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Phytochemical Investigation and Characterization of the Chemical Constituents from Root Extracts of *Tephrosia Vogeli*

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Abstract: ***Tephrosia vogelli*** **is an important medicinal plant used for various traditional healing purposes such as fever, diarrhea, skin diseases, malaria and meningitis. Phytochemical screening of the root CH_2Cl_2:CH_3OH (1:1) extract revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenes and absence of anthraquinones. Column chromatographic separation of the root CH_2Cl_2:CH_3OH (1:1) extract yielded two flavonoids, compounds 13 and 14. The structures of these compounds were identified based on spectroscopic techniques (UV-Vis, IR and NMR).**

Keywords: **Phytochemical Screening, Column,** ***Tephrosia vogelii*****, TLC, Extraction.**

1. INTRODUTION

From the very first moment of his creation, man has utilized environment around him for his survival. Since prehistoric times, food, shelter and cure from diseases have been the essential human needs. In this context, all life forms on earth depend upon unlimited treasure of the Almighty, ranging from oxygen to breath and drugs to heal [1]. Nature has grown a lot of plants of medicinal importance. From ages, mankind have been the beneficiary of natural product remedies and discovered wide variety of herbs which cure various diseases [2, 3].

According to an estimate of the World Health Organization, approximately 80% of the people in developing countries depend on traditional medicine for primary health care needs; a major portion of these involves the use of medicinal plants [5]. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. The different parts of several medicinal plants were used to cure specific ailments has been in vogue from ancient times in India [6]. Plants also have been used in ethnopharmacy for various diseases such as hypertension, and diarrhea for centuries and today their scientific validation was provided by identification and isolation of bioactive phytochemicals [7].

Natural products, as the term implies, are those chemical compounds derived from living organisms, plants, animals, insects, and the study of natural products is the investigation of their structure, formation, use, and purpose in the organism [8]. Natural products isolated from plants arid microorganisms have been providing noble, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedures [9]. Natural product chemistry covers the chemistry of naturally occurring organic compounds, their biosynthesis, function in their own environment, metabolism, and more conventional branches of chemistry such as structure elucidation and synthesis [10].

Phytochemicals are the secondary metabolites that have several subgroups possessing various bioactivities such as antioxidant [11], antimicrobial [12, 13], antivirus, anticancer etc [14]. These chemical products of plants and animals can

be classified into primary and secondary metabolites. Primary metabolites are those which are common to all species and can be subdivided into proteins, carbohydrates, lipids and nucleic acids. These four groups of materials are defined according to the chemical structures of their members. The secondary metabolites are often referred to as "natural products". These can be subdivided into terpenoids, alkaloids, shikimates and polypeptides. The classification is based on the means by which the materials were made. The reaction path leading to a particular natural product is called the biosynthetic pathway, and the corresponding event is known as the biogenesis [15]. Different plant and animal species can employ dramatically different biosynthetic pathways to produce the same metabolite [10].

Exploration of the chemical constituents of the plants and pharmacological screening is of great importance which leads for development of novel agents [16]. First, it is very likely that the phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year [17]. It is estimated that there are 250,000 to 500,000 species of plants on Earth [18]. Terpenenes or terpenoids are active against bacteria [19] and fungi [20]. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties.

Ethiopians have used traditional medicines for many centuries, the use of which has become an integral part of the different cultures in Ethiopia. The indigenous peoples of different localities in the country have developed their own specific knowledge of plant resource uses, management and conservation [21].

In Ethiopia, 80% of the people use medicinal plants and plant remedies selected over centuries. Moreover, medicinal plants remain the most important and sometimes the only source of therapeutics [22]. A study by Hamilton [23] attributed the dependence on medicinal plants to the low proportion of medical doctors to patients in Africa (Ethiopia 1:33,000; Kenya 1:7142; Tanzania 1:33,000; Uganda 1:25,000, Malawi 1:50,000; Mozambique 1:50,000; South Africa 1:1639; Swaziland 1:10,000).

Furthermore the expensive cost of modern medicine, to treat various infections and the acquisition of drug resistant by pathogens, particularly in third world countries necessitates the search for an alternative anti-infective agent from natural products [24]. Many previous studies conducted in Ethiopia have shown the antimicrobial activities of many indigenous plants used in traditional medicine [25, 26].

Individual secondary metabolites may be common to a number of species or may be produced by only one organism. Relative species often have related patterns of secondary metabolite production and so a species can be classified according to the secondary metabolite they produce. Such a classification is known as chemical taxonomy. Occasionally, two plants are found to have identical physical aspects which botanists use for classification, but differ in the secondary metabolites they produce. For example, two flowers may look identical but one is odorless while the other possesses a strong scent due to the production of a fragrant terpenoid chemical. Such different strains are known as chemotypes [15].

Originally teas or decoctions (aqueous extracts) or tinctures or elixirs (alcoholic extracts) were used to prepare and administer herbal remedies - these were usually the starting points for isolation work. These days various extraction protocols are applied *n*-coupled with modern isolation techniques which include all types of chromatography, often guided by bioassay, to isolate the active compounds. Up until the 1950's, the structures of natural products were determined by degradative techniques, and a structure was not proven until the compound had been synthesized in an unambiguous manner. Stereochemistry was not often determined. Now, structures are elucidated primarily by spectroscopic techniques, and the stereochemistry is an important feature of the structure [8].

In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants. These biologically important secondary metabolites play a crucial role in meeting this demand through the continued investigation of the world's biodiversity, much of which remains unexplored. Development of several new and highly specific *in vitro* bioassay techniques, chromatographic methods, and spectroscopic techniques, especially nuclear magnetic resonance, have made it much easier to screen, isolate, and identify potential drugs [27-30].

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Fig 1: *Tephrosia vogelii* (Picture taken by Dagne on March 12th, 2018)

1.1. Description of genus of *Tephrosia*

The genus *Tephrosia* belongs to the family Leguminosae and subfamily Papilionaceae. There are approximately 400 species included in this genus. The plants in this genus are widely distributed in tropical, sub-tropical and arid regions of the world [31]. The plants are prostate or erect herbs or in the form of soft or woody shrubs. The base chromosome number of this genus is X=11 and are placed in the tribe Galegeae of the family Leguminosae [32]. Many plants from this genus have been used traditionally for the treatment of diseases like rheumatic pains, syphilis, dropsy, stomach ache, diarrhea, asthma, abortifacient, respiratory disorders, laxative, diuretic, and inflammation etc [33]. *Tephrosia purpurea*, an important plant of the genus is used as tonic, laxative, antivenom, antiulcer, antidiarhheal, and in leprosy [34].

Even though, the roots of *Tephrosia vogelii* are used widely across Africa as a fish poison, pesticide and soil enrichment [35], still now few phytochemical analysis has been carried out on the genus and only two studies were reported on the biological activity of the crude extracts of the plant despite the fact that the plant has been used for years for various traditional healing purposes [36, 37]. Thus, this project was designed to investigate the chemical constituents of the plant.

2. MATERIALS AND METHODS

2.1 Plant material

The roots of the plant were collected from Wondo Genet, found in Sidama Zone, SNNPR State, 45 km away from Hawassa. The plant material was identified by Mr. Wege Abebe of the Department of Botany, Addis Ababa University. The plant specimen was deposited at the herbarium of Science Faculty of Addis Ababa University. The plant was dried in an open air protected from direct exposure to sun light. The dried plant materials were then separately powdered to suitable site and 400 g the sample was made ready for extraction.

2.2 Extraction and Isolation

2.2.1 Extraction

400 g of the powdered root material was soaked CH_2Cl_2:CH_3OH (1:1) in three successive extractions (three times every 24 hr, 0.5L solvent amount). The marc left was further extracted with 100% methanol (3 times with 0.5L of methanol). The mixture was filtered and then concentrated with the help of rotary evaporator under reduced pressure at 40^oC to yield brownish crude extract (40 g, 10%).

2.2.2. Isolation

The CH_2Cl_2:CH_3OH (1:1) crude extract (30 g) was subjected to column chromatography (60 g silica gel) using increasing gradient of ethyl acetate in *n*-hexane as eluent. A total of 28 fractions were collected. Fractions 1-19 were discarded because their TLC results showed unclear spots and long thin yellow color respectively in different solvent systems. Fraction 20 showed single spot with R_f value of 0.63 in *n*-hexane/ethyl acetate (7:3) solvent system. This fraction was further purified by washing with *n*-hexane to give 100 mg deep yellow solid

Fractions 21-28 each showed the same two pure spots on their TLC (*n*-hexane/ethyl acetate, 6:4). These fractions were mixed and concentrated on rotary evaporator and subjected to a small column chromatography for further purification with increasing gradient of ethyl acetate in *n*-hexane as eluent. A total of 28 fractions were collected, however, the purification was not successful to obtain a pure compound.

Table 1: Solvent systems and fractions collected from crude extract.

Fraction	Solvent	Ratio (%)	Volume (mL)	Remark
1	*n*-Hexane	100	100	
2	*n*-Hexane/EtOAc	99:1	100	
3	*n*-Hexane/EtOAc	98:2	100	
4	*n*-Hexane/EtOAc	97:3	100	
5	*n*-Hexane/EtOAc	96:4	100	
6	*n*-Hexane/EtOAc	95:5	100	
7	*n*-Hexane/EtOAc	94:6	100	
8	*n*-Hexane/EtOAc	93:7	100	
9	*n*-Hexane/EtOAc	92:8	100	
10	*n*-Hexane / EtOAc	91:9	100	
11	*n*-Hexane / EtOAc	90:10	100	
12	*n*-Hexane / EtOAc	85:15	100	
13	*n*-Hexane / EtOAc	80:20	100	
14	*n*-Hexane / EtOAc	75:25	100	
15	*n*-Hexane / EtOAc	70:30	100	
16	*n*-Hexane / EtOAc	65:35	100	
17	*n*-Hexane / EtOAc	60:40	100	
18	*n*-Hexane / EtOAc	55:45	100	
19	*n*-Hexane / EtOAc	50:50	100	
20	*n*-Hexane / EtOAc	45:55	100	TV-1
21	*n*-Hexane/ EtOAc	40:60	100	
22	*n*-Hexane/ EtOAc	35:65	100	
23	*n*-Hexane/ EtOAc	30:70	100	
24	*n*-Hexane/ EtOAc	25:75	100	
25	*n*-Hexane/ EtOAc	20:80	100	
26	*n*-Hexane/ EtOAc	15:85	100	
27	*n*-Hexane/ EtOAc	10:90	100	
28	EtOAc	100	100	

2.3 Preliminary pphytochemicals iinvestigations

Phytochemical screening was carried out on the crude extracts (CH_2Cl_2:CH_3OH (1:1) and 100% CH_3OH). The presence secondary metabolites were determined following standard procedures [38-41].

2.31 Phytochemical Screening

Phytochemical examination was carried out for all the extracts as per the following standard methods.

2.3.1.1 Detection of alkaloids (Dragendroff's Test).

Extracts dissolved individually in dilute Hydrochloric acid and filtered [42]. Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate proves the presence of alkaloids.

2.3.1.2 Detection of glycosides

Modified Borntrager's Test. The extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides. The extracts was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides [43].

Legal's Test. The extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

2.3.1.3 Detection of saponins (Froth Test). The extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins [44, 45].

2.3.1.4 Detection of phytosterols (Salkowski's Test). The extracts was treated with chloroform and filtered. The filtrates was treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes [46].

2.3.1.5 Detection of phenols (Ferric Chloride Test). The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols[43].

2.3.1.6 Detection of tannins (Gelatin Test). To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins [47, 48].

2.3.1.7 Detection of flavonoids (Alkaline Reagent Test). The extract was treated with few drops of ammonia solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids [49].

3. RESULTS AND DISCUSSION

Phytochemical screening of the crude CH_2Cl_2:CH_3OH (1:1) extract of the roots of *Tephrosia vogelli* was carried out following literature protocols [43-47]. The test result showed the presence of tannins, terpenoids, flavonoids, and absence of anthraquinones and alkaloids (see Table 2). Chromatographic separation of the CH_2Cl_2:CH_3OH (1:1) root extract of *Tephrosia vogelli* afforded two flavonoids (**13** and **14**). The structures of these compounds were determined using spectroscopic technique (UV-Vis, IR and NMR).

Table 2: Phytochemical screening tests of CH_2Cl_2:CH_3OH (1:1) root extract of *Tephrosia vogelii*

Plant Constituent	Reagent used	Present (+) /Absent(-)
Alkaloids	Mayer's reagent + Dragendroff's reagent	-
Anthraquinones	HCl + $CHCl_3$ + NH_3	-
Saponins	Warming in water bath	+

Terpenoides	Chloroform + Conc. Sulphuric acid	+
Flavonoides	Dilute ammonia solution + dil. HCl	+
Phlobatannins	1% Aqueous HCl	+
Steroids	Acetic anhydride + Chloroform + Concentrated H_2SO_4	-
glycosides	Hydrolyzed with dil. HCl + Ferric Chloride +benzene + NH_3	-
tannins	1% gelatin + sodium chloride	+
phenols	Ferric chloride	+

3.1 Characterization of compound 13

Compound **13** is a deep yellow solid with R_f value of 0.63 in *n*-hexane/ethyl acetate (7:3). The UV-Vis spectrum (Appendix-1) showed absorption maxima at λ_{max} (in $CHCl_3$) 250 and 300 nm attributed to a flavonoid skeleton having bathochromic shift indicating the presence of *n*-π* transition of carbonyl and ether group. The IR (KBr) spectrum (Appendix-2) showed absorption band at 3334 cm^{-1} attributed to hydroxyl group, a medium absorption band around 1700 cm^{-1} showing the presence of carbonyl moiety, a medium absorption band around 1600 and 1520 cm^{-1} attributed to C=C stretching of an aromatic ring, strong absorption bands around 2950 and 2850 cm^{-1} due to C-H stretching of aliphatic CH_3 and CH_2 groups, a weak absorption band at 3080 cm^{-1} and a medium absorption bands around 1400 and 1350 cm^{-1} attributed to C-H stretching of an alkene group and that of CH_3 and CH_2 bending respectively. Moreover, the absorption bands at 1286, 1274, 1121 and 1073 cm^{-1} indicated C-O stretching vibrations.

The ^1H NMR (400 MHz, $CDCl_3$, Table 3) spectrum revealed six singlet peaks observed at δ 1.48, 1.49, 0.89, and 1.20 attributed to methyl groups. The downfield chemical shift of the two methyl indicates that the two methyl are attached to an sp^2 carbon. The presence of protons at δ 5.23 (1H, *t*, *J*=3.6*)* and 5.5 (1H, brs) revealed the presence of a phenyl olefinic protons. The above two NMR evidence revealed that the compound have two prenyl groups. Protons at δ 5.90 (2H) is a characteristic of methylenedioxy group, also supported by DEPT-135 pointing down at δ 101.2 in agreement with a methylene connected to two oxygen atoms. There is a pair of doublets coupled to each other at δ 5.6 and 7.3 (*d*, *J*= 10) suggesting the presence of a pyran moiety in the skeleton.

The ^{13}C NMR spectrum with the aid of DEPT-135 (Table 3) showed a total of forty seven carbon signals; seven sp^2 oxygenated quaternary carbons, two sp^3 oxygenated quaternary carbons, two carbonyls carbons at δ 171.1 and 196.6, eleven methine carbon signals, four methylene at δ 44.30, 101.24, 22.68, and 66.37, six methyl signals at δ 21.05, 28.12, 28.48, 14.16, 29.36 and 29.10. The presence of methylene carbons signals at δ 101.24, 65 and 44 attributed to oxymethylene and methylene attached to heteroatoms and carbonyl respectively, also supported by DEPT-135 spectrum. The presence of two carbonyl carbons at δ 171.0 and 189.6 coupled with the presence of two sp^3 oxygenated quaternary carbons at δ 72 and 78 suggest that the spectra refers to a mixture of two flavonoids having a very close to skeleton to each other. The sp^3 oxygenated quaternary carbon at δ 78 is a characteristic of pyran moiety sp^3 oxygenated quaternary carbons. The carbonyl carbon at δ 189.6 should be a carbonyl carbon which is not α,β-conjugated where as the carbonyl at δ 171.0 is attributed to carbonyl carbons which is α,β-conjugated. Thus, considering the above spectroscopic evidence the difference between the two compounds possible is in A ring of the flavonoid skeleton one having a pyran cyclization where as the other one having a C-prenyl group at C-9 of ring A and O-prenyl group at C-8 of ring A. The NMR evidence revealed that the most differences observed between the two structures is mostly attributed to the difference on ring A of the flavonoid skeleton where as the ring B shows overlap of the NMR signals. One supporting evidence for this conclusion is the high intensity of oxymethylene signals at δ 101.24 as evidenced by the DEPT-135 spectrum. Thus, based on the above spectroscopic evidence and comparison with literature values compounds **13** and **14** were proposed as follows.

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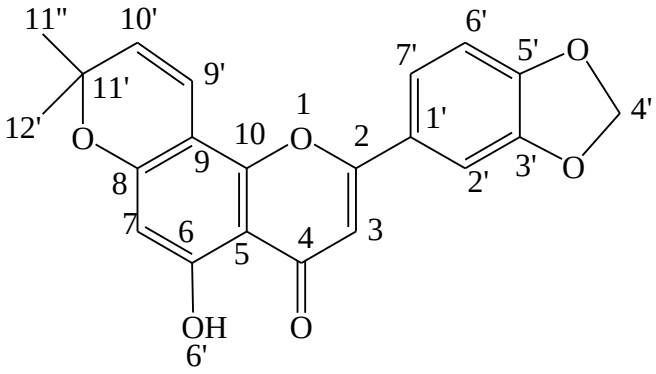

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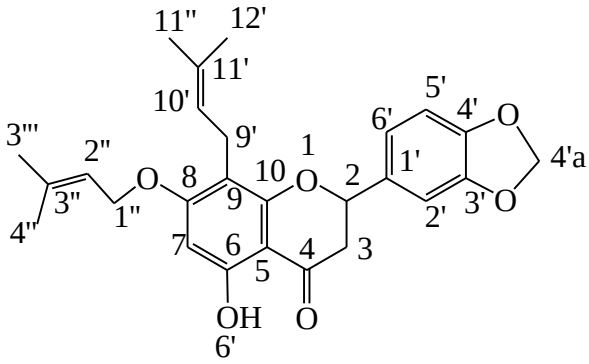

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Figure 2: The proposed structure of TV-1

Table 3: The ^{1}H NMR ^{13}C NMR and DEPT-135 spectra of compound 13

Position	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)	DEPT-135
2		157.97	Quaternary
3	7.44 (1H, *s*)	104.74	CH
4		171.0	Quaternary
5		104.70	Quaternary
6		157.04	Quaternary
6'	5.0 (1H, *s*)	-	-
7	5.78 (1H, *s*)	100.94	CH
8		157.97	Quaternary
9		109.13	Quaternary
10		154.19	Quaternary
9'	6.62 (1H, *d*, *J*=10)	118.02	CH
10'	5.50 (1H, *d*, *J*=10)	128.68	CH
11'	-	72.39	Quaternary
11''	0.89 (3H, *s*)	29.36	CH_3

12'	1.20 (3H, *s*)	29.10	CH_3
1'		128.59	Quaternary
2'	6.70 (1H, *s*)	111.52	CH
3'		148.02	Quaternary
4'	5.90 (2H, *s*)	101.2	CH_2
5'		148.02	Quaternary
6'	6.61(1H, *d*, *J*=8)	115.68	CH
7'	6.75 (1H, *dd*, *J*=8, 1.2)	118.02	CH

Table 4: The ^{1}H NMR ^{13}C NMR and DEPT-135 spectra of compound 14

Position	**^{1}H NMR (δ in ppm)**	**^{13}C NMR (δ in ppm)**	**DEPT-135**
2	4.58 (1H, *t*)	78.61	CH
3	2.0 (1H, *d J*=10)	44.30	CH_2
4		189.6	Quaternary
5		103.62	Quaternary
6		156.51	Quaternary
6'	5.0 (1H, *s*)	-	-
7	5.44 (1H, *s*)	93.77	CH
8		160	Quaternary
9		110	Quaternary
10		156.51	Quaternary
1'		134.94	Quaternary
2'	6.59(1H, *s J*=8.8)	112.65	CH
3'		149.41	Quaternary
4'a	5.90 (2H,*s*)	101.24	CH_2
4'		147.41	Quaternary
5'	6.59 (1H, *d*)	115.68	CH
6'	6.64 (1H, *d*)	128.4	CH
9'	2.1 (H, *d J*=8.8)	22.68	CH_2
10'	5.80 (1H, *t*)	128.59	CH
11'		131.94	Quaternary
11''	1.48 (3H, *s*)	21.05	CH_3
12'	1.49 (3H, *s*)	28.12	CH_3
1''	6.30 (2H, *d*)	66.37	CH_2
2''	5.39 (1H, *t J*=6.8)	128.59	CH
3''		131.94	Quaternary
3'''	0.89 (3H, *s*)	28.48	CH_3
4''	1.20 (3H, *s*)	14.16	CH_3

Spectral data of compound 13

Deep yellow solid, (40 mg), UV-Vis λ_{max} ($CHCl_3$) nm: 250 & 300; IR (KBr) ν_{max} cm^{-1}: 3334, 3073, 2920,2849, 1716, 1667, 1618,1596, 1457, 1270, 1213, 1114, 1029, &729 & ^{1}H NMR (100 MHz, $CDCl_3$) δ in ppm: 7.44 (1H, *s*, H-3) , 5.0 (1H, s, H-6'), 5.78 (1H,*s*, H-7) , 6.62 (1H, *d* H-9'), 5.50 (1H, *d*, H-10'), 0.89 (3H, *s*, H-11''), 1.20 (3H, *s*, H-12'), 6.70 (1H, *s*, H-2'), 5.90 (2H, *s*, H-4'), 6.61 (1H, *d*, H-6'), 6.75 (1H, *d*, H-7'), ^{13}C-NMR (100 MHz, $CDCl_3$) δ in ppm: 157.97 (C-2), 104.74 (C-3), 171 (C-4), 104.70 (C-5), 157.04 (C-6), 100.94 (C-7), 157.97 (C-8), 109.13 (C-9), 154.19 (C-10), 118.02 (C-9'), 128.68 (C-10'), 72.39 (C-11'), 29.36 (C-11''), 29.10 (C-12'), 128.59 (C-1'), 111.52 (C-2'), 148.02 (C-3'), 101.2 (C-4'), 148.02 (C-5'), 115.68 (C-6'), and 118.02 (C-7').

Spectral data of compound 14

Deep yellow solid, (40 mg), UV-Vis λ_{max} ($CHCl_3$) nm: 250 & 300; IR (KBr) ν_{max} cm^{-1}: 3334, 3073, 2920, 2849, 1716, 1667, 1618,1596, 1457, 1270, 1213, 1114, 1029, &729 & ^{1}H NMR (100 MHz, $CDCl_3$) δ in ppm: 4.58 (1H, *t*, H-2) , 2.0 (1H, *d*, H-3), 5.0 (1H,*s*, H-6') , 5.44 (1H, *s* H-7), 6.59 (1H, *s*, H-2'), 5.90 (2H, *s*, H-4'a), 6.59 (1H, *d*, H-5'), 6.64 (1H, *d*, H-6'), 2.1 (1H, *d*, H-9'), 5.80 (1H,t , H-10'), 1.48 (3H, *s*, H-11''), 1.49 (3H, *s*, H-12'), 6.30 (2H, *d*, H-1'') , 5.39 (1H,t , H-2''), 0.89 (3H, *s*, H-3'''), 1.20(3H, s H-4'')] ^{13}C-NMR (100 MHz, CDCl3) δ in ppm: 78.61 (C-2), 44.30 (C-3), 101.24 (C-4), 103.62 (C-5), 156.51 (C-6), 93.77 (C-7), 160 (C-8), 110 (C-9), 156.51 (C-10), 134.94 (C-1'),112.65 (C-2'), 149.41 (C-3'), 101.24 (C-4'a), 147.41 (C-4'), 115.68 (C-5'), 128.4 (C-6'), 22.68 (C-9'), 128.59 (C-10'), 131.94 (C-11'), 21.05 (C-11''), 28.12 (C-12'), 66.37 (C-1''), 128.59 (C-2''), 131.94 (C-3''), 28.48 (C-3'''), and 14.16 (C-4'').

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

To the best of our knowledge there was no prior report on the phytochemistry of *Tephrosia vogelii* on the contrary to its high use traditionally. Results of the phytochemical screening of the roots of *Tephrosia vogelii* revealed the presence of terpenoids, flavonoids, tannins, saponins, and phlobatannins and absence of anthraquinones. Based on spectroscopic data and comparison with literature data two flavonoids (**13** and **14**) were isolated and characterized. These compounds are very close to each other in structure and final complete separation of the two compounds couldn't be achieved due to lack of advanced purification techniques such a RP-HPLC.

TLC analysis of the rest fractions revealed presence of more polar compounds in the plant, nevertheless, due to solvent constraint and lack of advanced chromatographic techniques such as RP-HPLC isolation of more compounds could not be achieved.

4.2 Recommendation

- The present study used gravity column using ethyl acetate and *n*-hexane as an eluent. However, as evidenced by the presence of more compound spots in the TLC profile, more phytochemical analysis needs to be carried out on the polar extracts of the plant with the help of reverse phase RP-HPLC (C8 or C18 column) using water/methanol or water/acetonitrile as mobile phase**.** This will help to identify more polar phenolic compounds which are not identified in the present study.

- The present study started with small amount of the plant material and could not achieve to isolated and identify some of the minor phenolic compound as supported by the literature reports, Literature reports indicated the genus is a rich source of flavonoids, Hence, future phytochemical work is recommended starting with higher amount of the plant material and also needs further optimization of extraction solvents and purification approach for better extraction yield.

- *In vitro* bioassay and structural activity relationship (SAR) and has to be done on the crude as well as isolated compounds on various strains to establish explanation for traditional uses of the plant.

REFERENCES

[1] Pir Muazzam Shaha, Studies on bioactive constituents from *Sphaeranthus indicus* and *Berberis parkeriana*, HEJ Reasearch Institute of Chemistry University of Karachi, Karachi-75270, 1991.

[2] Flores, S.E.; Medina, J.D. Acta Cientifica Venezolana, 1971, 22(5), 147.

[3] Albro, P.W. *Rev. Biochem. Toxicol*. 1987,8,73.

[4] Kumari, P., Singh B.K., Joshi G.C and Tewari L.M., Veterinary Ethnomedicinal Plants in Uttarakhand Himalayan Region, India, *Ethnobotanical Leaflets*. (2009), 13: 1312-1327

[5] Bhattacharjee S.K., Handbook of Medicinal Plants, Pointer Pub, Jaipur-03, India., 1998, 1-6.

[6] Littleton, J., Rogers T. and Falcone D., Novel approaches to plant drug discovery based on high throughput pharmacological screening and genetic manipulation, Life Sciences 2005, 78, 467 – 475.

[7] http://www.people.vcu.edu

[8] http://www.arbec.com.my/top

[9] Koskinen, A., Asymmetric synthesis of natural products, John Wiley & Sons Ltd, University of Oulu, Oulu, Finland 1993.

[10] Rajan, S., Mahalakshmi, S., Deepa, V. M., Sathyal, K., Shajitha, S., Thirunalasundari,T., Antioxidant potentials of Punica granatum fruit rind extracts, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011, 3(3), 197.

[11] Jaiganesh, K.P., Arunachalam, G., Preliminary Phytochemical Screening And Antimicrobial Potential Of Pterospermum Canescens Roxb, (Sterculiaceae), *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011, 3(3), 140.

[12] Syed H., Keshava, C.K., Chandrashekar, K.R., Phytochemical Evaluation And Antibacterial Activity Of Pterospermum diversifolium Blume, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011,3(2), 240.

[13] Duffy, C.F, Power, R.F., Antioxidant and antimicrobial properties of some Chinese plant extracts, *International Journal of Antimicrobial Agents*.2001, 17,527-529.

[14] http://www.rsc.org/ebooks/archive/free/BK9780854046812/BK9780854046812- 00001.pdf

[15] Goyal, B.R., Goyal, R.K., Mehta, A.A., Phyto-pharmacology of Achyranthes aspera : A *Review. Pharmacognosy Reviews*, 2007, 1, 170.

[16] Clark A. M., Natural products as a resource for new drugs, Pharm. Res., 1996, 13.

[17] Borris R.P., Natural products research: perspectives from a major pharmaceutical company, J. *Ethnopharmacol*. 1996, 51, 29-38

[18] Ahmed A. A., Mahmoud A.A., Williams H.J., Scott I.A., Reibenspies J.H., Mabry T.J. New sesquiterpene α-methylene lactones from the Egyptian plant *Jasonia candicans, J. Nat. Prod*. 1993, 56, 1276-1280

[19] Casteel H.W., Wender S.M., Identification of flavonoid compounds, Rf valuesand colour tests. Anal. Chem. 1953, 25, 508.

[20] Nychas, G.J., Natural antimicrobials from plants, In: New Methods of FoodPreservation, (Gould, G. W., ed.), Chapman and Hall, Glasgow, 1995, 58-89.

[21] Hamilton A., Medicinal Plants and Conservation: issues and approaches London: Panda House, Catteshall Lane; 2003.

[22] Pankhurst R: A Historical Reflection on the Traditional Ethiopian pharmacopeias. Journal of Ethiopian Pharmaceutical Association 1965, 2:29-33.

[23] Tefer, G., Hahn, H., The use of medicinal plants in self care in rural centralEthiopia. J. Ethnopharmacol. 2003, 87, 155-161.

[24] Tesfaye, S., Kaleab, A., Tsige, G., Ethno botanical and ethno pharmaceutical studies on medicinal plants of Chifra Disrict, Afar Region, North-Eastern Ethiopia. J.Ethiopian Pharmaceutical. 2006, 24(1), 41-58.

[25] Angiosperm Phylogeny Group (2009). "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III" (PDF). Botanical Journal of the Linnean Society161 (2): 105–121. doi:10.1111/j.1095-8339.2009.00996.x. Retrieved 2013–07–06.

[26] Ben Sassi, A., Barzallah-Skhiri, F., Aouni, M. , Investigation of some medicinal plants from Tunisia for antimicrobial activities. *Pharmaceut. Biol.* 2007, 15(5), 421-428.

[27] Coruh, I., Gornez, A.A., Ercisli, S., Total phenolics, mineral elements, antioxidant and antibacterial activities of some edible wild plants in Turkey. *Asian.J.Chem.* 2007, 19(7), 5755-5762.

[28] Baraka, D. M., Ecological and Phytochemical Study on One Species of Polygonaceae. Faculty of science, Zagazig University, Egypt. 1985, 171.

[29] Iwu, M. W., Duncan, A. R., Okunji, C. O., New antimicrobials of plant origin. In: Janick, A.S. (Ed.), Perspectives on New Crops and New Uses. *ASHS Press.* 1999, 457-462.

[30] Willis, J. C., The Dictionary of Flowering Plants and Ferns. 8th ed. Cambridge University Press, Cambridge, UK, 1973, 1135.

[31] Atchison, E., Studies in the Leguminosae. VI. Chromosome Numbers Among Tropical Woody Species. American Journal of Botany,1951, 538-546.

[32] Qureshi, R., Bhatti, G.R., Memon, R.A., Ethnomedicinal uses of herbs from northern part of NARA desert, Pakistan. Pak. J. Bot 2010, 42, 839-851.

[33] Virupanagouda, P.P., Shivakumar, H., Nanjappaiah, H.M., Navanath, K., Mohan, C. P., Phytopharmacology of *Tephrosia purpurea Linn*: An Overview. Pharmacologyonline 2011, 3, 1112-1140.

[34] Burkill, H.M.,The Useful Plants of West Tropical Africa, second ed. Royal Botanic Gardens, Kew, 1995, 3, 460–462.

[35] Ingham, J.L., Naturally occurring isoflavonoids (1855–1981). Prog. Chem. Org. Nat. Prod. 1983, 43, 1–266.

[36] Marston, A., Msonthi, J.D., Hostettmann, K., On the reported molluscicidal activity from Tephrosia vogelii leaves. Phytochemistry 1984, 23, 1824–1825.

[37] Kokate, C. K., Practical pharmacognosy , 4th edn.(Vallabh Prakashan Pune) 1996, 107.

[38] Harbone, J.B., Phytochemical methods.1973.

[39] Plaisted, P. H., Contributions from Boyee Thompron Institute. 1958; 9: 231- 44.

[40] Sethi, P. D., HPTLC Quantitative Analysis of Pharmaceutical Formulations, 1st ed. CBS Publishers and Distributors, NewDelhi. 1996, 3-73.

[41] Roopashree, T. S., Dang, R., Rani, S. R. H., Narendra. C., Antibacterial activity of anti-psoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. International Journal of Applied Research in Natural Products 2008; 1(3): 20-28.

[42] Segelman, A. B., Farnsworth, N. R., Quimby, M. D., False-negative saponins test results induced by the presence of tannins. Lloydia 1969; 32: 52 - 8.

[43] Evans, W. C., Evans P., General methods associated with the phytochemical investigation of herbal products. 2002; 139-143.

[44] Kapoor, L. D., Singh, A., Kapoor, S. L., Srivastava, S. N., Survey of Indian plants for saponins, alkaloids and flavonoids. *I. Lloydia* 1969; 32: 297 - 304.

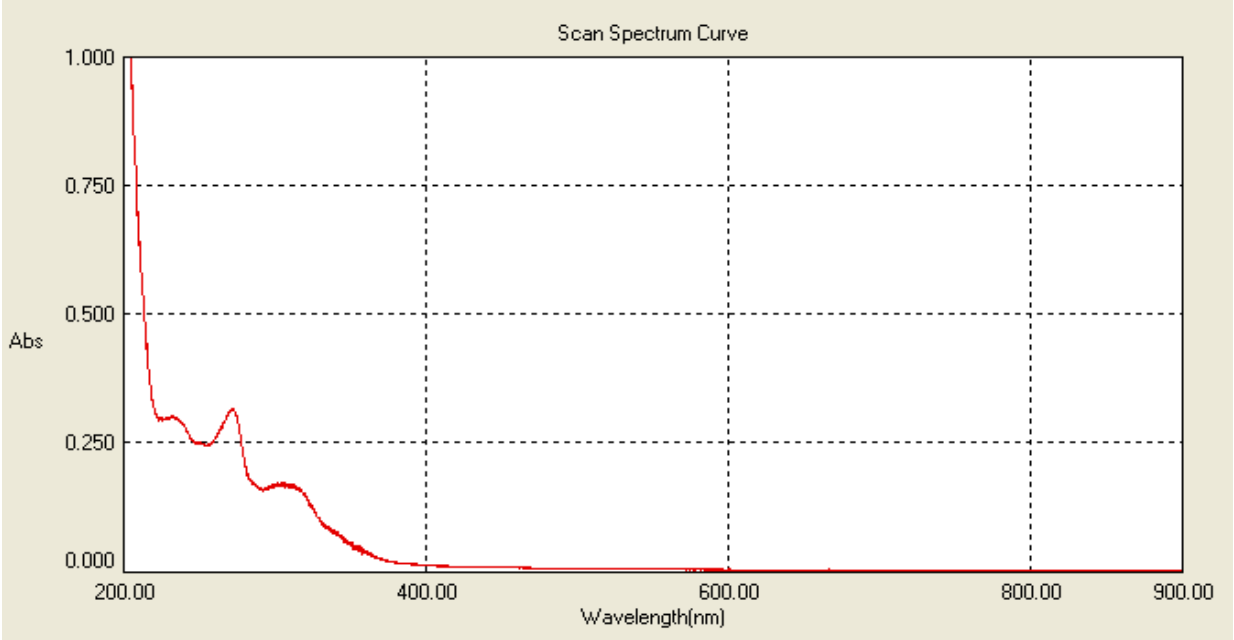
[45] Roopashree, R., Dang, S., Rani, N.C., Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. International Journal of Applied Research in Natural Products, 2008, 1(3) 20-28.

[46] Salehi, S. MH., Aynehchi, Y., Amin, G.H., Mahmoodi, Z., Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins. IV. Daru 1992, 2, 281 - 291.

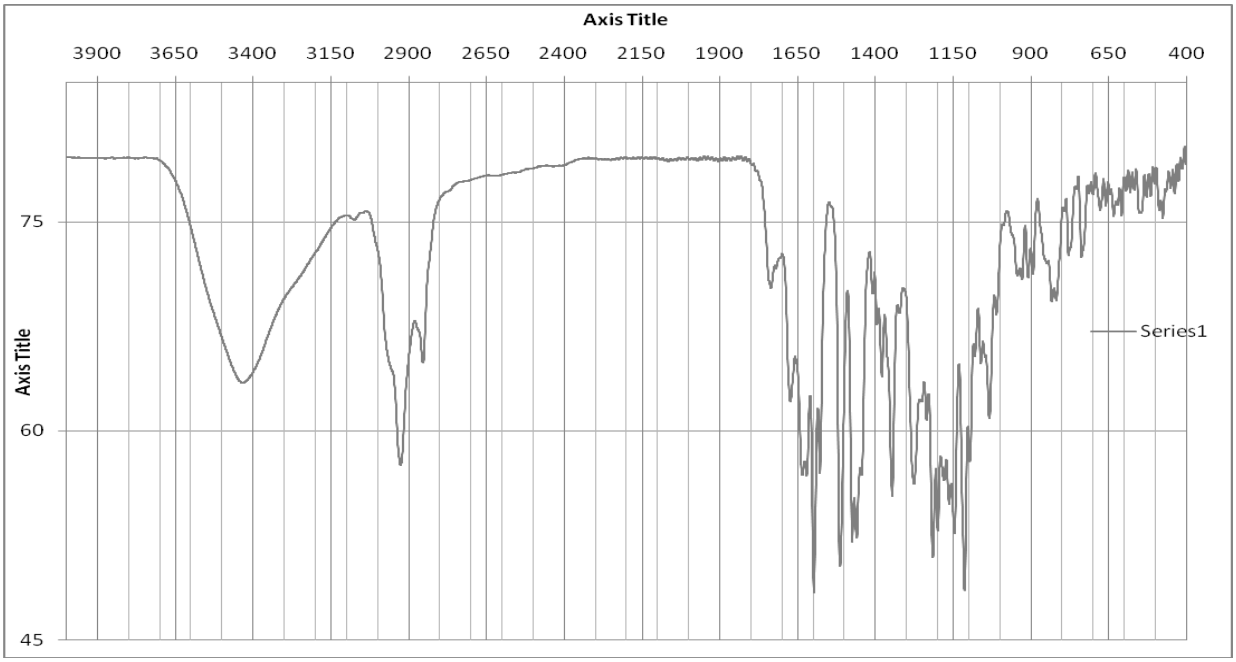
[47] Segelman, A.B., Farnsworth, N.R. Biological and phytochemical screening of plants. IV. A new rapid procedure for the simultaneous determination of saponins and tannins. Lloydia 1969, 32, 59 - 65.

APPENDICES

APPENDIX A: UV OF COMPOUNDS 13 AND 14



APPENDIX B: IR SPECTRUM OF COMPOUNDS 13 AND 14



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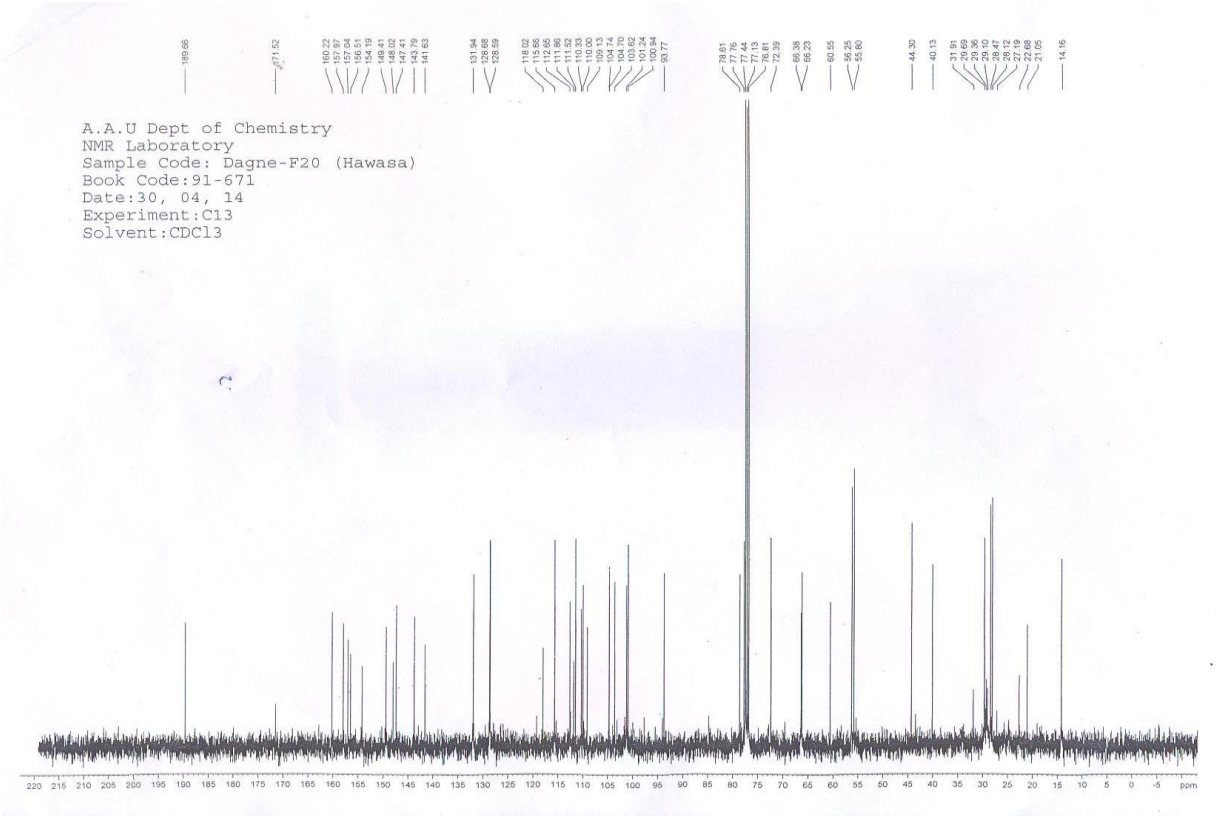
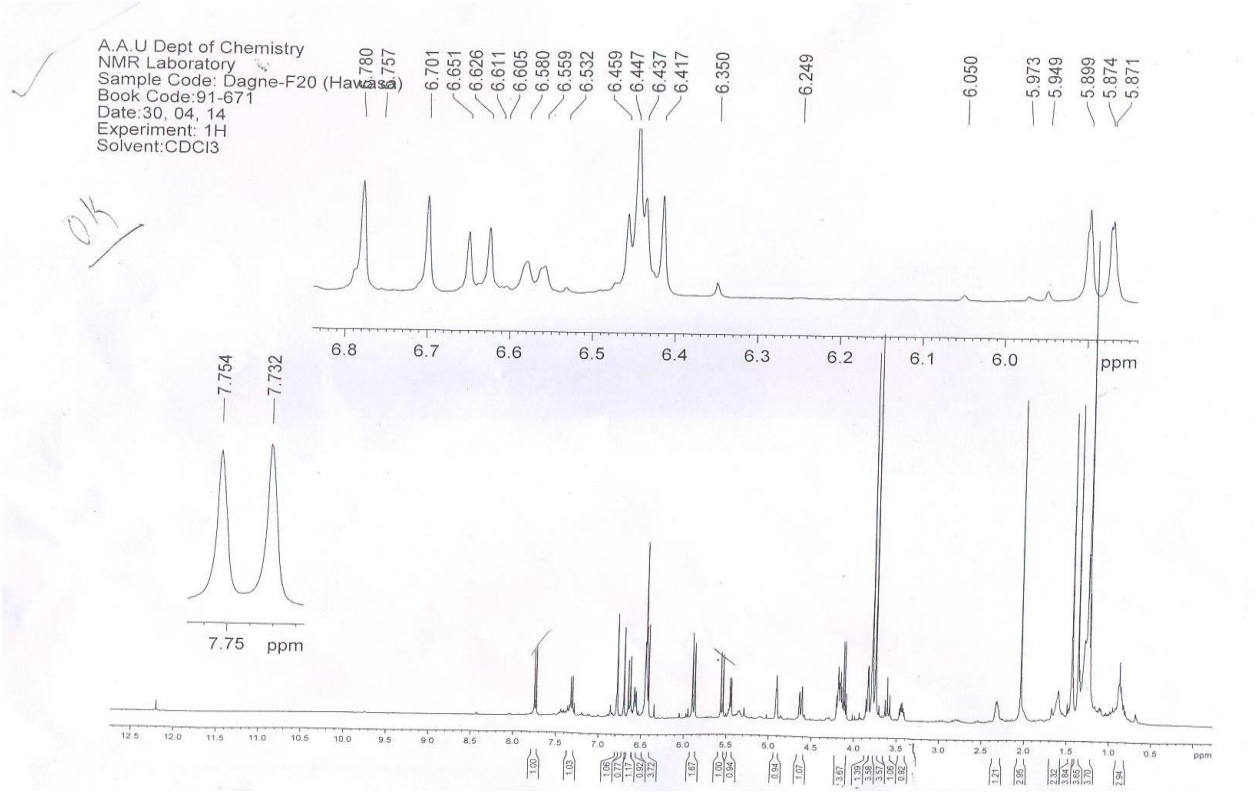
APPENDIX C: ^{13}C-NMR OF COMPOUNDS 13 AND 14 IN $CDCl_3$



APPENDIX D: ^{1}H-NMR OF COMPOUNDS 13 AND 14 IN $CDCl_3$



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APPENDIX E: DEPT SPECTRUM OF COMPOUNDS 13 AND 14 IN $CDCl_3$