

# Chemical Studies of *Tephrosia vogelii* and *Commiphora schimperi* Occurring in Ethiopia

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**Abstract:** The objective of this study is to extract, screen, isolate, and characterize the chemical constituents of *Tephrosia vogelii* and *Commiphora schimperi*. In the course of this study the stem bark of *T. vogelii* and resin of *C. schimperi* were collected from Areka Agricultural Research institute, Wolaita Zone and Konso, Gamo Gofa Zone, respectively. Phytochemical screening of the crude extract of *T. vogelii* revealed the presence of tannins, saponins, flavonoids, and terpenoids. Chromatographic separation of the methanolic crude extract of *T. vogelii* yielded the compound 8 (4, 5-dihydro-5, 5-dimethyl-4-oxofuran-3-yl)-5-hydroxy-7-methoxy-2-phenyl-4H-chrome-4-one. The essential oil from the resin of *C. schimperi* was isolated by hydro-distillation and some of the components identified by means of GC and GC/MS analysis. The main components of the essential oil from *C. schimperi* were:  $\alpha$ -pinene (73%) and  $\beta$ -pinene (17%). The resin was also subjected to extraction by petrol, ethyl acetate, and methanol. The components of *C. schimperi* were isolated using chromatographic techniques and attempts were made to identify the isolated substances. Analysis of the petrol extract of the resin of *C. schimperi* showed to be an excellent source of the industrially important fragrant compounds,  $\alpha$ - and  $\beta$ -pinene. The structures of the isolated compounds were characterized by comparing IR,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR chromatographic data of the compounds with literature.

**Keywords:** Chemical Studies, *Commiphora schimperi*, Ethiopia, Resin, Stem Bark, *Tephrosia vogelii*

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## 1. Introduction

Medicinal plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases. The knowledge of their healing properties has been transmitted over the centuries within and among human communities [1]. The use of Medicinal plants has a long history throughout the world. They are known to provide a rich source of raw materials and have been used in traditional medicine for numerous human diseases for thousands of years, in different parts of the world, particularly those living in rural areas of the developing countries [2]. Traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary healthcare delivery, not only in Africa but also, in all countries of the world. It is the first choice healthcare treatment for at least 80% of Africans who suffer from high fever and other common ailments [3]. Ethiopians have used

traditional medicines for many centuries, the use of which has become an integral part of the different cultures in the country. The indigenous peoples of different localities in the country have developed their own specific knowledge of plant resource uses, management and conservation [4].

Originally teas or decoctions (aqueous extracts) or tinctures or elixirs action protocols and applied and coupled with modern isolation techniques were used to prepare and administer herbal remedies. These were usually the starting points for isolation work. These days, various extraction protocols are applied and coupled with modern isolation techniques which include chromatography, often guided by bioassay, to isolate the active compounds.

In Ethiopia, about 80% of the people use medicinal plant remedies selected over centuries. Exploration of the chemical constituents of the plants and pharmacological screening is of great importance which leads for developments of novel agents [5]. A systematic study of a crude drug from

medicinal plants embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The compounds that are responsible for medicinal properties of the drug are usually secondary metabolites such as flavonoids, alkaloids, saponins, tannins, terpenoids, etc. and their derivatives [6].

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 [7]. Many plants from this genus have been used traditionally for the treatment of rheumatic pains, syphilis, dropsy, stomach ache, diarrhea, asthma, abortifacient, respiratory disorders, laxative, diuretic and inflammation [8]. In Pakistan the roots are used to treat typhoid fever [9].

In China, *Tephrosia vogelii* is used as a botanical insecticide and fly repellent [10]. It has been shown to have toxic and repellent effects against certain insect pests of stored grains [11]. *Tephrosia* has been used as a rat poison by compounding with ground nut [12]. Powders of *Tephrosia vogelii* are effectively used in the Congo against the stored ground nut pest *Carye donsertatus* [9]. In Nigeria, it is used as a seed dresser for cereals and legumes [13]. It is also applied directly to treat head lice, fleas, scabies and other eco-parasites [14].

Recently, naturally occurring elata-dihydrochalcone, isopongaflavone, obovatol methyl ether, and deguelin were isolated from seed pods of *Tephrosia elata* [15]. Purpuritin and semiglabin are isolated from *Tephrosia purpurea* seed extract [16]. Phytochemical analysis of *Tephrosia candida*, dichloromethane: methanol (1:1) extract of the air-dried aerial parts afforded new prenylated flavonoids, namely, Tephro-candidins, candida chalcone, a sesquiterpene and a flavonoid phytoalexin, pisatin [17]. Semiglabin, apollinine, pseudo semiglabin, and lanceolatin A are isolated from the roots of *Tephrosia nubica* [18]. Emoroidenone, tephro, ovalichacone, and hildecarpin are isolated from the root bark of *Tephrosia aquilata* [19].

The sesquiterpene ( $1\beta$ ,  $6\alpha$ ,  $10\alpha$ )-guai-4 (15)-ene-6, 7, 10-triol, the lignan (+)-lariciresinol 9'-stearate, the larvicidal rotenoid deguelin and the flavonoid quercetin are secondary metabolites isolated from *Tephrosia vogelii* [20, 21]. Chen *et al.*, reported the isolation of four flavonols, four flavans, a flavanone and a sesquiterpene from *T. vogelii* [22]. The rotenoid and deguelin content of the leaf extracts of the white and purple varieties of *T. vogelii* showed similar acaricidal activity [22]. A 100% larval mortality of *Rhipicephalus sanguineus* was observed at the concentration of 20% the ethanolic extract of the leaf of *T. vogelii* [23]. Rotenoids from *T. vogelii* Hook. F. (*Leguminosae*) including deguelin, rotenone, sarcolobine, tephrosin and  $\alpha$ -toxicarol are required for pest control and soil enrichment agent for poorly resourced small scale farmers in Southern and Eastern Africa [24, 25].

The ethyl acetate extract of the fruit of *Piper aduncum* and *T. vogelii* leaf extract showed a strong synergistic toxic effect on larvae of *C. pavonana* at 5:1 mixture respectively [26].

Similarly the ethyl acetate-methanol (9:1) and ethyl acetate extract of the leaf of *T. vogelii* showed insecticidal activity against the cabbage head caterpillar, *Crociodolomia pavonana* [27].

Although investigation of phytochemicals and biological activities of the leaf of *T. vogelii* was done in different places, there is no report on stem bark of *T. vogelii*, thus far. More specifically to the best of our knowledge there is no investigation that has been done in Ethiopia. Thus it is worthwhile to investigate the phytochemicals found on the stem bark of the medicinally important *T. vogelii* occurring in Ethiopia.

The Burseraceae is a large family with 17-20 genera and 500-600 species, widespread in tropical and subtropical regions [28, 29]. Engler [30] subdivided the Burseraceae into three tribes on the basis of fruit structure: Protieae (4 genera), Boswellia or Bursereae (eight genera which include *Boswellia* and *Commiphora*) and Canarieae (9 genera).

The genus *Commiphora* with 150-200 species is wide spread in the drier parts of tropical Africa and Madagascar, also from Arabia to India and with few species occurring in South America. The genus is a very conspicuous and dominant element in the dry bush lands of North East Africa, where a large number of species are endemic to this area [31].

*Commiphora myrrha* is the chief source of myrrh today. The plants grow wild in the North-Eastern province of Kenya and adjoining areas of Somalia and Ethiopia. These plants yield economically important gum exudates which have been collected for centuries as medicinal and perfumery substances [32].

Holmes [33, 34] apparently was the first to propose that the myrrh of the Bible was the perfumed myrrh or "bissabol" and not the medicinal myrrh or "heerabol" from *C. myrrha*. Common myrrh (heerabol) is obtained from *C. myrrha*; this is the species from which "oil of myrrh", or stacte, was obtained. Other species sometimes passing as myrrh or bdellium include *C. africana*, *C. anglosomaliae*, *C. gileadensis* (*C. opobalsamum*), *C. hildebrandtii*, *C. kataf*, *C. mukul*, and *C. schimperi* [35]. The odor of myrrh is described as warm-balsamic, (sweet, and somewhat spicy aromatic, sharp and pungent when fresh [36].

The gifts presented by the Maji to the infant Christ symbolized His life: "gold for royalty, rank incense for divinity, and myrrh for suffering" [36]. Myrrh was also in the final drink offered to Christ on the cross: "And they gave Him to drink wine mingled with myrrh; but He received it not" (Mark 15:23). Myrrh was in addition, used to embalm the body of Christ: "And there came Nicodemus, which at first came to Jesus by night, and brought a mixture of myrrh and aloes, about a hundred pound weight" (John 19:39). Myrrh was also included in the "oil of holy ointment" (Exodus 30: 23-24). Many herbalists recommend tincture of myrrh as an astringent for the mucous membranes of the mouth and throat [37]. Myrrh is found in salve used in treating bed sores, hemorrhoids, and wounds. Internally, myrrh is also used for indigestion, ulcers, and bronchial

congestion and as an emmenagogue [35].

Among local African traditional medicines, the resinous gums of *C. myrrha* and *C. guidottii*; which are locally known as "malmal" and "habak-hadi" in the Somali vernacular, respectively, are used on livestock against ticks [32]. The major use of myrrh is for burning as incense in religious ceremonies. The resin is distilled to yield volatile oils and these have their own characteristic balsamic odor which finds use in perfumery [29]. The resin of *C. guidottii* is the second major type of myrrh and it is commonly known in commerce as gum "bissabol" (Hindi) or "opopanax". Opopanax occurs also widely in Ethiopia from which the resin is collected for export to India, China and Europe. Thulin *et al.* [38] suggested that the myrrh of the Bible and the incense of the ancient Egyptians and of Classical times [35] was most likely the "perfumed myrrh" or "bissabol" from *C. guidottii* and not the medicinal myrrh from *C. myrrha*. "Bissabol" according to Holmes [39] meant buffalo myrrh, "as it is mixed with food given to milch cows and buffaloes to improve quality and quantity of their milk." Thulin and Claeson [38] confirmed that the tree called "hadi" in Somali is *C. guidottii* and produces the resin "habak-hadi" also known by several other names including "opopanax", "bissabol", "scented myrrh", "abeked" (Amharic). Tucholka [40], in a thesis on the chemical composition of "bissabol", reported that "habak-hadi" was used during female circumcision, by bathing in water in which the resin was emulsified. A similar bath was taken by Somali women after giving birth to a child. "Habak-hadi" is used in Somalia for the treatment of stomach complaints and diarrhea [41]. It is also used topically for the treatment of wounds.

Extraction of the resins by organic solvents furnishes a "resinoid" or an "absolute." The "resinoid" is prepared by extraction of the crude resin with a hydrocarbon solvent such as hexane or petrol. The "resinoid" contains almost all the available essential oils of the resin. The "absolute" is prepared by extraction of the resins with alcohol [29]. Essential oils on the other hand are separated by steam or hydro-distillation at atmospheric pressure.

Gas chromatography is an excellent tool for the separation, characterization, and quantitative analysis of essential oils. The combined gas chromatogram-mass spectrometer (GC/MS) method provides a facile, sensitive and convenient system for the separation and identification of complex mixtures [42]. Spectroscopic methods like UV, IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR are among the most powerful techniques for the characterization of isolated compounds.

The components of the essential oils obtained from only few *Commiphora* species have been investigated and these include: *C. terebinthina* and *C. cyclophylla* [43], *C. myrrha* [44, 45] and *C. rostrata* [46]. The oils from *C. rostrata* are distinguished by the presence of the homologous ketones starting from 2-octanone, 2-nonanone, 2-decanone etc [46]. The other *Commiphora* species are rich both in the structures of monoterpenes and sesquiterpenes. Oxygenated terpenoids are the components of essential oils most often responsible for their distinctive aroma and flavor, even though they are

often minor constituents of the oil [47].

It is interesting to note that as most of the previous reports on resin of *C. myrrha* are based on the study of materials obtained from commerce, it is highly likely that the resins are derived from different *Commiphora* species. This shows the significance of working on resins from properly identified trees.

## 2. Materials and Methods

### 2.1. General

Hydro-distillation of resin was done at atmospheric pressure using 4 L round bottom flask fitted with Clevenger apparatus and glass condenser. Optical rotation of the hydro-distillate was measured with Perkin-Elmer 241, Polarimeter, at room temperature using sodium D line.

GC was run using Hewlett-Packard 6890 GC series equipped with FID and HP-5 capillary column (cross linked 5% diPh, 95% dimethylpolysiloxane, 30 m x 0.32 mm i.d. x 0.25  $\mu\text{m}$  film thickness). The column temperature was programmed at 50-210°C at a rate of 3°C/min. The injector and detector temperatures were 220°C and 270°C, respectively. Samples (0.5  $\mu\text{L}$  of the oil solutions in  $\text{CHCl}_3$ , 2 mg/mL) were injected by the splitless technique. Nitrogen was used as carrier gas (10 Psi or 2.3 mL/min).

GC/MS was performed on a Fisons GC model 8000 series coupled to a mass spectrometer, MD 800 quadrupole analyzer operating at 70 eV. The capillary column type was DB-17 (50% Ph, 50% methylpolysiloxane, 30 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$  film thickness) with helium as the carrier gas (5 Psi or 1.15 mL/min). Samples (0.6  $\mu\text{L}$  of the oil solutions in  $\text{CHCl}_3$  5 mg/mL) were injected by the split technique.

Identification of the constituents of the essential oils was performed by matching MS data of each constituent With Wiley, NIST and user generated mass spectral libraries.

Refractive index was measured at room temperature using Atago Abbe refractometer, No 99996, Japan.

Column chromatography (CC) was done with column size 3 cm x 30 cm packed with silica gel 60, size 0.063-0.200 mm (70-230 mesh ASTM) and thin layer chromatography (TLC) on aluminum sheets, silica gel 60 F<sub>254</sub>, and layer thickness 0.2 mm (Merck). Preparative thin layer chromatography (PTLC) plates were prepared on 20 cm x 20 cm glass, silica gel 60 PF<sub>254+366</sub>, 7748 (Merck) layer thickness 0.5 mm. Spot detection on TLC was performed by using UV (254 nm, 365 nm) and spray reagent 1% vanillin in  $\text{H}_2\text{SO}_4$ .

NMR data was generated with 300 MHz for  $^1\text{H}$  and 75 MHz for  $^{13}\text{C}$ , for compounds isolated from *C. schimperi* and 400 MHz for  $^1\text{H}$  NMR and 100 MHz for  $^{13}\text{C}$  NMR, for the compound isolated from *T. vogelii*, TMS as internal standard and  $\text{CDCl}_3$  (for *C. schimperi*) and  $\text{DMSO}-d_6$  (for *T. vogelii*) solvent. IR spectrum was measured with Perkin-Elmer, 1600 series FTIR, using KBr pellets.

## 2.2. Extraction and Compound Isolation from the Stem Bark of *Tephrosia vogelii*

### 2.2.1. Plant Materials

The stem-bark of *Tephrosia vogelii* was collected in November, 2017 from Areka Agricultural Research institute, Wolaita Zone, Ethiopia. The plant was identified by botanist Retta Regasa Department of Biology, Hawassa College of Teachers Education. Plant specimen was deposited at the herbarium of Hawassa College of Teacher Education, with voucher number MB/0087.

### 2.2.2. Extraction

The collected stem bark of *Tephrosia vogelii* was chopped into small pieces and air dried under shade for 30 days and milled to suitable size by using mortar and pestle. About 500 g of the powdered stem bark of *Tephrosia vogelii* was soaked in 3 L petrol in a round bottom flask, at room temperature. The round bottom flask was put on an orbital shaker and left for 24 hours at a speed of 120 revolutions per minute. After 24 hrs the solution was filtered using Whatman filter paper. The filtered solution was concentrated using rotary evaporator at reduced pressure and a temperature of about 40°C. The marc left was further extracted with chloroform and methanol consecutively, likewise.

### 2.2.3. Phytochemical Screening

Phytochemical screening tests were carried out on the crude extracts (petroleum ether, chloroform and methanol) following the standard procedures of Ganesh and Vennila [48] and Prashant *et al.*, [49] in order to investigate the types of secondary metabolites present in the plant species under investigation.

### 2.2.4. Compound Isolation

TLC profile analyses were carried out in chloroform and methanol extract in different solvent systems to identify the appropriate solvent for column chromatography. TLC profile of methanolic extract was conducted in n-hexane-ethyl acetate, ethyl acetate-chloroform, and methanol-ethyl acetate, solvent combinations by varying solvent ratios. Ethyl acetate-n-hexane combination showed good TLC profile. Twelve gram of methanolic extract was adsorbed on 15 g of silica gel and was subjected to column chromatography. The column was packed by n-hexane to achieve least polarity to the mobile phase during the beginning of elution. Elution was conducted with increasing gradient of n-hexane-ethyl acetate. A total of 65 fractions (25 ml each) were collected and analyzed by TLC for identification of the pure components. Fractions 16-27 showed two spots and were combined and further fractionation was conducted in small column in n-hexane-ethyl acetate (6:4, 4:6, 7:3, 3:7, ethyl acetate) solvent system and a total of 34 fractions were collected. TLC analysis of fractions 10-17 showed a single spot with R<sub>f</sub> value (0.63) in (7:3) n-hexane-ethyl acetate solvent system having minor impurity. These fractions were combined and further purified by washing in n-hexane for several times. A white solid compound (40 mg) was obtained and coded BA-

5. Spectroscopic data of BA-5 was generated using UV, IR, <sup>1</sup>H and <sup>13</sup>C NMR techniques, for structure elucidation.

## 2.3. Extraction and Compound Isolation from the Resin of *C. schimperi*

### 2.3.1. Plant Material

Plant materials of *C. schimperi* were collected on two occasions in January, and April, 2016 from Gamo Gofa (Konso). Konso is located 587 km South of Addis Ababa. The local name of all the trees at Konso is "Qahatita". The trees are approximately 4-6 m, with bark that peels into flakes. When bark is incised milky exudate flows out and solidifies within 30 min and becomes dark-brown after one day. Naturally exuded resins were collected. Leaves and bark were collected to aid in the botanical identification of the species. The plant was identified by Kaj Vollesen (Kew Royal Botanical Garden, U.K.) and voucher specimens have been deposited at the National Herbarium, Addis Ababa University with collectors' number Tegene 3 and herbarium numbers 072773 and 072774.

### 2.3.2. Ethnobotany

Ethnobotanical information was gathered by interviewing the local people and through observation.

### 2.3.3. Hydro-distillation

The dry resin (15 g) was first crushed as much as possible into smaller pieces and placed in a round bottom flask fitted with Clevenger apparatus and was hydro-distilled for 3 h at atmospheric pressure. The strongly aromatic oil was separated from the water layer by separatory funnel and dried by adding anhydrous Na<sub>2</sub>SO<sub>4</sub> and then weighed.

### 2.3.4. Extraction

Resin of *C. schimperi* (62 g) was first crushed and extracted with petrol (200 mL) to give crude extract (30 g, 48%). The marc was soaked with MeOH (150 mL), filtered, and the solvent removed to give 3.5 g (6%). The total amount of extract obtained was 33.5 g (54%).

### 2.3.5. Compound Isolation

The petrol extract (28 g) of the resin of *C. schimperi* was applied on column packed with silica gel (petrol) and eluted with petrol-CHCl<sub>3</sub> gradients. Out of the 31 fractions collected, fractions number 4 and 5 (petrol: CHCl<sub>3</sub>, 49: 1) gave nearly pure colorless liquid compound coded 82-1A (1 g). Subsequent fractions gave colorless liquids with two spots having the same R<sub>f</sub> values and was coded 82-1B.

## 3. Results and Discussion

### 3.1. Chemical Analysis of the Stem Bark of *Tephrosia vogelii*

#### 3.1.1. Extraction

The yields of the crude extracts of *Tephrosia vogelii* are presented in Table 1 below.

**Table 1.** The percent yield of crude extracts.

| Solvent         | Extract (g) | Yield (%) |
|-----------------|-------------|-----------|
| Petroleum ether | 2           | 0.4       |
| Chloroform      | 5           | 1         |
| Methanol        | 12          | 2.4       |

As can be seen in Table 1 above the crude extract yield increased up on increasing the solvent polarity. This indicates that most of the chemical constituents in the plant stem bark are polar. The small yield of petroleum ether and chloroform extracts only allowed phytochemical screening.

### 3.1.2. Phytochemical Screening

A variety of herbs and herbal extracts contain different secondary metabolites with biological activity that can be of valuable therapeutic index. These secondary metabolites are natural products that are responsible for medicinal properties of plants to which they belong. Plants of the genus *Tephrosia* are known as rich source of secondary metabolites such as alkaloids, terpenoids, saponins, tannins and other aromatic compounds [50]. Phytochemical screening tests of different solvent extract of stem bark of *Tephrosia vogelii* revealed the presence of medicinally active secondary metabolites including tannins, saponins, terpenoids, and flavonoids. Anthraquinones, steroids and alkaloids are absent, however. The phytochemical screening results of the methanolic and aqueous extracts of the leaf of *T. vogelii* by Inalegwu and Sodipo [51] showed the presence of cardiac glycosides, alkaloids, saponins, and phlobatannins and absence of anthraquinones and tannins. Similarly the ethanolic leaf extract of *T. vogelii* showed the presence of catechol tannins, saponins, sugars, leuco-anthocyanins, polyterpenes, and sterols [32].

### 3.1.3. Structure Elucidation of Compound BA-5

Compound BA-5 is deep yellow solid, melting point 128°C and Rf0.63(n- hexane-ethyl acetate; 70:30). The UV-VIS spectrum showed  $\lambda_{\text{max}}$  343 nm (in  $\text{CHCl}_3$ ) attributed to the presence of n- $\pi^*$  transition of carbonyl.

In the IR spectrum, a broad absorption band at 3397  $\text{cm}^{-1}$  attributed to hydroxyl group; 1743  $\text{cm}^{-1}$  presence of carbonyl group. Absorption bands at 2965  $\text{cm}^{-1}$  and 2918  $\text{cm}^{-1}$  shows the presence of  $\text{sp}^3$  and  $\text{sp}^2$  C-H stretching vibrations. The medium bands at 1618 and 1516  $\text{cm}^{-1}$  attributed to aromatic ring C=C stretching. The absorption bands at 1138  $\text{cm}^{-1}$  and 1092  $\text{cm}^{-1}$  indicated C-O stretching.

$^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$  = 3.96 (3H, s, H-7') and 1.55 (3H, d, H-6'') correspond to methyl and methoxy groups, respectively. The signals at  $\delta$  = 7.70 - 6.20 are aromatic; 7.66 (1H, d, J = 8.8 Hz, H-4''), 7.33 (1H, d, J = 8 Hz, H-2' and 6'), 6.92 (1H, d, J = 7.6 Hz, H-3' and 5'), 6.80 (1H, d, J = 8.4 Hz, H-5), 6.51 (1H, d, J = 6.4 Hz, H-6), 6.34 (1H, m, H-5'').

The  $^{13}\text{C}$  NMR and DEPT-135 spectra of compound BA-5 shows a total of nineteen carbon signals  $\delta$ : 24.8 (C-6'') and 56.2 (C-7') for the methyl and methoxy carbons, respectively;  $\delta$ : 147.99 (C-4''), 130.8 (C-4'), 128.7 (C-5), 127.5 (C-3' & 5'), 126.3 (C-2' & C-6'), 110.9 (C-6), 103.0 (C-3), 79.7 (C-5'') are CH carbons;  $\delta$ : 190.6 (C-4), 174.9 (C-2''), 165.1 (C-7), 163.6 (C-2), 158.1 (C-8a), 130.3 (C-1'), 128.7 (C-3''), 115.6 (C-4a), 111.6 (C-8) are quaternary carbons.

Based on UV, IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data and comparison with previous reports (Table 2), compound BA-5 shares most of the spectroscopic data of 8 (4, 5-dihydro-5, 5-dimethyl-4-oxofuran-3-yl)-5-hydroxy-7-methoxy-2-phenyl-4H-chrome-4-one (Figure 1 below). This compound was isolated for the first time from *Tephrosia vogelii*.

**Table 2.** Comparison of  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra of compound BA-5 with previous report [50].

| Position | $^{13}\text{C-NMR}$ ( $\delta$ in ppm) | $^1\text{H-NMR}$ ( $\delta$ in ppm) | DEPT           | Reported [50]          |                           |
|----------|--|-------------------------------------|----------------|------------------------|---------------------------|
|          |  |                                     |                | $\delta^1\text{H-NMR}$ | $\delta^{13}\text{C-NMR}$ |
| 1        | -                                      | -                                   | -              | -                      | -                         |
| 2        | 163.63                                 | -                                   | Quaternary     | -                      | 161.69                    |
| 3        | 103.04                                 | 7.01 (1H, s)                        | CH             | 6.74(s)                | 107.35                    |
| 4        | 190.64                                 | -                                   | Quaternary     | -                      | 177.72                    |
| 4a       | 115.61                                 | -                                   | Quaternary     | -                      | 118.04                    |
| 5        | 128.7                                  | 6.5(1H,d,H-5)                       | CH             | 6.4(d)                 | 128.13                    |
| 6        | 110.98                                 | 7.3(1H, d)                          | CH             | 7.08(d)                | 109.36                    |
| 7        | 165.08                                 | -                                   | Quaternary     | -                      | 163.18                    |
| 8        | 111.58                                 | -                                   | Quaternary     | -                      | 109.7                     |
| 8a       | 158.05                                 | -                                   | Quaternary     | -                      | 154.87                    |
| 1'       | 130.33                                 | -                                   | Quaternary     | -                      | 131.92                    |
| 2', 6'   | 126.3                                  | 7.63(1H, d)                         | CH             | 7.43(m)                | 126.2                     |
| 3', 5'   | 127.52                                 | 7.3(1H, d)                          | CH             | 7.74(m)                | 128.99                    |
| 4'       | 130.80                                 | 6.9(1H, d)                          | CH             | 7.43 (m)               | 131.5                     |
| 6''      | 24.8                                   | 1.55(3H, s)                         | $\text{CH}_3$  | 1.65(6H,s)             | 25.83                     |
| 7'       | 56.1                                   | 3.96 (3H,s)                         | $\text{OCH}_3$ | 3.94(3H,s)             | 56.6                      |
| 2''      | 174.99                                 | -                                   | Quaternary     | -                      | 170.62                    |
| 3''      | 128.69                                 | -                                   | Quaternary     | -                      | 124.24                    |
| 4''      | 147.99                                 | 7.1(1H, s)                          | CH             | 7.52(s)                | 159.89                    |
| 5''      | 79.71                                  | 6.34 (1H,s)                         | CH             | 6.29 (s)               | 84.92 (q)                 |

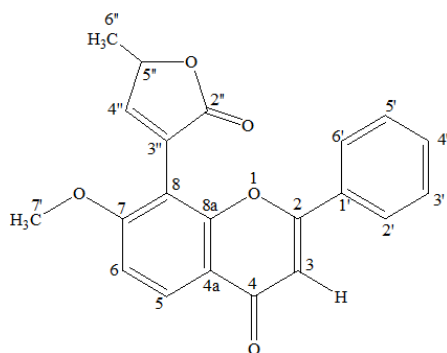


Figure 1. A proposed structure of compound BA-5.

### 3.2. Ethnobotany and Chemical Analysis of *Commiphora Schimperi*

#### 3.2.1. Ethnobotany

The *Commiphora* plant is widely grown in Arbaminch and Konso because it is suitable for hedge and fence. In Arbaminch the tree is known as "Tsedaki" (Amharic), the Konso people call it "Qahatita". The name "Qahatita" means that the plant drives away wild animals that destroy vegetables in the garden. The ease with which the plant is propagated from cutting accounts for its wide use for fences and hedges in Konso and Arbaminch. Furthermore the leaves are used for cattle feed and the wood for building purposes. However, interview made with several residents in Arbaminch and Konso revealed that the residents have very little knowledge of the resins produced by the trees. The resins are not collected for use as incense. The resin and plant

specimens used in this study were collected from Konso.

#### 3.2.2. Isolation and Analysis of the Essential Oil

The resin is collected in such a way that the milky liquid exudate coming out from the tree hardens on exposure to air into droplets or "tears" which are then easily detached by a collector. Occasionally, some "tears" are produced by accidental injury or from splits which occur in the stems or branches of the tree.

The essential oil of the resin of *C. schimperi* was obtained by hydro-distillation. GC and GC/MS analysis of the oil was undertaken and the result is presented below (Table 3).

Table 3. Physical data on the isolated essential oil of *C. schimperi* resin.

| Species             | Resin (g) | Oil (mg) | % yield | $[\alpha]_D$ | Ref. Ind. |
|---------------------|-----------|----------|---------|--------------|-----------|
| <i>C. schimperi</i> | 14        | 662      | 5.0     | -41.6        | 1.472     |

The total number of components is 15 (98.6%) with  $\alpha$ -pinene and  $\beta$ -pinene accounting for 89% of the oil, making this resin an excellent source of these industrially important chemicals. Other constituents found in the essential oil include: camphene (0.6%), *d, l*-limonene (0.9%),  $\beta$ -phelandrene (0.5%), *p*-cymene (0.9%), pinocarveole (0.5%), *t*-verbenol (0.3%), *p*-menth-1-en-4-ol (0.5%), myrtenol (0.2%), *Z*-citral (1.4%), 2-pinene-4-one (0.6%), *E*-citral (1.5%), *t*-caryophyllene (0.8%), and caryophyllene oxide (0.8%).  $\alpha$ -Pinene (6.2%),  $\beta$ -pinene (6.7%), and *t*-caryophyllene (33.4%) exist in the essential oil extracted from *Commiphora kataf* resin [52].

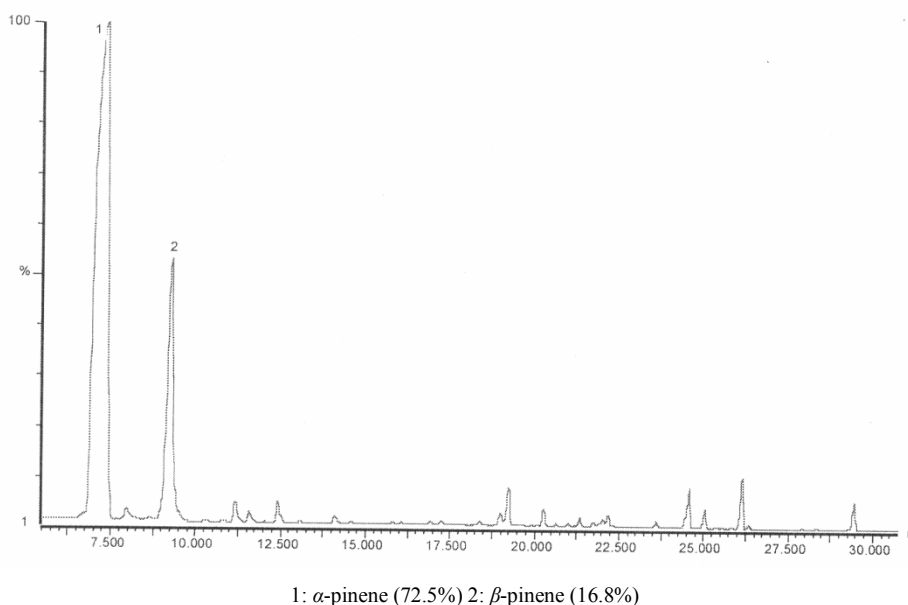


Figure 2. Gas chromatogram of hydro-distillate of *C. schimperi*.

GC-MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the essential oil confirmed that the major components are  $\alpha$ -pinene and  $\beta$ -pinene. Also fractionation of the petrol extract of the resin resulted in isolation of  $\alpha$ -pinene and a mixture of  $\alpha$ -pinene (69%) and  $\beta$ -pinene (20%).

$\alpha$ -Pinene is the main constituent of turpentine, widely distributed in conifers and other plants. It is important intermediate in the manufacture of synthetic fragrant compounds and also used as a flavoring ingredient. It undergoes cationic polymerization to give terpene resins. In



Kenya, *C. schimperi*, traditionally known as “Osilalei” is used for treatment of malaria. The methanolic extract of the resin of *C. schimperi* showed anti malarial activity [53]. The stem extract of the same species exhibited antioxidant effect in the DPPH assay [54].

### 3.2.3. Characterization of Compounds Isolated from the Resin of *Commiphora Schimperi*

The resin of *C. schimperi* was first extracted with petrol (48%) and then with MeOH (6%). Fractionation of the petrol extract resulted in the isolation of the two major constituents of the essential oil which make up 89%. The isolated compounds are coded 82-1A and 82-1B.

IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR values are compared with literature [55] for characterization of the isolated compounds in addition to GC.

#### i. Compound 82-1A

IR:  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ) 3024 (*w*) H-C str. of unsaturated compounds, 2918 (*s*) H-C- str. of saturated compounds, 1380 and 1355 (both *m*) H-C- def. of branched saturated compounds.

$^1\text{H}$  NMR:  $\delta$  5.15 (1H, *t*) imply vinyl proton, 1.63 (3H, *s*), 1.23 (3H, *s*), and 0.79 (3H, *s*) are methyl protons at carbon number 8, 9 and 10, respectively.

$^{13}\text{C}$  NMR: The total number of carbons in the spectra is 10. Therefore the compound is a monoterpene. Comparing NMR data with literature [55], all chemical shifts match with that of  $\alpha$ -pinene (1). Therefore the compound is  $\alpha$ -pinene as a result of its NMR data and by its retention time indicated in the GC.

#### i. Compound 82-1B:

$^1\text{H}$  and  $^{13}\text{C}$  NMR data comparison with literature [55], GC, and GC/MS data are used for characterization.

GC and GC/MS: The gas chromatogram and GC/MS clearly showed that, fraction 82-1B is a mixture of  $\alpha$ -pinene (1, 69%) and  $\beta$ -pinene (2, 20%).

$^1\text{H}$  NMR: 84.58 (1H, *td*) and 4.52 (1H, *td*) are vinyl protons of  $\beta$ -pinene. In addition to these all the chemical shifts of  $\alpha$ -pinene are found in the spectrum.

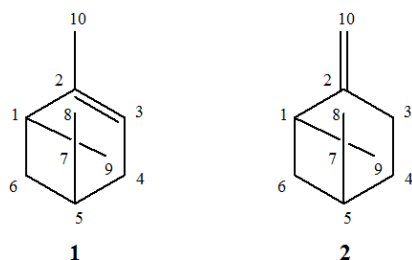


Figure 3. Compounds isolated from the resin of *C. schimperi*.

$^{13}\text{C}$  NMR: The chemical shift values of the  $^{13}\text{C}$  NMR spectra match with  $\alpha$ -pinene (1) and  $\beta$ -pinene (2), when compared to literature [55]. Therefore, 82-1B is a mixture of  $\alpha$ -pinene and  $\beta$ -pinene.

## 4. Conclusion

To the best of our knowledge there is no prior report on the chemical constituents of stem bark of *Tephrosia vogelii*

contrary to its high traditional use. Preliminary phytochemical screening of the extracts of the stem bark revealed the presence of terpenoids, flavonoids, tannins, and saponins and the absence of anthraquinones, steroids, and alkaloids. Fractionation of the methanolic extract resulted in the isolation of the terpenoids 8 (4, 5-dihydro-5, 5-dimethyl-4-oxofuran-3-yl)-5-hydroxy-7-methoxy-2-phenyl-4H-chrome-4-one which is isolated for the first time, from the species. It, however, is important to investigate its biological activity.

Analysis of the essential oil isolated from the resin of *Commiphora schimperi* revealed the presence of  $\alpha$ -pinene (69%) which is an important intermediate in the synthetic fragrant compounds and also used as a flavoring ingredient and  $\beta$ -pinene (20%) as the major components. here is no previous report on the analysis of the essential oil from the resin of *C. schimperi*, in Ethiopia. Fractionation of the petrol extract of the resin of *C. schimperi* further confirmed the presence of  $\alpha$ -pinene and  $\beta$ -pinene, which together make up 89% of the essential oil. In both species isolation and characterization of other compounds needs to be done.

## Conflict of Interest

The authors have not declared conflict of interest.

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