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## Characterization of Essential Oils Extracted from *Artemisia Absinthium* and their physicochemical properties

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**Abstract:** *Artemisia absinthium* is a medicinal plant used by traditional herbal practitioners in whole part of the world for treating several diseases. This study was carried out to examine the chemical composition of essential oil of *Artemisia absinthium*. Chemical composition of hydro-distilled essential oils obtains from aerial part of *Artemisia absinthium* was analyzed by using column chromatography, IR and GC-MS. Freshly extracted essential oil was dark blue, soluble in common organic solvents and insoluble in water. Twenty five compounds representing 100 % of total oil were identified. The main component of the oil were 2-(5-ethenyltetrahydro-5-methyl-2-furanyl-6-methyl-[2S-[2 $\alpha$  (R), 5 $\alpha$ ]]-5-hepten-3-one (71.501 %), bornyl acetate (5.117 %), ethyl-2-propenoate (5.059 %), mercenyl acetate (1.371 %), 7-ethyl-1, 4-dimethylazulene, (4.861 %) and 4,11,11-trimethyl-8-methylene-[1R-(1R, 4Z, 9S)]bicyclo[7, 2, 0]undec-4-ene (3.56 %); these together constituted 94.18 % of the extracted oil. The results of the study suggested that the chemical composition of essential oils *Artemisia absinthium* obtained from study area is different from previous reports.

**Keywords:** *Artemisia absinthium* L., GC-MS, and IR.

### Introduction

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way, indigenous medicinal plants play significant role of an economy of a country. One of these medicinal plants is *Artemisia absinthium* (wormwood). *Artemisia absinthium* is a species of wormwood native to warm Mediterranean countries, usually found growing in dry waste places such as roadsides, preferring a nitrogen-rich stony and hence loose soil. The plant grows naturally in northern and central parts of Ethiopia<sup>1</sup>. The plant's essential oil and bitter principles underlie its medicinal and commercial significance<sup>2</sup>. The medicinal use of this plant dates back to at least Roman times<sup>3</sup>. The plant was used as ant diarrhea<sup>4</sup>, antimicrobial<sup>5</sup> and anthelmintic<sup>6</sup>. Others uses include the treatment of dyspepsia, colds and rheumatism, esophageal reflux, inflammation and joint pain, hypertension and cardiac, as well as diabetes mellitus, insomnia, epilepsy and menstrual problems<sup>7</sup>. Wormwood is also used as a tonic, antiseptic, carminative and febrifuge and antispasmodic and to relief pain during child birth<sup>8</sup>. The extracts of the plant have shown to inhibit strong antimicrobial activity<sup>9</sup>. The oil of the plant can be used as a cardiac stimulant to improve blood circulation<sup>10</sup>. The pure wormwood oil is very poisonous, but with proper dosage poses little or no danger<sup>11</sup>. The oil is a potential source of novel agents for the treatment of leishmaniasis<sup>6</sup>. The investigation of the essential oil this plant will help to verify the reason behind the use of this plant as drug to treat these illnesses.

### Materials and Methods

#### Plant material collection

Aerial parts of *Artemisia absinthium* Linn was collected during spring (March) 2014 from different part of Mekoni, Tigray regional states of the northern part of Ethiopia. The botanical identification of plant sample was

carried out by Addis Ababa University national herbarium, Addis Ababa, Ethiopia and voucher specimens (Herbarium No. CNCS-CN-No-001) were deposited at the institute.

#### Extraction of essential oil

First the collected plant parts was washed by tap water in order to remove dust and other contamination then air dried at room temperature in a dust free environment. They were pulverized into fine powder by using pestle and mortar. The air-dried sample (150 g) was subjected to hydro distillation using a Clevenger-type apparatus for 4 hour to obtain the essential oils. The oil was removed every one hour from the distillation apparatus to decrease evaporation of the oil due to heat. After the distillation was over, the oil was collected, filtered, weighed and kept in a stopper bottle. The oil extracted from each sample was found containing fractions of water, which was removed by adding small amount of anhydrous sodium sulfate and kept in refrigerator at 4 °C for further analysis.

#### Determination of physicochemical parameters

Physical and chemical properties such as color, solubility, refractive index, specific gravity, pH value, carbon residue, saponification value, acid value, ester value, glycerol content, free fatty acid and iodine value were determined according to the method described in<sup>12</sup>.

#### Fractionation and purification of essential oil

Fractionation and purification of essential oil of *Artemisia absinthium* was done by using column chromatography and thin layer chromatography. For partitioning of the components of the essential oil, petroleum ether and dichloromethane (1:1) was selected as solvent.

#### Column chromatography

Isolation of constituents of the essential oil of *Artemisia absinthium* was carried out by column chromatography using silica gel (mesh 70-230). The column has been run on a silica gel column (20 cm × 2.0 cm i.d.), which was preconditioned with petroleum ether. 0.4 g of essential oil was packed on column and eluted with petroleum ether and CH<sub>2</sub>Cl<sub>2</sub> (1:1). The eluent was collected in fraction of 1 mL and tentative identification has been carried

out using TLC. The fractions containing single spot were dried by rotary evaporation.

#### TLC detection

The spots, which are obtained from column, were applied onto a Merck silica gel 60 GF<sub>254</sub>, 20 × 20 cm per coated plate and developed with 1:1 petroleum ether/dichloromethane, as a mobile phase over a path of 20 cm chamber. The spots on TLC were initially examined with under UV- light (254 nm) and then visualized with iodine fume. Further characterization of the component of oil was performed by IR techniques.

#### Infrared spectroscopy (IR) analysis

Infra red spectral study was made for the fraction of essential oils which is obtained from column chromatography. Each fraction was mixed with potassium bromide (IR grade) powder. The mixture then pressed in a special dye to yield a transparent disc. The disc was then holding in the instrument beam for spectroscopic examination and the resulting IR spectrum was recorded.

#### GC-MS analysis of essential oil of *Artemisia absinthium* L.

The identification of different chemical components of the essential oil was done by Agilent 6890N Network GC with Agilent 5975 inert MSD (E.I.Quadrapole), equipped with a DB-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as the carrier gas at a rate of 1.5 mL/min. The inlet and transfer line temperatures were set at 250 °C and 280 °C respectively. Initial column temperature was 70 °C and increased to 120 °C at rate 5 °C/min, then programmed to 280 °C at rate 10 °C/min, ending with a 4 min isothermal at 280 °C. The essential oil was diluted with methylene chloride and 0.2 μL of the diluted sample was injected automatically into GC in the split ratio 90:1. Total GC running time was 30 min. The MS detection was carried out in the electron impact mode with a scan range of 50-1000 amu. Identification of components in the sample was based on the retention index (RI), similarity index (SI), National Institute of Standards and Technology (NIST) MS spectral library and literature survey. The relative percentage amount of each component of the essential oil was calculated by comparing its average peak area to the total areas.

#### Result and Discussion

##### Physicochemical properties of the essential oil

**Color and smell:** The essential oil was noted as dark blue opaque liquid with strong aromatic fragrance. This result was somewhat different to the color of *Artemisia* oil obtained from Iran, which is dark green to brown liquid<sup>13</sup>. However, this oil was similar to that reported from Tajikistan, which is blue and dark blue liquid<sup>14</sup>. The color and fragrance of the oil can be used in bath soaps, cosmetics and perfumery items.

**Density:** The value of the density of essential oil was 0.924 g/cm<sup>3</sup>. This shows that the essential oils of *Artemisia absinthium* are rich in a complex composition.

**pH value:** The pH value of the essential oil of *A.absinthium* was measured as 6.203, which indicated that, the oil have weak acid behavior. The low acidity of oils is considered as neutralized and safe for making skin care products though high acidity of oils may be harmful for skin. Therefore, the finding can be used in preparation skin care products.

**Solubility:** Freshly extracted essential oil is soluble in common organic solvents and very slightly soluble in water.

This shows that the essential oil of *Artemisia absinthium* might be slightly polar behavior.

**Refractive index:** The measured refractive index of *A.absinthium* was 1.472 at 25 °C. This value is within the range given by<sup>15</sup> (1.4660 to 1.4750) for the essential oil of *Artemisia absinthium* and is high to value given for the essential oil of *Artemisia nilagirica* (1.442)<sup>16</sup>. Therefore the result shows is evidence that the sample might be long carbon chain with double bond.

**Specific gravity:** Specific gravity of the oil was found 0.924 at 25 °C. This value is within the range given by<sup>15</sup> (0.900 to 0.955) for the essential oil of *Artemisia absinthium*. It is slightly larger than the value obtained for essential oils of *Artemisia nilagirica* (0.907)<sup>16</sup>. Therefore, the oil can be used in preparation of different products since it is pure.

**Optical rotation:** The specific rotation of essential oil of *A.absinthium* was found to +8°, 954'. It allowed researcher to infer that the essential oil is dextrotatory.

**Carbon residue:** Carbon residue values for *A.absinthium* were found 4.225, which is higher than for essential oil for *zanthoxylum armatum*<sup>17</sup>.

**Acid value:** Acid value of the essential oil of *A.absinthium* was calculated as 7.48. It is in the range given by<sup>15</sup> for the oil of the same plant (0 to 18) and is high that of *Artemisia nilagirica* (2.2)<sup>16</sup>. This value indicates the oil could not be suitable for dietary purposes, as they contain higher acid contents.

**Saponification:** The saponification value of essential oil of *A.absinthium* was found to be 39.2. This value is within the range given by<sup>15</sup> (12 to 167) for the essential oil of the same plant and is far below that of *Artemisia nilagirica*<sup>16</sup>. Result showed that oil is just right quantity required for making bath soap.

**Ester value:** Ester number of Essential oil of *A.absinthium* was found to be 31.72. It is close to the range given by<sup>15</sup> (12 to 185) for the oil of the same plant and is far below that of *Artemisia nilagirica* (151.86)<sup>16</sup>. This result showed oil has good quality and can be used for preparation different product.

**Iodine value:** Iodine value of the essential oil of *Artemisia absinthium* L. was 64.13 ± 0.173/100 g. This shows that the oil is non-drying oil and apart from this, it implies that the oil is more of unsaturated fatty acid and it does not congeal at ordinary temperature. Hence, it can be used in the production of soap.

**Glycerol Content:** Glycerol content of the essential oil was found 1.737 %.

**Free Fatty Acids:** The free fatty acids content were found 3.74 %. This value indicates the oil could not suitable for eating, as it contain high free acid content.

##### Chemical composition of essential oil of *Artemisia absinthium*

A resulted in the separation of 24 compounds was obtained from *Artemisia absinthium*. This is almost similar with result obtained from GC-MS analysis. 44 fractions were collected. The fraction of oil exhibit a variety of colours: yellow and dark blue. The fraction F<sub>1</sub>-F<sub>6</sub> gave a dark blue spot with R<sub>f</sub> = 0.812 and expected to be a single compound because due to their similar R<sub>f</sub> values. This dark blue color of spot is attributed to the presence of Azulene derivatives, which is 7-ethyl-1, 4-dimethylazulene and was identified by reference<sup>18</sup>. In the oil of *Artemisia absinthium*, fraction - 40 could be identified as a single yellow spot at R<sub>f</sub> = 0.086.

## GC-MS studies

The chemical (and percentage) compositions of the essential oils of the aerial part of *Artemisia absinthium* are presented in Figure-1, 2 and Table-1 respectively. The constituents of *Artemisia absinthium* from Ethiopia are listed in order of their elution on the column test report (Figure-1).

The essential oil obtained using Clevenger-type apparatus of the dried aerial part was analyzed and oil percentage was found to be 1.996 %. A total volatile compound percentage present in aerial parts of the plant was found to be 100 %.

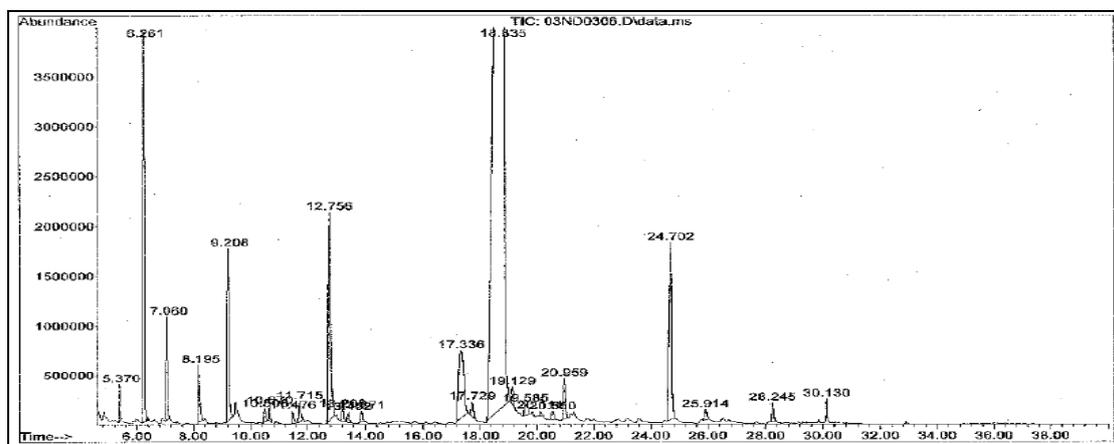


Figure-1 Gas chromatogram of essential oil of *Artemisia absinthium*

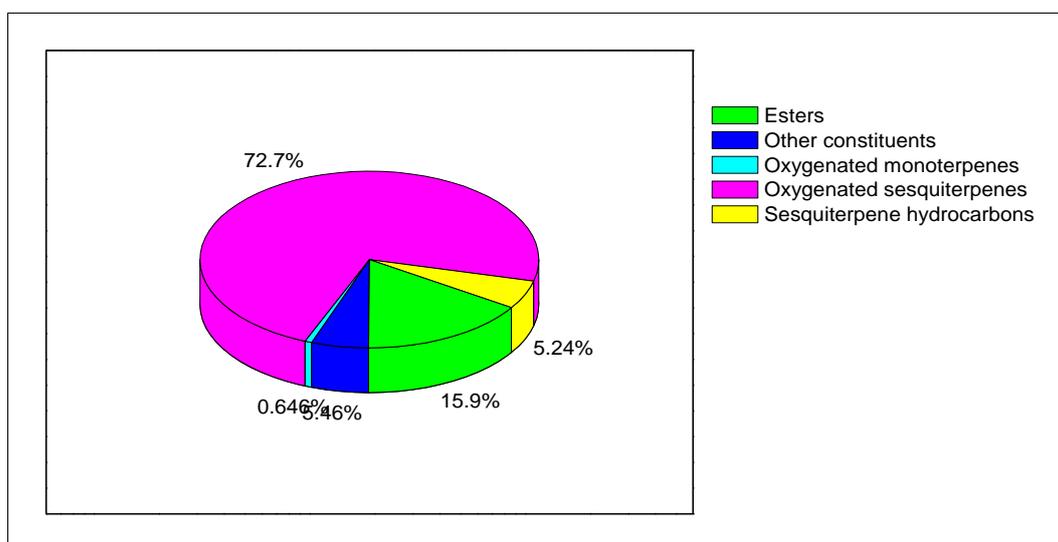


Figure-2 Composition of essential oil of *Artemisia absinthium* L. residues by class

This total oil composition (100 %) is higher than other *Artemisia absinthium* study in Iran (93.3 %) <sup>13</sup>, in west Antonia (79.78 %) <sup>19</sup> And in Tajikistan (72-94 %) <sup>14</sup>. GC and GC-MS analysis of essential oil resulted in the identification of twenty-five components. The number of chemical component of *Artemisia absinthium* oil relatively lower than those indicated by other studies <sup>20,21</sup>. The essential oil was found to be rich in 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-[2S-[2 $\alpha$ (R), 5 $\alpha$ ]]-5-hepten-3-one (Rt18.83, 71.501 %), bornyl acetate (Rt 6.26, 5.117 %), ethyl-2-propenoate, (Rt12.75, 5.059 %), 7-ethyl-1,4-dimethyl azulene (Rt 24.71, 4.861%), 4, 11, 11-trimethyl-8-methylene-[1R-(1R\*, 4Z, 9S\*)]bicyclo[7, 2, 0]undec-4-ene, (Rt 17.34, 3.56 %) and mercenyl acetate (Rt 6.26, 1.371 %) (Table-1). All the remaining compounds add up to 5.819 %.

The essential oil was found to be dominated by oxygenated sesquiterpene (72.742 %), esters (15.906 %), and sesquiterpene hydrocarbons (5.241 %) and oxygenated monoterpenes (0.646 %) (Figure-4.4). Besides this, other components belonging to different classes were found to be (5.465 %). A comparison of the present results with that of literature indicated that in the essential oil of *Artemisia absinthium*, oxygenated monoterpenes were not identified as major component. In the present study, the researcher has also reported a few oxygenated sesquiterpenes and esters which were unreported by many investigators. Therefore, the main components which were identified in the chemical composition of the locally available *Artemisia* oil differed from that of the reported constituents in the literature. For instance, in the present study 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-[2S-[2 $\alpha$ (R), 5 $\alpha$ ]]-5-hepten-3-one (71.50 %) was identified as principal compound from the

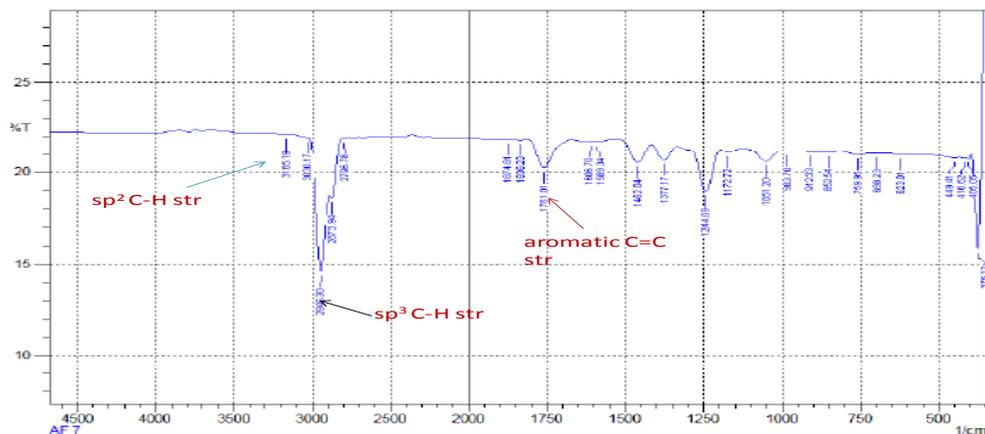
volatile components of the extract. Five other compounds, bornyl acetate, ethyl-2-propenoate, 7-ethyl-1, 4-dimethylazulene, 4, 11, 11-trimethyl-8-

methylene [1R (1R\*, 4Z, 9S\*)] bicyclo[7, 2, 0] undec-4-ene and Mercenyl acetate were also identified as the major volatile components of the extract.

**Table-1** Chemical constituents of aerial essential oil from *Artemisia absinthium*

Peak No	Rt	Compounds identified	RI	Molecular Formula	% composition
1	5.37	$\beta$ -pinene	960	C <sub>10</sub> H <sub>16</sub>	0.40
2	6.26	Bornyl acetate	978	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	5.117
3	7.06	Mercenyl acetate	1064	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	1.371
4	8.19	ethyl-3-phenyl propanoate	1169	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	0.812
5	9.21	ethyl-3-phenyl-2-propenoate	1235	C <sub>11</sub> H <sub>12</sub> O <sub>2</sub>	2.712
6	10.51	1, 3, 5-Tris(methylene) cycloheptane	1252	C <sub>10</sub> H <sub>14</sub>	0.204
7	10.67	Caryophyllene	1378	C <sub>15</sub> H <sub>24</sub>	0.232
8	11.48	2-methyl-5-(1-methylethenyl)-2-Cyclohexen-1-ol	1290	C <sub>10</sub> H <sub>16</sub> O	0.221
9	11.71	Same as 8	1299	C <sub>10</sub> H <sub>16</sub> O	0.425
10	12.75	Same as 5	1421	C <sub>11</sub> H <sub>12</sub> O <sub>2</sub>	5.059
11	13.21	(-)-Isoledene	1633	C <sub>15</sub> H <sub>24</sub>	0.213
12	13.40	1, 2, 3, 4, 4 $\alpha$ , 5, 6, 8 $\alpha$ -octahydro-4 $\alpha$ , 8- dimethyl-2-(1-methylethenyl)-[2R(2 $\alpha$ , 4 $\alpha$ , 8 $\alpha\beta$ )]naphthalene,	1649	C <sub>15</sub> H <sub>24</sub>	0.178
13	13.87	Seychellene	1689	C <sub>15</sub> H <sub>24</sub>	0.187
14	17.34	4, 11,11-trimethyl-8-methylene-[1R-(1R*, 4Z, 9s*)]bicyclo[7, 2, 0]undec-4-ene,	1955	C <sub>15</sub> H <sub>24</sub>	3.56
15	17.73	Ledene oxide-(II)	1981	C <sub>15</sub> H <sub>24</sub> O	0.366
16	18.84	2-(5-ethenyltetrahydro-5- methyl-2-furanyl)-6-methyl-5-hepten-3-one	2058	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>	71.501
17	19.13	6-(1-hydroxymethylvinyl)-4, 8 $\alpha$ -dimethyl-3, 5, 6, 7, 8, 8 $\alpha$ -hexahydro-1H- naphthalene-2-one	2076	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	0.286
18	19.58	Methyl-5, 8, 11-heptadecatriynoate	2308	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	0.402
19	20.1	Same as 18	2353	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	0.093
20	20.55	Isoaromadendrene epoxide	2162	C <sub>15</sub> H <sub>24</sub> O	0.174
21	20.96	Eudesma-4 (14), 11-diene	2186	C <sub>15</sub> H <sub>24</sub>	0.871
22	24.70	7-Ethyl-1, 4-dimethylazulene	2256	C <sub>14</sub> H <sub>16</sub>	4.861
23	25.91	1, 7-dimethyl-4-(1-methylethyl) Spiro[4,5]dec-6-en-8-one	2440	C <sub>15</sub> H <sub>24</sub> O	0.277
24	28.24	Methyl hinokiate	2683	C <sub>16</sub> H <sub>24</sub> O <sub>2</sub>	0.34
25	30.13	6-(p-tolyl)-2-methyl-2-heptenol	2621	C <sub>15</sub> H <sub>22</sub> O	0.138
Total					100 %

## IR spectral studies



**Figure-3** IR spectrum for fraction-7

These major compounds amount to 94.181 % of the total constituents. In this study, 7-ethyl-1, 4-dimethylazulene was the fourth most abundant compound. This component may be produced from unstable sesquiterpene lactone artabsin during distillation of the process. The dark blue color of this essential oil is attributed to the presence of azulene derivatives namely 7-ethyl-1, 4-dimethylazulene.

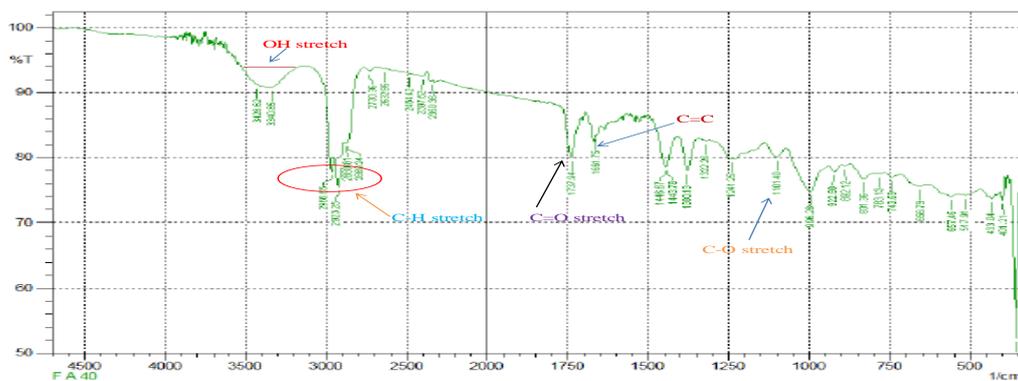
It is observed that ethyl-2-propenoate was eluted at two different retention times 9.21 and 12.75 min suggesting its isomeric forms and third large abundant compound.

Except, bornyl acetate all the major components of the extract, which are listed in Table-1 were proved to be present only in *Artemisia absinthium* collected from Tigray region and not in the previous reports of Iran, Tajikistan and west Antonia. It indicates that this *Artemisia absinthium* is a new chemo type and restricted only to Ethiopia. So, the observation about the presence 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-[2S-[2 $\alpha$  (R), 5 $\alpha$ ]]-5-hepten-3-one, ethyl-2-propenoate, 7-ethyl-1, 4-dimethylazulene, 4, 11, 11-trimethyl-8-methylene-[1R-(1R, 4Z, 9S)] bicyclo[7,2,0]undec-4-ene and mercenyl acetate in *Artemisia absinthium* have not been reported earlier in the same species, but only bornyl acetate reported in the oil *A. argyi* and *A. frigida*. In the present study, the concentration of bornyl acetate was lowest (5.117 %) when

the researcher compare with the concentration of oils of *A. argyi* (29.8 %) and *A. frigida* (22 %) from China and Turkey respectively. This also shows that, *A. absinthium* collected from southern part of Tigray is a new species.

Infra-red spectrum for fraction-7 (Figure-3) shows weak band at 3030.17  $\text{cm}^{-1}$  which is due to the presence of  $\text{SP}^2$  C-H stretch in the compound and that at 2945.30  $\text{cm}^{-1}$  is characteristic for C-H stretching vibration indicating aliphatic chain. The band at 1761.01  $\text{cm}^{-1}$  for C-H stretching gives evidence for the presence of aromatic ring. Therefore, based on IR and GC-MS data this spot may be belong to 7-ethyl-1, 4-dimethylazulene.

Infra-red spectrum for fraction-40 (Figure-4) shows broad band at 3428.02  $\text{cm}^{-1}$  which is due to the presence of moisture or hydroxyl groups in the compound and that at 2966.65  $\text{cm}^{-1}$  is characteristic for C-H stretching vibration indicating aliphatic chain. The band at 1101.40  $\text{cm}^{-1}$  for C-O stretching gives evidence for the presence of alcohol and the band at 1737.94  $\text{cm}^{-1}$  for C=O stretching vibration indicates carbonyl group. The weak band at 1661.75  $\text{cm}^{-1}$  for C=C shows evidence for alkenes. Then we can say this compound contains three functional groups O-H, C=C and C=O. Therefore, based on IR and GC-MS data the fraction-40 may be belongs to 6-(1-hydroxymethylvinyl)-4, 8 $\alpha$ -dimethyl-3, 5, 6, 7, 8, 8 $\alpha$ -hexahydro-1H-naphthalene-2-one.



**Figure-4** IR spectrums for fraction-40

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