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Identification of chemical components from the Rhizomes of *Acorus calamus L.* with gas chromatography-tandem mass spectrometry (GC-MS\MS)

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ABSTRACT: In current work our main objective was to search the chemical components of volatiles in *Acorus Calamus L.* was identified by gas chromatography-tandem mass spectrometry (GC-MS\MS) combined with headspace (HS) technique. The technique is a very valuable in terms of the extraction yield, sample work up time, and profiling analysis. According to the our study data, about 78 chemical components were detected from the rhizomes of *Acorus calamus L.* by headspace method. The major components are as follows: 75.8% asarone, 79% benzen, 25,8 % trans-β-Ocimene, 20,5% Isocalamendiol, 20,1 % Methyleugenol, 22,6% 3-Carene 17.40% ß-asarone and 17.1% α-Pinene. In terms of the characteristics of the components contained in this plant and studies show that *Acorus calamus* could be a potential source of novel antibacterial, antioxidant ve anticancer agent.

Key words: ß-asarone; GC-MS/MS; *Acorus calamus L.*

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INTRODUCTION

Chemical composition and content of aromatic plants have been studied in present works extensively (Zou et al., 2015). Essential oils contens of plants and their other compounds from secondary metabolism have a significant usage area in folk medicine, pharmaceutical industries fragrance and food flavoring, (Alma and Ertas, 2007; Satil et al., 2003; Dıgrak et al., 1999; Kusmenoglu et al., 1995). For centuries, plant extracts are consumed as a remedy to treat inflammation and various serious diseases (Aktumsek et al., 2013, Wei and Shibamoto, 2010).

For almost 80% of the local population medicinal and aromatic plants are still primary way in treatment because of their biological activities. Essential oil in plants has been studied by many researchers (Hamdy et al., 2012; Uddin et al., 2011; Farnsworth and Soejarto, 1991). Due to essential oil contents, plantsare commonly used in folk medicine as well as they have been accepted to be good model for new synthetical products (Houghton et al., 2000).

Acorus calamus L. species is one of the member of the *Araceae* family is widely spreaded throughout the northern hemisphere mostly in temperate and sub-tropical climates including North America, Europe, far–east countries and Indian subcontinent.

In Indian climate, it is found above 2200 m in the Himalayas. In the medicine, the rhizomes of this plant are known to have antimicrobial, antispas-modic, anthelmintic and carminative features; they have used to treat many mental problem such as memory impairment and epilepsy. (Bisht et al., 2011; Kim et al., 2011; Rajputet al., 2014), and digestive disorders such as bloating, colic, gas and poor digestive functions (Balakumbahan et al., 2010; Kumar et al., 2016).

Because they have believed that the rhizomes of *Acorus calamus L.* is *e*ffective in treating some diseases some diseases, this plant rhizomes have been extensively used especially in Eastern Asia (Cho et al., 2000; Lee, 2007). It have reported that mainly chemical components of this species come about primarily essential oils and other compounds such as flavonoids, phenols (Singh, 2012), terpenoids, tannins and polysaccharides (Wagner, 2011).

The rhizomes of *Acorus Calamus* and their essential oil are extencively used in the production of alcoholic beverages and flavoring industry. Many pharmacological properties of *Acorus Calamus*, anticancerous antibacterial, antioxidant, antimicrobial, antispasmodic, antidiarrhoeal, anticonvulsant, cardiovascular, anti-inflammatory and antidiabetic has been reported by scientist (Kumar et al., 2016; Sharma et al., 2014; Rajput et al., 2014).

Many chemical constituents from the rhizomes of *Acorus calamus* has been reported for the presence of essential oil containing calamen, clamenol, calameon and asarone. This plant includes previously some constituents which is composed of β-asarone, α-asarone, elemicine, cisisoelemicine, cis and trans isoeugenol, camphene, acorone and acrenone (Imam et al.,2013). Furthermore, components of the essential oil of the plant rhizomes was mainly defined a higher percentage of βasarone and second majority of camphene (Dong et al., 2010).

MATERIAL AND METHOD

Extraction

20 g of dried rhizomes of *Acorus calamus L.* were powdered by a blender for preparation of propanol extract, and added to 500 mL propanol. This extraction mixture was stirred at room temperature for 24 h by a magnetic stirrer. The mixture was filtered with filter paper. In order to dry the evaporated sample was used the lyophilization metod at -50 °C but it were placed in an ice-bath for 24 hours prior to lyophilization. After drying, sample was evaporated with a rotary evaporator (Heidolph 94200, Bioblock Scientific) and was stored -30**°C** until using on the experiments.

Chemical Analysis

2 g of evaporated sample were taken for analyses with headspace metod were placed in a 20-mL headspace vials. Afterwards, HS–GC–Tandem Mass Spectrometry method conditions were optimized for the analysis (given in Table 1). after optimization, the sample is characterized by using HS–GC-MS/MS (Agilent 7890A system) method.

Table 1. General Experimental Conditions for Chemical Analysis by GC-Tandem Mass Spectrometry

1.GC		
Carrier gas	Helium	
Inlet flow (column 1)	~ 1 mL/min	2.0 psi (during backflush)
PUU flow (column 2)	column 1 flow + 0.2 mL/min	
Inlet temperature	280°C	
Injection volume	1 µL	
Injection mode	splitless	
Gas saver	On (20 mL/min at 2.0 min)	
Purge flow to split vent	30 mL/min at 0.75 min	
Oven temperature program		60°C 1 min
	40°C/min	170°C 0 min
	10°C/min	310° C 3 min
2. Capillary flow technology		
Aux EPC gas	Helium	
Aux EPC pressure	3.0 psi during run, 50.0 psi during backflush	
Analytical columns	HP-5ms UI 15 m × 0.25 mm × 0.25 µm	
Retention time locking	locked to 9.143 min	
3. Tandem Mass Spectrometer		
Mode	Electron impact	
Transfer line temperature	280 °C	
Source temperature	300 °C	
Quadrupole temperature	Q1 and Q2 = 180 °C	
4. MRM Mode Conditions		
Collision gas flow	Nitrogen at 1.5 mL/min,	
Quenching gas flow	Helium at 2.25 mL/min	
Detector Gain	10	
5.Backflushing Conditions		
Timing	5 min duration during post-run	
Oven temperature	310 °C	
Aux EPC pressure	50 psi	
Inlet pressure	2 psi	

RESULTS AND DISCUSSION

Relative percentage and RI values of main compounds of extracts from *Acorus calamus L.* rhizomes are reported in Table 1, As can be seen from this table, the analysis of the SPME headspace of the rhizomes of *Acorus calamus L.* 78 compounds, representing about 95% of the essential oil from the rhizomes, were characterized. The majör components are as follows: 75.8% asarone, 25.8 % trans-β-Ocimene, 20.5% Isocalamendiol, 20.1 % Methyleugenol, 22.6% 3-Carene 17.40% ß-asarone, 9.87% Cetene.16.5% β-Guaiene. 79% benzen. 1.2.4-trimethoksy-5-(1-propenyl). 15.8 % β-copaene. 15.5% Tridecane and 17.1% α-Pinene.

A typical gas chromatogram the chemical compositions from the rhizomes of *Acorus calamus* is shown in Figure. 1.

Table 2. Chemical Composition of the Essential Oil from the rhizomes of *Acorus calamus*

Compound	RI	Peak Area%	RT	MW	Molecular Formula
Asarone	1646	75.8	7.4	208	$C_{12}H_{16}O_3$
β-Asarone	1561	17.4	7.51	208	$C_{12}H_{16}O_3$
Azaron	1568	9.69	7.4	208	$C_{12}H_{16}O_3$
Elemicin	1531	2.85	7.4	208	$C_{12}H_{16}O_3$
Hexadecen-1-ol. trans-9-	1811	6.72	7.132	224	$C_{16}H_{32}$
3-Hexadecene, (Z)-	1587.4	4.21	7.132	242	$C_{16}H_{34}O$
1-Nonadecene	1891	3.57	7.132	240	$C_{18}H_{36}$
1-Docosene	2192	2.03	7.132	224	$C_{16}H_{32}$
1-Pentadecene	1486	2.03	7.132	226	$C_{19}H_{38}$
n-Heptadecanol-1	1941	1.29	7.132	308	$C_{22}H_{44}$
E-15-Heptadecenal	2083.2	1.87	7.132	210	$C_{15}H_{30}$
Dichloroacetic acid	2259	1.29	7.132	182	$C_{13}H_{26}$
n-Pentadecanol	1791	1.24	7.132	256	$C_{17}H_{36}O$
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2282.3	8.86	7.132	252	$C_{17}H_{32}O$
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	2470.7	8.17	7.132	352	$C_{18}H_{34}C_{l2}O_2$
Doconexent	2520.9	5.92	7.132	228	$C_{15}H_{32}O$
Acetic acid, 2-[[3-cyano-4 (methoxymethyl)-6-methyl-2-pyridinyl]thio]-	2174	1.75	7.36	316	$C_{21}H_{32}O_2$
Mannosamine	1714	4.9	7.36	342	$C_{22}H_{32}O_2$
Deoxyspergualin	3516	4.33	7.36	328	$C_{22}H_{32}O_2$
9-Octadecenoic acid	3238	3.19	7.36	302	$C_{20}H_{30}O_2$
1,2,4-Triazole, 4-[N-(2-hydroxyethyl)-N-nitro]amino-	1526	1.66	7.36	252	$C_{11}H_{12}N_2O_3S$
L-Glucose	1765	3.13	2.976	179	$C_6H_{13}NO_5$
Stearic acid, 2-hydroxy-1-methylpropyl ester	2474	1.85	2.976	387	$C_{17}H_{37}N_7O_3$
Octanoic acid, 7-oxo	1309	2.32	2.976	444	$C_{28}H_{44}O_4$
17-Octadecynoic acid	2165	2.63	2.976	173	$C_4H_7N_5O_3$
β-curcumene	1480	3.52	2.98	180	$C_6H_{12}O_6$
10,12-Octadecadiynoic acid	2202	3.38	2.98	356	$C_{22}H_{44}O_3$
trans-α-Bergamotene	1433	5.51	3.186	158	$C_8H_{14}O_3$
3-Carene	1005	22.6	3.186	280	$C_{18}H_{32}O_2$
α-Pinene	931	17.1	4.114	204	$C_{15}H_{24}$
D-Limonene	1018	3.65	4.114	276	$C_{18}H_{28}O_2$
Limonen-6-ol, pivalate	1560	8.96	4.117	204	$C_{15}H2_4$
Farnesene epoxide, E-	1540	5.37	4.416	136	$C_{10}H_{16}$

β-Pinene	970	4.97	4.136	136	$C_{10}H_{16}$
2-Dodecenal, (E)-	1442	8.85	4.143	136	$C_{10}H_{16}$
Cyclododecane	1316	4.5	4.408	236	$C_{10}H_{16}$
1-Dodecene	1187	14.3	4.412	220	$C_{15}H_{24}O_2$
Cetene	1587	10.9	4.427	136	$C_{15}H_{24}O$
1-Tridecene	1287	5.29	4.809	182	$C_{10}H_{16}$
Caryophyllene	1424	7.92	4.813	168	$C_{12}H_{22}O$
Aromandendrene	1439	6.06	4.824	168	$C_{12}H_{24}$
Longifolene-(V4)	1387	8.82	5.844	242	$C_{12}H_{24}$
(-)-Aristolene	1417	3.47	7.139	224	$C_{16}H_{34}O$
Naphthalene	1515	17.1	5.847	182	$C_{16}H_{32}$
Benzene, 1,2-dimethoxy-4-(1-propenyl)-	1461	79	6.241	204	$C_{13}H_{26}$
Methyleugenol	1402	20.1	6.242	204	$C_{15}H_{24}$
γ-HIMACHALENE	1479	21.2	6.252	204	$C_{15}H_{24}$
β-Guaiene	1523	16.5	6.835	204	$C_{15}H_{24}$
α-ylangene	1221	4.39	6.834	204	$C_{15}H_{24}$
Retinol, acetate	2531	3.79	6.289	178	$C_{15}H_{24}$
Erucic acid	1530	6.02	6.30	178	$C_{11}H_{14}O_2$
trans-13-Octadecenoic acid	2163	2.32	6.845	204	$C_{15}H_{24}$
cis-10-Nonadecenoic acid	2256	2.23	6.72	204	$C_{15}H_{24}$
cis-Vaccenic acid	2116	2.06	6.72	204	$C_{15}H_{24}$
Oleic Acid	2113	1.75	6.815	328	$C_{15}H_{24}$
1-Hexadecanol	1864	10.8	6.904	238	$C_{22}H_{32}O_2$
β-copaene	1216	15.8	6.97	222	$C_{15}H_{26}O_2$
Isocalamendiol	1725	20.5	6.97	282	$C_{15}H_{26}O$
β-Vatirenene	1489	11.1	6.97	296	$C_{18}H_{34}O_2$
Isolongifolene, 4,5,9,10-dehydro-	1544	12	6.97	282	$C_{19}H_{36}O_2$
trans-calamenene	1537	7.93	6.97	282	$C_{18}H_{34}O_2$
alfa-Copaene	1221	17.1	7.272	204	$C_{16}H_{34}O$
Tridecane	1300	15.5	7.721	238	$C_{15}H_{24}$
Coumarin. 3,4-dihydro-4,4,7-trimethyl-	1640	2.51	7,526	202	$C_{15}H_{26}O_2$
trans-β-Ocimene	1034	25.8	7,526	202	$C_{15}H_{20}$
Octanoic acid, 7-oxo-	1309	9.03	8.192	202	$C_{15}H_{22}$

RI: Linear Retention Index
RT: Retention Time
MW: Molecular Weight

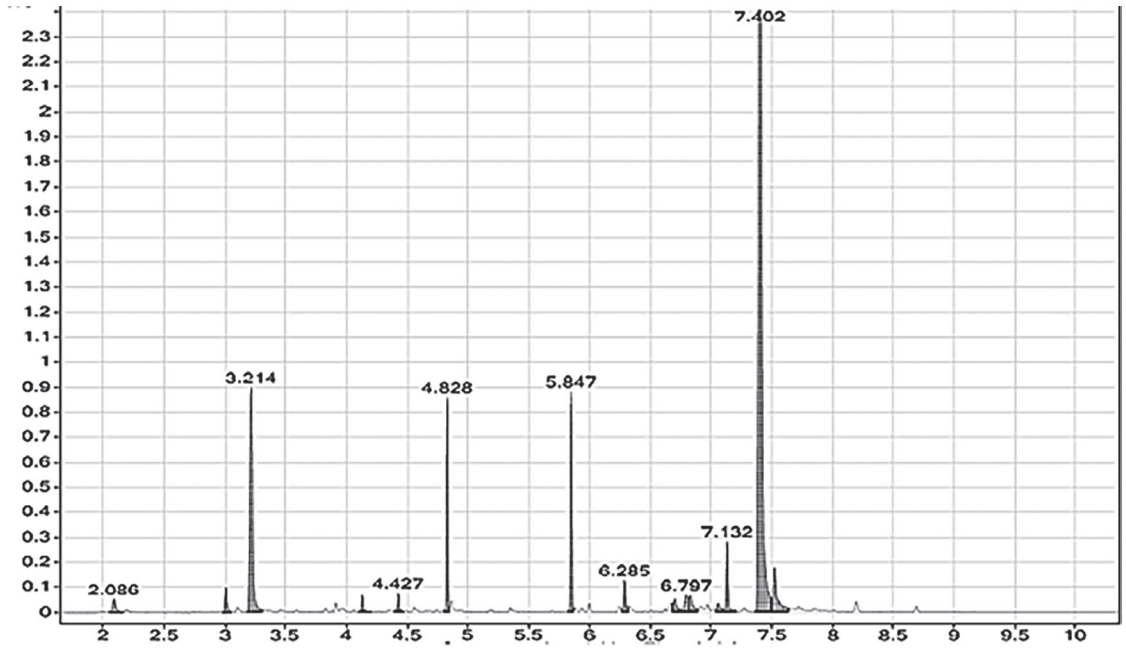

Figure 1. Typical gas chromatogram of the essential oil from the rhizomes of *Acorus Calamus*

In previous studies, the essential oil contents from leaves of *Acorus calamus* were reported by Radusˇiene et al. (2006); as asarone (15.7–25.5%), isoeugenol (2.0–4.9%). In our research, we found 75.8% asarone the essential oil contents from the rhizomes of *Acorus calamus* by gas chromatography-mass spectrometry (GC-MS\MS) combined with headspace. In addition to, Röst and Bos determined 31-44% of β-asarone in the leaf oil. Maza (1985) obtained the essential oils from the rhizomes and leave, in terms of its major constituents β-asarone (83.2% and 77.7%) and α -asarone (9.7% and 6.8%). The other common constituents were α-pinene, α-ocimene, α–caryophyllene, methyl isoeugenol, curcumene, elemicin, calacorene, isoelimicin was reported by Maza (1985).

CONCLUSION

In this study, essential oil from *Acorus calamus* was obtained from steam distillation method, and its chemical composition was determined by GC and GC-MS/MS. In addition chemical composition of sample was investigated and reported as well. The results revealed that extracts of the plant has potential of essential oil on the solvent and the technique that used. According to the ourstudy data the essential oils mainly contained about 75.8% asarone, 25,8 % trans-β-Ocimene, 20.5% Isocalamendiol, 20.1 % Methyleugenol, 22.6% 3-Carene 17.40% ß-asarone, 9.87% Cetene,16.5% β-Guaiene and 12.30% benzen, 1,2,4-trimethoksy-5-(1-propenyl)-. Further studies should be performed in order to investigate possible features of *A. Calamus* in food industry and pharmacology. Mukherjee et al., (2007) reported that α-asarone and β-asarone was activity of acetylcholinesterase (AChE) inhibitory. Furthermore, rhizome of *Acorus calamus* could be a potential source of novel antibacterial agent because of the significant antimicrobial effect has been seen in the clinical isolates tested in some study. some studies were reported also that *Acorus calamus* have control over antibacterial(Chopra et al., 1992) and antifungal (Ghosh 2006) activity (Hassan et al., 2006). Moreover, it has been reported to be used in infantile fever, cough, bronchitis and asthma (Hassan Gilani and Jabbar Shah 2009). This research shows that chemical composition of *Acorus calamus* such as β-asarone is potential compounds for therapies that prevent cancer formation and inhibit its development (Liu et al.,2013).

Furthermore, the presence of polyphenolic compound such as flavonoids and flavonols in the structure of this plant recommended that plant part can be considered to show antioxidant activity.

Conflict of Interest: The authors declare that there is no conflict of interest.

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