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Chemical Investigations of the Essential Oils of Some Artemisia species of Ethiopia

Mukul Chauhan
Department of chemistry, college of Natural and computational science, Ambo university, Ethiopia

Abstract: *The compositions of the areal parts of essential oils of Artemisia abyssinica, A. absinthium (Ariti), and A. annua have been studied. The oils were examined by GC, GC/MS, ^{1}HNMR, and ^{13}CNMR techniques.The total constituents of the oils 59%, 56%, and 44% have been identified from A. abyssinica, A. absinthium(Ariti), and A. annua respectively. The major components were yomogi alcohol and artemisia alcohol acetate for A. abyssinica; camphor,davanone and chamazulene for A. absinthium and camphor for A. annua. Four compounds were isolated from A. abyssinica and A. absinthium and their structure were elucidated based on spectroscopic techniques.*

Key words: *Artemisia, A. absinthium (Ariti),anisaldehyde reagent ,chamazulene,essential oils, monoterpenes*

I. Introduction

Artemisia is Annual or perennial herbs with strong aromatic leaves. The genus *Artemisia* is one of the largest genera in the tribe *Anthemideae* (*Asteraceae*) [2,3,10]. These four species (*A. afra, A. annua, A. absinthium* and *A. abyssinica*) are found in the Ethiopian flora [11,14]. They include glycosides, terpenes, alkaloids and essential oils [1].Three species were analyzed for comparison of essential oils of *A. abyssinica, A. absinthium, and A. annua respectively*. A. annua wondrous anti-malarial plant [3, 5, 10] is native of Asia [3] and has been successfully introduced into many African countries including Ethiopia, Europe, America and Australia [5, 11, 12]. The major components in *A. absinthium (Ariti)* (Ethiopian Wormwood) essential oil are camphor and davanone [7,11].

The essential oil has potential to be used in perfumery, cosmetics and aromatherapy and has been reported that it has an antifungal and antimicrobial effects [5]. *A. absinthium*. for which the botanists suggest the name "Ethiopian Wormwood", (Ariti) is cultivated in home gardens in the Northern and Central Parts of Ethiopia. It is used to for its aroma at coffee ceremonies and in rituals called "Adbar" and for flavoring a locally distilled alcoholic drink called Areki. *Artemisia abyssinica* found on farm fields after harvest in Ethiopia is being used as a remedy for heart troubles and cough.

II. Experimental, observations and discussion

The areal part of A. abyssinica was collected from African Laboratory from Natural Products (ALNAP) garden. *A. absinthium (Ariti) and A. annua* collected from local area. Fresh aerial parts of the three plants were crushed by liquid nitrogen. Each of the three powdered aerial parts of the plant material were subjected to hydro-distillation for 1 ½ h, using a Clevenger-type apparatus.

Isolation of constituents of the essential oils was performed by column chromatography using silica gel (mesh: 70-230). Analytical TLC were run on a silica gel 60 F_{254} (Merck), 20 x 20 cm per coated plate and components were detected by spraying anisaldehyde reagent (anisaldehyde: H_2SO_4: AcOH:EtOH ; 0.5:0.5:0.1:9) and further characterization by using ^{1}HNMR and ^{13}CNMR sophisticated techniques.

The identification and percentage composition of the essential oils was computed from GC and GC/MS peak areas. Qualitative analysis was based on comparison of retention times with the standard or isolated compounds and the corresponding data in the literature [2, 6, 8, 9] GC analysis of the essential oils were performed using an instrument (Model: - HP6890 GC, coupled with an auto sampler) equipped with a capillary column HP-5 (5%phenyl methyl siloxane), 30.0 m x 320 µm i.d., film thickness 0.25µm. Nitrogen was used as the carrier gas at a flow rate of 0.8 ml/min. The oven temperature was held at 50 ^{0}C, and then programmed to 210 ^{0}C at a rate of 3^{0}C/min. The injection and FID temperatures were kept at 210 ^{0}C and 270 ^{0}C, respectively.

The essential oils isolated by steam distillation of the areal parts of *A. abyssinica, A. absinthium (Ariti)*and *A. annua* . Yield and color of the oils were summarized in Table-1.

Table-1.

Percentage yield of essential oils of *A. abyssinica*, *A. absinthium (Ariti)* and *A. annua*.

Plant	Wt. of plant material	Wt. of the Oil	Yield	Color of the Oil
A. abyssinica	45 g	400 mg	0.9 %	yellowish
A. absinthium	100 g	460 mg	0.5 %	Deep blue
A. annua	300 g	144 mg	0.05 %	greenish

The essential oils isolated from *A. abyssinica* were examined by GC and GC/MS. The results can be seen in Table-2. The components are listed in order of their elution on the HP-5 column. Chromatogram of the analysis is shown in appendix. The most compositions of the oil isolated around the first 20 min in GC/MS.

Table-2.

Percentage composition of the essential oil of *A. abyssinica* aerial parts.

Compounds	(%)	Method of identification
Yomogi alcohol	32.2	GC, GC/MS, NMR
1,8-Cineole	2.1	GC/MS
α-Terpinene	1.8	GC/MS
Nonanone	6.4	GC/MS
Artemisia alcohol acetate	26.7	GC, GC/MS, NMR

Theessential oil of *A. abyssinica* contains the irregular monoterpenes yomogi alcohol (32%) and artemisia alcohol acetate (26.7%) as its major constituents. These two accounts 59% of the oil. The major peaks in the GC/MS spectra (at RT 12 and 18) indicate the presence of Yomogi alcohol and Artemisia alcohol acetate. Table-3 summarizes the major peak and the possible fragmented ion. The MS of the two compounds Yomogi alcohol and Artemisia alcohol acetate are shown in appendix.

GC/MS Relative Intensity 10, Sensitivity 10^{-6} amps/sec, First mass 1 amu, Last mass 300 amu, Integral start 40 amu, and Electron energy 70ev. Yomogi alcohol (M^+/e: 154) Mass: 43, 59, 85, 121,136, 139, Relative intensity: 100, 84, 57, 29, 22, 5. Artemisia alcohol acetate (M^+/e: 196)Mass: 85, 127, 137,153,182,Relative intensity: 100, 82, 68, 2. (see appendix A,B and E)

Table-3

Major peaks and possible fragmented ions of Yomogi alcohol and Artemisia alcohol acetate.

Yomogi alcohol		Artemisia alcohol acetate	
Peak (m/z)	Fragmented ion	Peak (m/z)	Fragmented ion
139	M-(CH_3)	182	M-(CH_3)
136	M-(H_2O)	153	M-(CH_3 + CO)
121	M-(CH_3 + H_2O)	137	M-(CH_3 + CO_2)
59	$(CH_3)_2$-C=OH^+	127	M-(C_5H_9)
43	$^+O \equiv C\text{-}CH_3$	85	$(CH_3)_2C$=CH-CH=OH^+

The presence of Yomogi alcohol and Artemisia alcohol acetate was further checked by comparing their NMR data with literature values [6, 8, 9]. The two major components of *A. Abyssinica* were investigated more throughly by two- dimensional NMR-techniques in order to assign the ^1H and ^{13}C NMR spectra. The spectra obtained Table-4 (see appendix F and G) and Table -5. (see appendix C and D)

Table-4 ^{1}HNMR spectra of Yomogi alcohol. **Table-5** ^{1}HNMR and ^{13}CNMR spectra of Artemisia alcohol acetate.

Position	^{1}HNMR Chemical shift, δ (ppm)		Position	^{1}HNMR Chemical shift, δ (ppm)	^{13}CNMR Chemical shift, δ (ppm)
1, 3’	1.29		1	4.96 (trans)	112.63
2 (OH)	1.58		1	4.99 (cis)	112.63
3	5.57		2	5.84	144.22
4	5.48		3	-	40.1
5	-		4	5.3	76.47
6	5.79		5	5.07	120.57
7	4.90 (trans)		6	-	137.15
7	4.91 (cis)		7, 5’	1.74	18.83
1’, 2’	1.09		1’	-	169.57
			2’	2	21.06
			3’, 4’	0.99	22.14

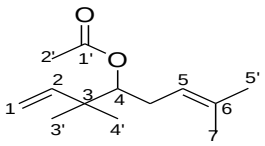
The percentage composition of the essential oils was computed from GC and GC/MS peak areas. Qualitative analysis was based on comparison of retention times with the standard or isolated compounds.[2, 6, 8, 9]. 1 g of the oil was separated on silica gel, which was successfully eluted with petroleum ether and CH_2Cl_2. 30 fractions were collected.

II. 1. Yomogi Alcohol

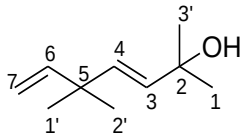
Fractions 19 to 28 (100 mg) were combined after chromatographed on a column of silica gel, mixtures of petrol-CH_2Cl_2 in the ratio (1:1) as eluent solvent. This was identified as Yomogi alcohol: ^{1}HNMR (400 MHz, $CDCl_3$ and CD_4): δ 1.09 (6H, s, 1'-CH_3, 2'-CH_3), 1.29 (6H, s, 1-CH_3, 3'-CH_3), 1.58 (1H, s, 2-OH), 4.90 (1H, d, 7-CH_2 trans), 4.91 (1H, d, 7-CH_2 cis), 5.48 (1H,d, 3-CH), 5.47 (1H, d, 4-CH), 5.79 (1H, dd, 6-CH) (see appendix F and G) .

II. 2. Artemisia Alcohol Acetate

The combined fractions 11 to 15 (160 mg) from CC contained the light yellow component and gave a single spot on TLC. This was identified as Artemisia alcohol acetate on the basis of its spectra: ^{1}HNMR (400 MHz, $CDCl_3$ and CD_4): δ 0.99 (6H, s, 3'-CH_3, 4'-CH_3), 1.74 (6H, s, 5'-CH_3, 7-CH_3), 2.00 (3H, s, 2'-CH_3), 4.96 (1H, d, 1-CH_2 trans), 4.99 (1H, d, 1-CH_2 cis), 5.02 (1H, d, 5-CH), 5.29 (1H, d, 4-CH), 5.84 (1H, dd, 2-CH). (see appendix C and D)



Artemisia alcohol acetate



Yomogi alcohol

The essential oils obtained from *A. absinthium (Ariti)* and *A. annua* were also analyzed by GC. Constituents of the oils were identified by using RT and NMR. The identified components account for 44% and 56% of the oils of *A. annua* and *A. absinthium (Ariti)*, respectively. Both oils were composed of camphor as a major constituent and account for 44% and 41% of the oil of *A. annua* and *A. absinthium (Ariti)* respectively.Chamazulene (0.6%) and Davanone (14%) were isolated from *A. absinthium (Ariti)* by CC and identified on the bases of their spectra ^{1}HNMR and ^{13}CNMR. The spectra obtained were identical with literature values Table-6 (see appendix H) and Table -7 (see appendix I) [7].

Table -6 ^{1}HNMR spectra of chamazulene.

Position	^{1}HNMR Chemical shift, δ (ppm)
3	6.9
4	7.38
6	8.14
9	7.2
10	7.6
1'	2.87
2'	1.38
3'	2.67
4,	2.85

Table -7 ^{1}HNMR spectra of davanone.

Position	^{1}HNMR Chemical shift, δ (ppm)
1	5.19 (cis)
1	4.98 (trans)
2	5.88
6	3.26
7	2.68
12	1.64
1'	1.27
2'	0.99
3'	1.77

II. 3. Isolation of Compounds from *A. absinthium (Ariti)*

400 mg of the oil was packed on column and eluted with petroleum ether and CH_2Cl_2. 30 fractions were collected.

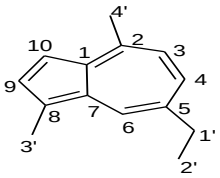
II. 3. 1. Chamazulene

The combined fractions 6 to 8 (46 mg) from CC contained the blue component and gave a single spot on TLC. This was identified as Chamazulene based on its spectra: ^{1}HNMR (400 MHz, $CDCl_3$ and CD_4): δ 1.38 (3H, t, 2'-CH_3), 2.67 (3H, s, 3'-CH_3), 2.85 (3H, s, 4'-CH_3), 2.87 (2H, q, 1'-CH_2), 6.90 (1H, d, 3-CH), 7.20 (1H, d, 9-CH), 7.38 (1H, dd, 4-CH), 7.60 (1H, d, 10-CH), 8.14 (1H, d, 6-CH). (see appendix H)

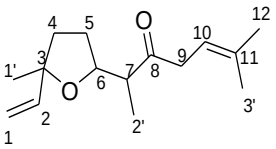
II. 3. 2. Davanone

Fractions 25, 26 and 27 were combined after chromatographed on a column of silica gel and petrol-EtOAc (8:2) solvent system, which was, identified as Davanone based on its spectra. ^{1}HNMR (400 MHz, $CDCl_3$

and CD_4): δ 0.99 (3H, d, 2'-CH_3), 1.27 (3H, s, 1'-CH_3), 1.64 (3H, s, 12-CH_3), 1.77 (3H, s, 3'-CH_3), 2.68 (1H, qn, 7-CH), 3.26 (1H, qd, 6-CH), 4.98 (1H, dd, 1-CH trans), 5.19 (1H, dd, 1-CH cis), 5.88 (1H, dd, 2-CH).



Chamazulene



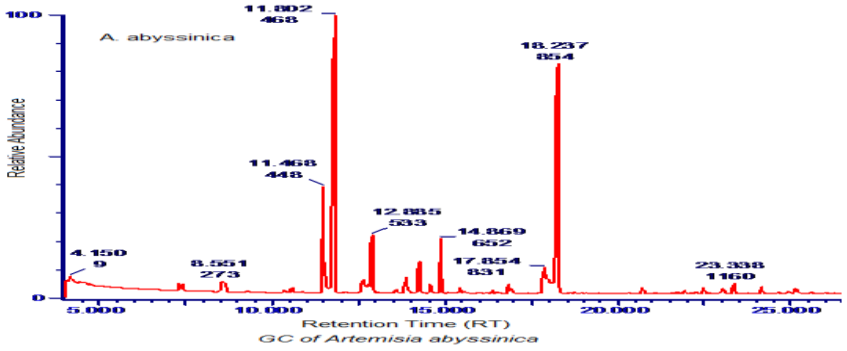
Devanone

III. Conclusions

The chemical constituent of *A. abyssinica* has not reported yet.However, several analyses [3, 4, 5, 7, 13] have done for the oils of *A. annua* and Ariti. The two major components of the oil of *A. abyssinica* are yomogi alcohol and artemisia alcohol acetate and both them are irregular monoterpenes.

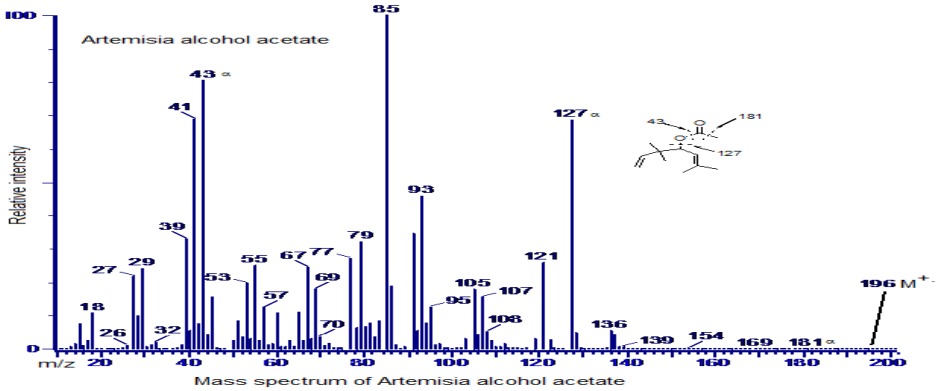
Even though the botanists lumped Ariti together with the well-known species *A. absinthium*, their chemical constituents shows some deviation. In the oil of Ariti (Ethiopian wormwood) we have found that camphor and davanone as a major components. However, thujone is the major component for the European wormwood. Therefore, it requires further investigation before mixing the two species.

Appendix (A)

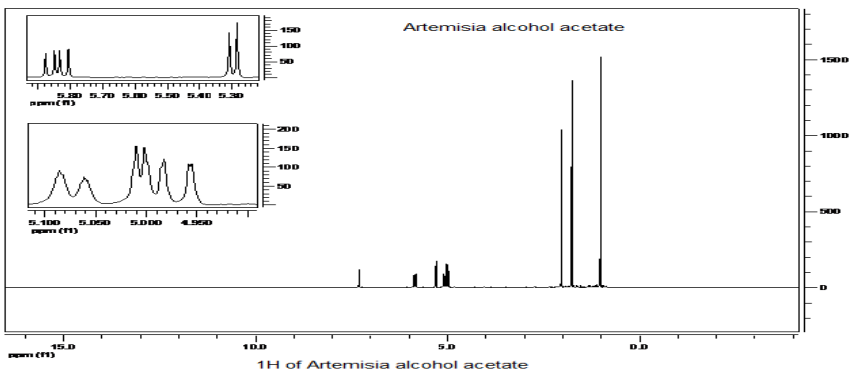


GC of Artemisia abyssinica

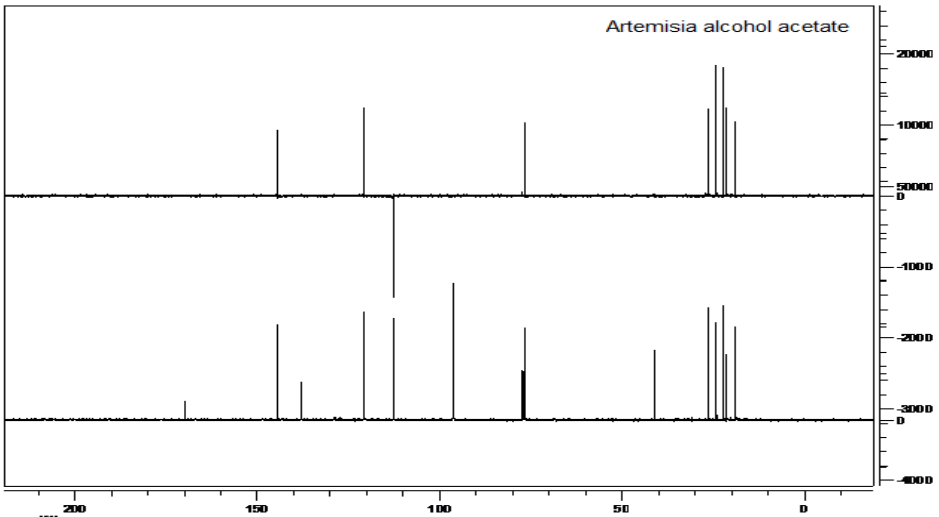
Appendix (B)



Appendix (C)

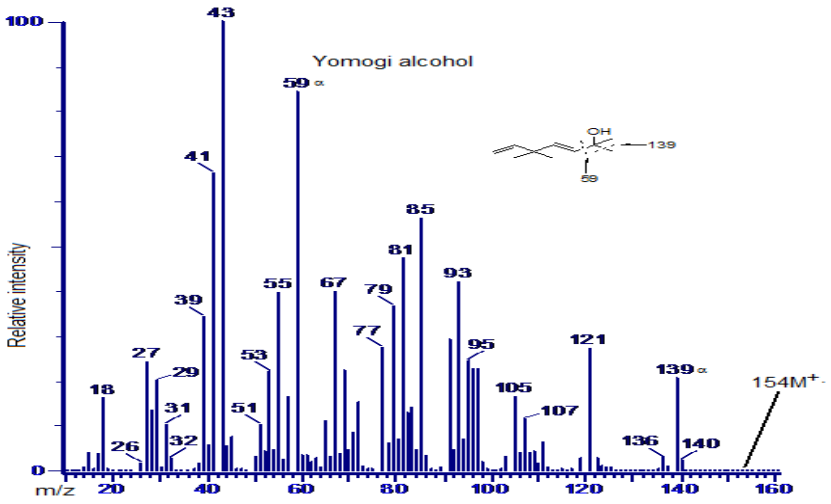


Appendix (D)



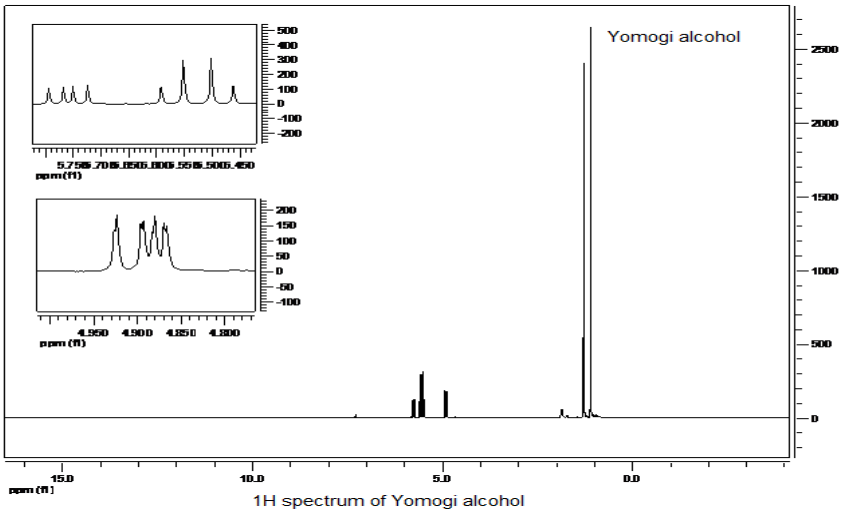
C-13 and DEPT spectra of Artemisia alcohol acetate

Appendix (E)



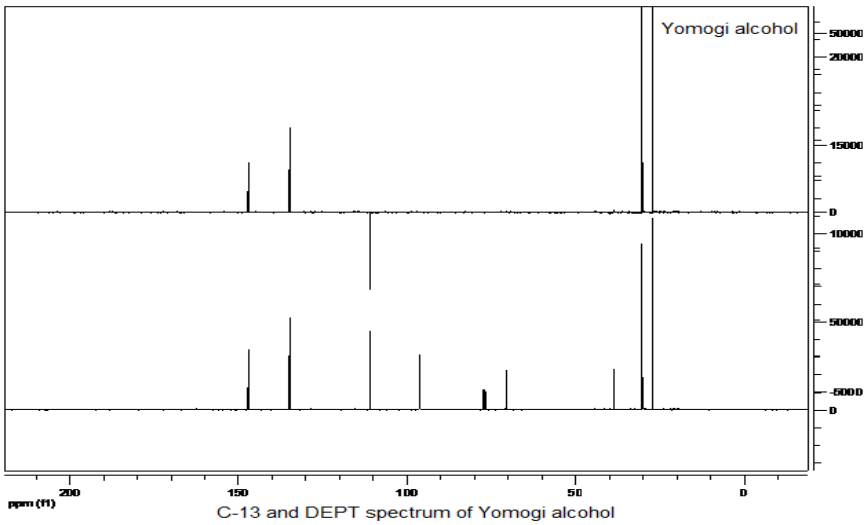
Mass spectrum of Yomogi alcohol

Appendix (F)



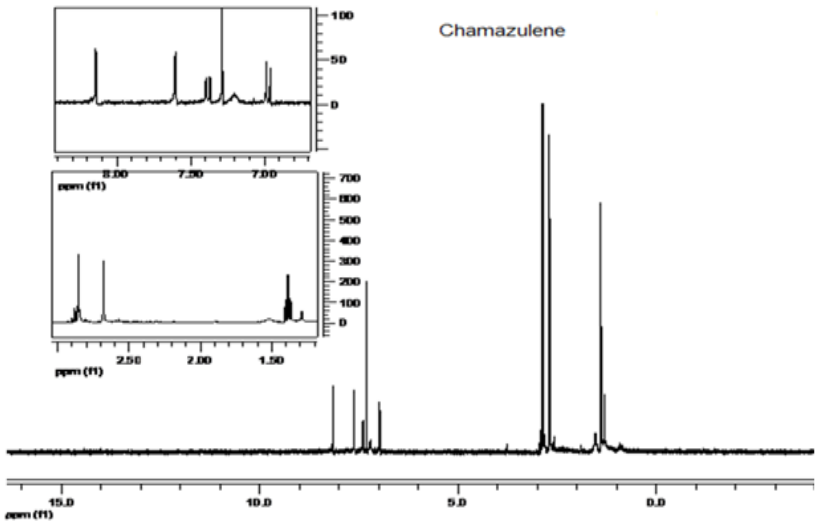
1H spectrum of Yomogi alcohol

Appendix (G)



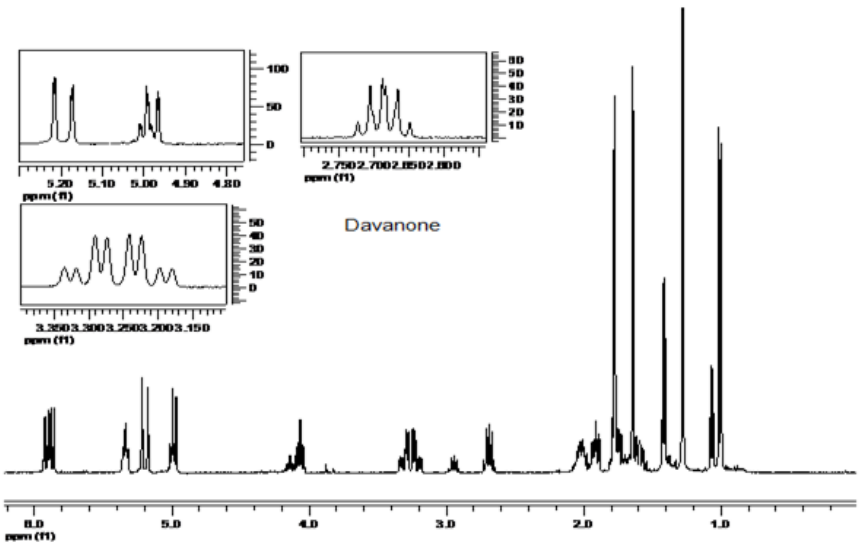
C-13 and DEPT spectrum of Yomogi alcohol

Appendix (H)



[1]HNMR of chamazulene

Appendix (I)



[1]HNMR of davanone

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