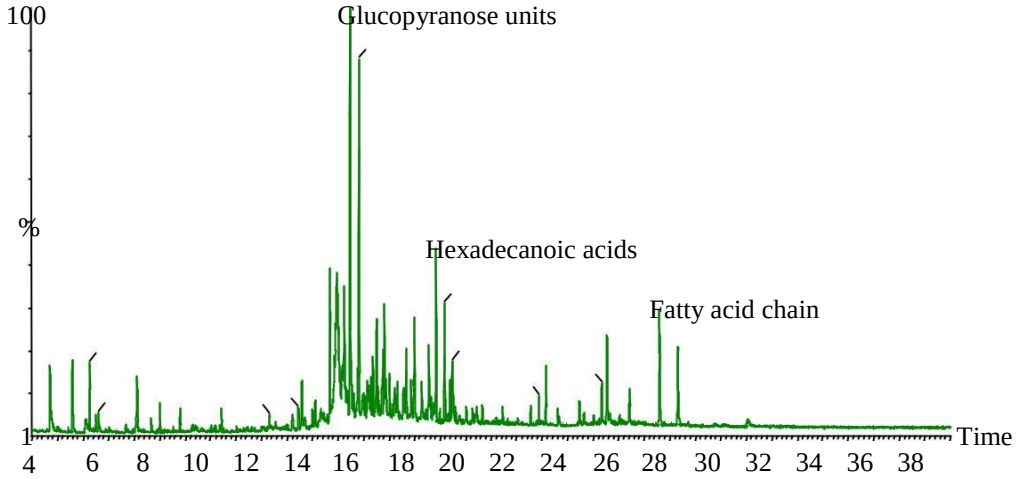


Figure 9. GC- MS chromatograms of different compounds in *Tephrosia vogelii* methanol leave extracts

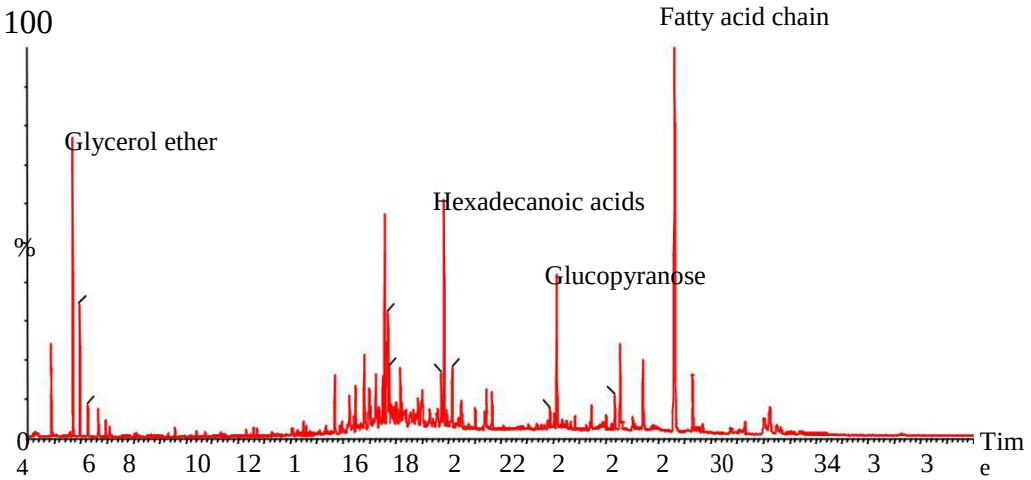


Figure 10: **GC- MS chromatograms of different compounds in *Tephrosia vogelii* ethyl acetate leave extracts**.

The molecular structures of *Vernonia amygdalina* elucidated from this study are as shown in figure 11.

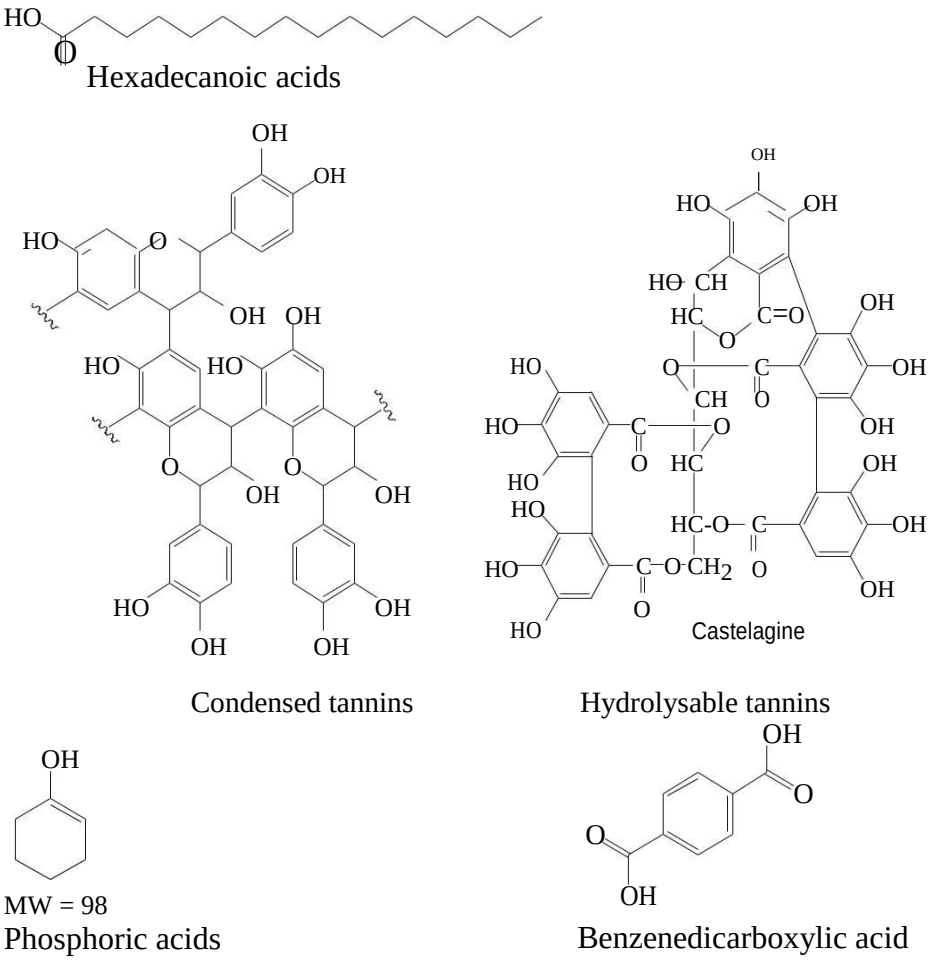


Figure 11: Probable Structures of the Compounds of *Tephrosia vogelii* Leaf Extracts

Seasonal Variation of V. amygdalinaand T. vogelii Extractives

Variation in yield among test plant species during wet and dry seasons in both aqueous and alcoholic extracts was observed (Table3). The highest yield was for the alcoholic extract of the leaves of *Vernonia*(30.53%) during the dry season and the lowest yield was recorded for the alcoholic extract of the leaves of *T. vogelii* (4.48%) during the wet season.

Table 3. Yield among test plants during different seasons

% Yield W/W	Plant extract (Wet season)		Plant extract (Dry season)	
	Tephrosia	***Vernonia***	***Tephrosia***	***Vernonia***
Water	14.3	11.8	17.6	19.5
Alcoholic	4.48	30.2	16.8	30.53

The major secondary metabolites detected in all plants were g lycosides, whereas, polyphenols (tannins) and Flavonoids (rotenoids) were detected in *Tephrosia* only. A higher amount of these compounds were observed during the dry season compared to the wet season. Sesquiterpene lactones were on the other hand detected only in *Vernonia* during both the wet and dry season at the same amount *(*Table 4).

Table 4. Chemical Constituents among test plants during different seasons

Chemical constituents	Plant extract (Wet season)		Plant extract (Dry season)	
	Tephrosia	***Vernonia***	***Tephrosia***	***Vernonia***
Rotenoids	+	-	++	-
Sesquiterpene lactones	-	+	-	+
Glycosides	+	+	++	++
Tannins	+	-	++	-

(+) - Present (-) –absent

Conclusion

Phytochemical results in the present study indicate that the two plants contain various c hemical compounds. The major secondary metabolites detected in both plants were glycosides, whereas, polyphenols (tannins) and Flavonoids (rotenoids) were detected in *Tephrosia* only. A higher amount of these compounds were observed during the dry season co mpared to the wet season. Sesquiterpene lactones were on the other hand detected only in *Vernonia* during both the wet and dry season at the same amount.

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