



**GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN THE
WHOLE PLANT OF *ANDROGRAPHIS ECHIOIDES* (L) NEES
(ACANTHAECEAE)**

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ABSTRACT

The investigation was carried out to determine the possible bioactive components of whole plant of *Andrographis echioides* using GC-MS analysis. The chemical composition of the ethanol extract of whole plant of *Andrographis echioides* was investigated using Perkin-Elmer Gas Chromatography- Mass matched with national institute of standard and Technology (NIST) library. Twenty one compounds were identified. The prevailing compounds were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol was found to be major component followed by

2-Tridecen-1-ol, (E)- 6,9,12- Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-, trans-2-Undecen-1-ol, Methoxyacetic acid, Squalene ,3-tetradecyl ester, Heptadecanoic acid, heptadecyl ester, 2-Cyclopentene-1-undecanoic acid, (+)-,Valeric acid, 4-pentadecyl ester, 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion, Heptadecane, 2,6,10,14-tetramethyl-, Pterin-6-carboxylic acid, Octadecane, 1-(ethenyloxy)-, Cyclo propane tetra decanoic acid, 2-octyl-, methyl ester, Z,Z,Z-4,6,9-Nonadecatriene, Z,Z-2,5-Pentadecadien-1-ol.

KEYWORDS: GC-MS, False water willow, Mass spectrum, Chemopreventive.

INTRODUCTION

Many naturally occurring chemicals from plants exhibit a broad spectrum of pharmacological profile. These plant chemicals are classified as primary or secondary metabolites. The primary metabolites include the common sugars, amino acids and chlorophyll. Secondary metabolites are the remaining chemicals which are produced from the primary metabolites. These include alkaloids, terpenoids, phenolics, tannins, steroid and volatile oil. ^[1]

Andrographis ecioides (Acanthaceae) which is commonly known as false water willow is an herb commonly found throughout India and Sri Lanka. The genus *Andrographis* is native of India and Sri Lanka contains 28 species of small annual shrubs essentially distributed in tropical Asia. Some of them are medicinally important. The plants from genus *Andrographis* used in goiter, liver diseases ^[2], fever, fertility problems, bacterial ^[3], malarial, helminthic, fungal, diarrhea and larvicidal disorders. ^[4,5] Leaf juice boiled with coconut oil is used to control falling and graying of hair. ^[6]

Taking into consideration of the medicinal importance of the plant, the ethanol extract of *Andrographis ecioides* was analyzed for the GC-MS. This work will help to identify the compounds of therapeutic value. GC-MS is one of the techniques used to identify the bioactive constituents of long chain, branched chain hydrocarbon, alcohols, acids, ethers, etc.

MATERIALS AND METHODS

Collection of plant sample

Whole plant of *Andrographis ecioides* (L) Nees was collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora the specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract: The whole plant of *Andrographis echioides* was cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC – MS analysis.

GC-MS Analysis: GC-MS analysis of ethanol extract was performed with GC clarus 500 Perkin Elmer system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite – 1 fused silica capillary column (30 mm x 0.25 mm 1D x 1 um df, composed of 100% Dimethyl poly siloxane). For GC-MS detection, and electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml / min and an injection volume of 2 ul was employed (Split ratio of 10:1); Injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbomass.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULT AND DISCUSSION

Table 1: Components detected in *A. echioides* whole plant

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	11.00	cisZ-11,12-Epoxytetradecan-1-ol	C ₁₄ H ₂₈ O ₂	228	0.46
2.	11.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	25.19
3.	11.34	trans-2-Undecen-1-ol	C ₁₁ H ₂₂ O	170	6.05
4.	11.55	2-Tridecen-1-ol, (E)-	C ₁₃ H ₂₆ O	198	8.58
5.	16.10	1,2:4,5:9,10-Triepoxydecane	C ₁₀ H ₁₆ O ₃	184	2.00
6.	16.44	2-Cyclopentene-1-undecanoic acid, (+)-	C ₁₆ H ₂₈ O ₂	252	4.21
7.	17.80	Octadecane, 1-(ethenyloxy)-	C ₂₀ H ₄₀ O	296	3.04
8.	19.18	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	C ₂₄ H ₄₅ N ₂ O ₃	409	3.76
9.	20.59	Valeric acid, 4-pentadecyl ester	C ₂₀ H ₄₀ O ₂	312	4.01
10.	21.95	Methoxyacetic acid, 3-tetradecyl ester	C ₁₇ H ₃₄ O ₃	286	5.26
11.	23.29	Heptadecane, 2,6,10,14-tetramethyl-	C ₂₁ H ₄₄	296	3.43

12.	23.48	Squalene	$C_{30}H_{50}$	410	5.58
13.	24.62	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508	4.32
14.	25.29	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	262	2.40
15.	25.91	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	2.31
16.	26.28	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	1.99
17.	26.83	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	1.36
18.	27.03	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$	368	7.04
19.	27.19	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	2.43
20.	27.72	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	3.51
21.	29.55	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	1.59
22.	30.62	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	0.64
23.	32.08	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	0.85

Table.2 Activity of phytochemicals identified in the ethanol extract of *A. echinoides* whole plant

No.	RT	Name of the compound	Molecular Formula	Compound nature	**Activity
1.	11.00	cisZ-11,12-Epoxytetradecan-1-ol	$C_{14}H_{28}O_2$	Epoxy compound	No activity reported
2.	11.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Unsaturated alcoholic compound	Antimicrobial Anti-inflammatory
3.	11.34	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	Unsaturated alcoholic compound	No activity reported
4.	11.55	2-Tridecen-1-ol, (E)-	$C_{13}H_{26}O$	Unsaturated alcoholic compound	No activity reported
5.	16.10	1,2:4,5:9,10-Triepoxydecane	$C_{10}H_{16}O_3$	Epoxy compound	No activity reported
6.	16.44	2-Cyclopentene-1-undecanoic acid, (+)-	$C_{16}H_{28}O_2$	Unsaturated cyclic fatty acid compound	No activity reported
7.	17.80	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	Alkane compound	No activity reported
8.	19.18	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	Nitrogen compound	Antimicrobial
9.	20.59	Valeric acid, 4-pentadecyl ester	$C_{20}H_{40}O_2$	Fatty acid ester	No activity reported
10.	21.95	Methoxyacetic acid, 3-tetradecyl ester	$C_{17}H_{34}O_3$	Acidic compound	Antimicrobial
11.	23.29	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$		No activity reported

12.	23.48	Squalene	$C_{30}H_{50}$	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
13.	24.62	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	Fatty acid ester compound	No activity reported
14.	25.29	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	Alkene compound	No activity reported
15.	25.91	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	Unsaturated alcoholic compound	No activity reported
16.	26.28	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	Sesquiterpene alcohol	Anti-tumor, Analgesic Antibacterial, Antiinflammatory, Sedative, Fungicide.
17.	26.83	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	Ketone compound	No activity reported
18.	27.03	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$	Linolenic acid ester	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
19.	27.19	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	Fatty acid ester compound	No activity reported
20.	27.72	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	Chemical nature	Used in folic acid estimation
21.	29.55	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	Steroid compound	Antiarthritic Hepatoprotective Antiasthma Anti-inflammatory Diuretic Cancer preventive
22.	30.62	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	Steroid compound	Antiarthritic Hepatoprotective Antiasthma Anti-inflammatory Diuretic Cancer preventive

23.	32.08	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C ₂₁ H ₃₈ O ₂	Ester compound	No activity reported
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Dr.Duke's Phytochemical and Ethnobotanical Databases

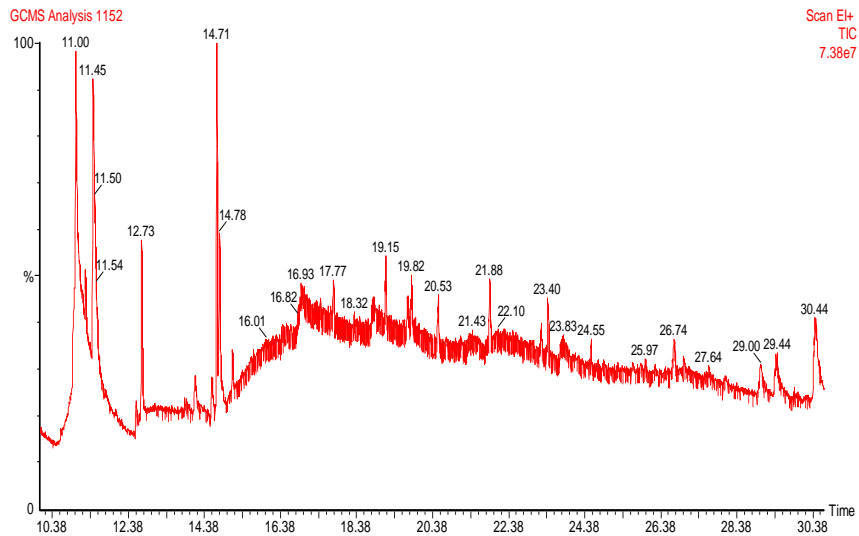


Fig-1: GC-MS chromatogram of the ethanol extract of *A. echioides* whole plant

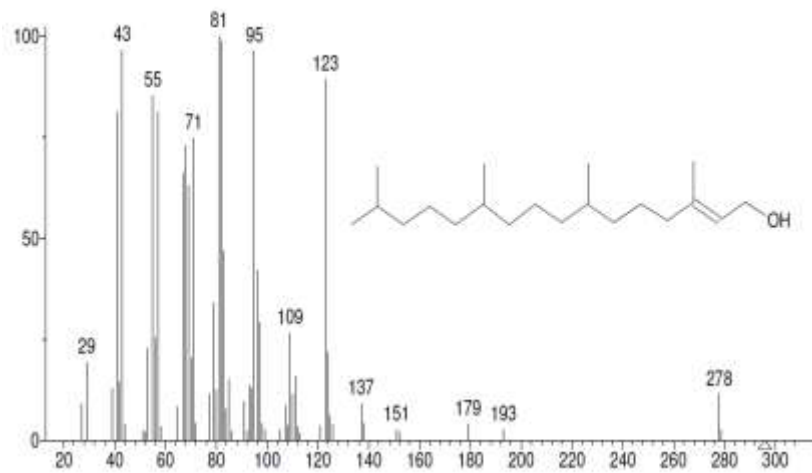


Fig-2: Mass spectrum of 3,7,11,15- Tetramethyl-2-hexadecen-1-ol,

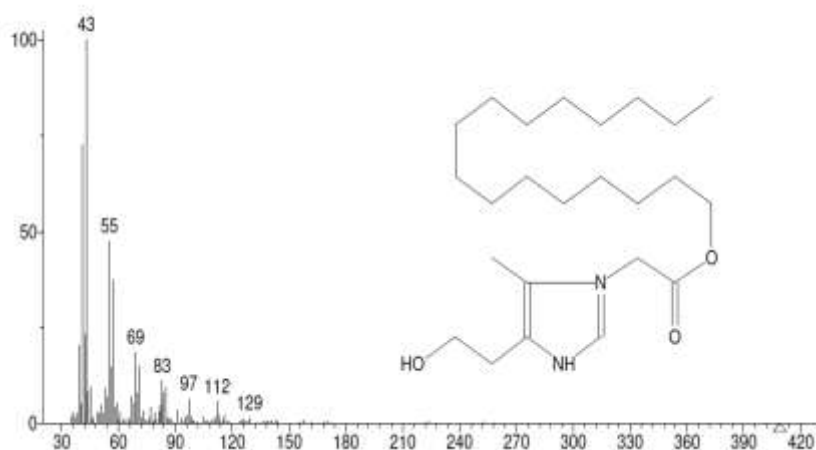


Fig-3: Mass spectrum of 3-Hexadecyloxycarbonyl 1-(2-hydroxyethyl)-4-methylimidazolium ion

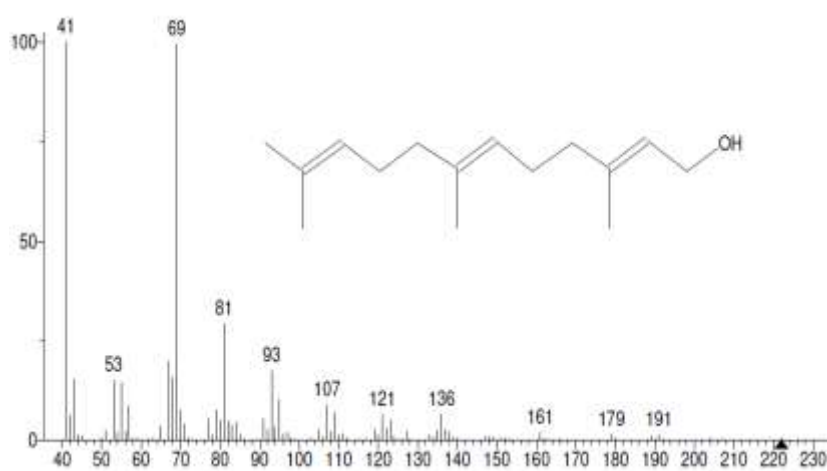


Fig 4: Mass spectrum of 2, 6, 10-Dodecatrien-1-ol, 3,7,11-trimethyl-

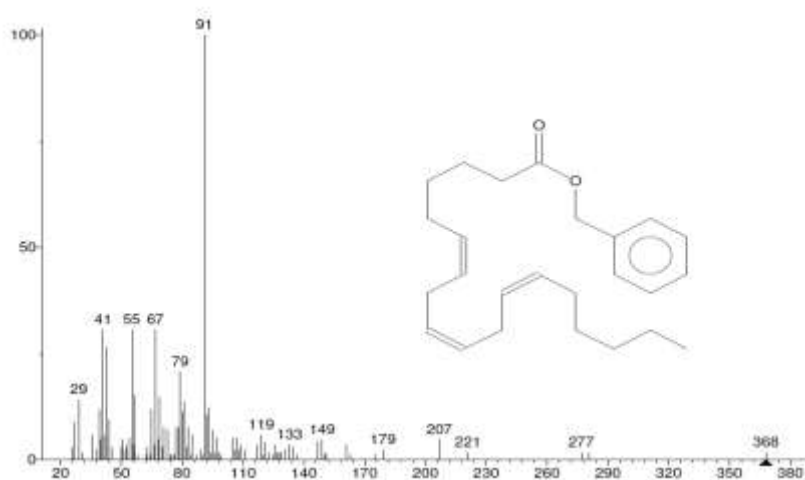


Fig – 5: Mass spectrum of 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-

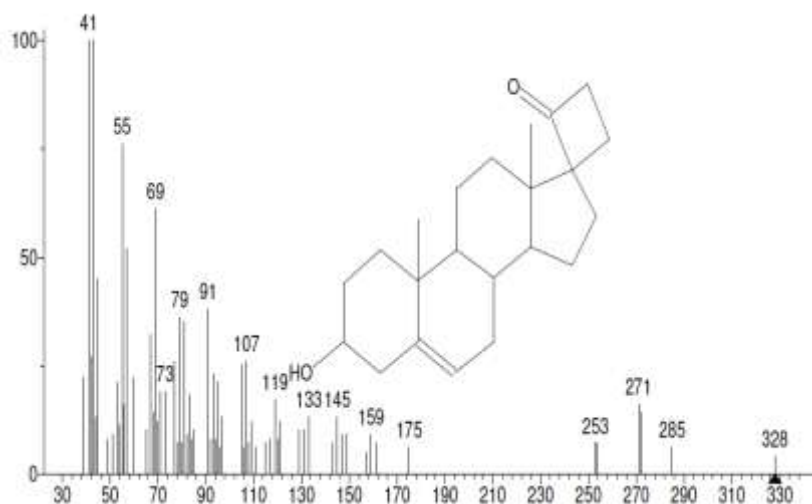


Fig- 6: Mass spectrum of Spiro [androst-5-ene-17,1'-cyclobutan]-2'-one, 3- hydroxy-, (3a,17a)-

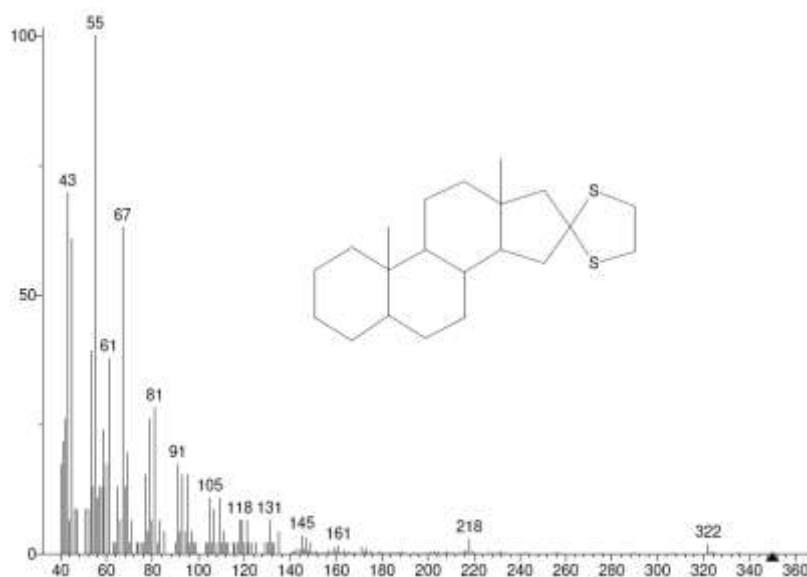


Fig-7: Mass spectrum of 5a-Androstan-16-one, cyclic ethylene mercaptole

Twenty three compounds were identified in *A. echioides* whole plant by GC –MS analysis (Fig-1). The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are presented in table.1. The prevailing compounds were 3,7,11,15- Tetramethyl-2-hexadecen-1-ol, 2- Tridecen-1-ol, 6, 9, 12-octadecatrienoic acid, phenylmethyl ester,(Z,Z,Z)-, trans-2-Undecen-1-ol, Squalene, Methoxyacetic acid, 3-tetradecyl ester, Heptadecanoic acid, heptadecyl ester, 2 Cyclopentene-1-undecanoic acid,(+)-, Valeric acid, 4-pentadecyl ester, 3 Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion, Heptadecane, 2,6,10,14-tetramethyl-, Pterin-6-carboxylic acid, Octadecane, 1-(ethenyloxy)-, Cyclo

propane tetra decanoic acid, 2-octyl-, methyl ester, Z,Z,Z-4,6,9-Nonadecatriene, Z,Z-2,5-Pentadecadien-1-ol.

Fig 2, 3, 4, 5, 6 and 7 shows the mass spectrum and structure of 3,7,11,15- Tetramethyl-2-hexadecen-1-ol, 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)- 4-methylimidazolium ion, 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-, Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)- and 5à-Androstan-16-one, cyclic ethylene mercaptole. Table.2 listed the major phytoconstituents and its biological activities obtained through the GC-MS study which contributes the medicinal activity of ethanol extract of whole plant of *A. echioides*. In the present study, 23 compounds were identified from the whole plant of *A. echioides* by Gas Chromatography- Mass Spectrometry (GC-MS) analysis. Among the identified phytochemicals, Squalene has the property of antioxidant. ^[7] Recently squalene possesses chemopreventive activity against colon carcinogenesis. ^[8,9] Many spiro compounds possess very promising biological activity of anticancer, antibacterial, anticonvulsant, antituberculosis antialzheimer's, pain relief, antidermatitis and microbial agents. ^[10] Recently spiro compounds have also used as antioxidants ^[11] 6,9,12- Octadecatrienoic acid, phenylmethyl ester (Z,Z,Z) also known as linolenic acid ester known to inhibit proliferation of ER –positive and ER- negative breast cancer cells. It is a potent antiangiogenic agent in colorectal cancer and in HUVEC cells ^[12]. 2, 6, 10- Dodecatrien-1-ol, 3,7 11- trimethyl is known as Farnesol. Farnesol is a natural 15-carban organic compound which is an a cyclic sesquiterpene alcohol. Farnesol has been suggested to function as chemopreventive and antitumor agent. ^[13]

CONCLUSION

The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results. It could be concluded that *A. echioides* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

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