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Der Pharmacia Lettre, 2015, 7 (12):138-147
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Identified bioactive constituents on leaf of *Andrographis echiodies* grown on Vellore District, Tamil Nadu

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ABSTRACT

In this paper we carried out the pharmacognostical evaluation, preliminary phytochemical and GC-MS analysis of A.echiodies leaf which was collected from Vellore district. In the present study crude extracts of petroleum ether, ethyl acetate, methanol and aqueous were prepared from the leaves of A.echioides by using Soxhlet apparatus hot continuous percolation method. The crude extracts were subjected to pharmacognostical and phytochemical analysis. Preliminary phytochemical screening reveals the presence of flavonoids, terpenoids, carbohydrate, protein, glycosides and phytosterols in A.echiodies. The physiochemical constants and its fluorescence properties of the leaf powder were observed and documented in this study. Further the leaf extracts were analysed by GC-MS. The GC-MS analysis reveals 3, 26 and 22bio active compounds from Pet ether, ethyl acetate and methanol respectively.

Keywords: *Andrographis Echiodies*, Pharmacognostical, Fluorescence, Phytochemical, GC-MSanalysis

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INTRODUCTION

Andrographisechiodies(L) nees belongs to the Acanthacae family. In local its known asGopuramthangi [1].This plantis an small herb widely located in dry places like Indiaand Srilanka. This *Andrographis* genus contains plenty of medicinal properties and used to treat goiter, liver diseases, fertility problems,bacterial,malarial and fungal disorders [2].Leafextract of *A.echiodies* is used as a medicine for fever.The leaf juice boiled and mixed with coconut oil used to prevent falling and graying of hair[3]. Leaf powder and rice water is used for snake bite and eczema [1]. This plant leaf extract is used for dengue against larvae of mosquito. [4] and also show diuretic activity [5]. In addition posses the anthelmintic activity to treat the various parasitic worms [6].*A.echioides* shows pharmacological activities such as diuretic, antimicrobial ,Anti-ulcer, Hepatoprotective and Antioxidant effect[7].The present study is focused on theevaluation ofPharmacognostical parameters and phytochemical analysis of leaves of the*A.echioides*.

MATERIALS AND METHODS

2.1 Plant collection

The plant materials were collected from Chendrampalli land Vellore district. The collected plant was identified and authenticatedfrom flora of madras presidency Reg. No: PARC/2014/2057.

2.2 Pharmacognostical Evaluation of *A.echioides*

Pharmacognostical parameters such as fluorescence analysis and physicochemical values like ash content and loss on drying were evaluated as per the standard protocol.[8]

2.3 Preparation of crude extracts from *A.echioides leaves*

The plant leaves were collected and washed with distilled water then the washed leaves were dried for two weeks and ground into coarse powder. The powder is extracted with solvents like petroleum ether, ethyl acetate, methanol and water using Soxhlet apparatus.

2.4 Phytochemicalscreeningof*A.echioides*

Phytochemical analysis of *A.echioides* was carried out as per the standard protocals. [9]

3. CHARACTERIZATION

GC-MS analysis was carried out on a Perkin elmer clarus 680 GC-MS instrument employing the following condition: column elite-5MS (30.0m, 0.25 mm ID, 250 µm, operating in electron impact mode; helium was used as a carrier gas at a constant flow and split ratio is 10:1 ; injector temperature is 250˚C; flow rate is 1 ml/min; oven temperature is initially 60˚C for 2 min, ramp 10˚C/min to 300˚C, hold for 6 min. Total run time is 32.00 min The molecular weight and structure of the compounds were ascertained by interpretation using the database of National Institute Standard and Technology (NIST).[10]

RESULT AND DISCUSSION

4.1 Pharmacognostical Evaluation of *A.echioides*

4.1.1 Physicochemical constants of *A.echioides*

Quantitative analysis of Pharmacognostical parameteris useful for setting standards for the crude drug. From results we found that 9.57, 81.12, 55.48 and 38.54 of LOD, total ash content water soluble ash content and acid insoluble ash content respectively**(Table.1)**. The moisture contentof *A.echioides*is not more than 14% (9.57% found) as per the African pharmacopoeia (1986).[11]

Table.1 Physicochemical constants of *A.echioides*

S.No	Parameters	Observations
1	Loss of drying	9.57%
2	Total ash content	81.12%
3	Water soluble ash content	55.48%
4	Acid insoluble ash content	38.51%

4.1.2 Fluorescence analysis of *A.echioides*

Fluorescence analysis of leaf powder was performed using UV short and long wavelength with different chemical reagents. The plant exhibit characteristic colors when it is treated with different reagents. This variation of color indicates the presence of active nutrients (functional groups) in the *A.echioides.* The results are given in **(Table.2)**

Table.2 Fluorescence analysis of*A.echioides*

S.No	Reagent	Visible light	Short UV(254nm)	Long UV(336 nm)
1	Powder	No change	Green	Black
2	Powder+con.HCL	No change	Dark green	Black
3	Powder+con.HNO_3	No change	Black	Dark black
4	Powder+conH_2SO_4	No change	Dark green	Black
5	Powder+NaOH	No change	Light green	Black
6	Powder+water	No change	Light green	Black
7	Powder+alcohol	No change	Green	Bluish green
8	Powder+acetic acid	No change	Greenish	Dark green

4.1.3 Nature and yield of crude extracts of *A.echioides* leaf

The extraction was carried out using Soxhlet apparatus (hot continuous percolation) with different polarity of solvent. Extractionyield of different solvent varied from 5.58% to 25.03% and ranked from low to high petroleum ether, ethyl acetate, methanol and aqueous extracts. The yield is increased with the ratio of solvents, sample extraction and temperature. The nature and yield of extracts are shown in **(Table.3)**.

Table.3 Nature and yield of crude extracts of *A.echioides*leaf

Crude Extracts	Color	Duration	Solvent consumed	Yield %
Petether	Brown	27days	8.1 lit	5.58%
Ethylacetate	Dark brown	13 days	3.9 lit	13.31%
Methanol	Green	8 days	2.4 lit	25.03%
Aqueous	Brownishblack	3 days	0.9 lit	19.31%

4.1.4 Phytochemical analysis of *A.echioides* leaves

Preliminary phytochemical screening of petroleum ether, ethyl acetate, methanol and aqueous extracts of were carried out as per the standard protocol. From the preliminary screeningchemical compounds like flavonoids, phytosterols,saponins,terpenoids and carbohydrate were detectedin this *A.echioides*. The results were tabulated in **(Table 4)**.

Table.4 Phytoconstituents of crude extracts of *A.echioides*

S.No	Phytoconstituents	PET extract	Ethylacetate extract	Methanol extract	Aqueous extract
1	**Phenols**	-	-	-	-
2	**Flavonoids**	-	+++	++	-
3	**Alkaloids**	-	-	-	+
4	**Carbohydrate**	+	++	+	++
5	**Proteins**	-	+	+++	++
6	**Glycosides**	+	+	+	+
7	**Phytosterols**	+++	+++	+++	++
8	**Saponins**	++	++	++	+
9	**Terpenoids**	++	++	++	++
10	**Tannins**	-	+++	+++	+
11	**Fats and oils**	+++	-	+	-

(-)- absent (+)-Weak (++)-Moderate (+++)-strong;

4.2.1 GC-MS analysis of PET ether crude extract

The GC-MS chromatogram of petroleum ether *A.echioides* leaf extractshows major peaks which is given in (**Fig.1)**. And the compound name retention time and molecular formula are given **(Table.5)**.

Figure 1.GC-MS Analysis of PET Ether Extract

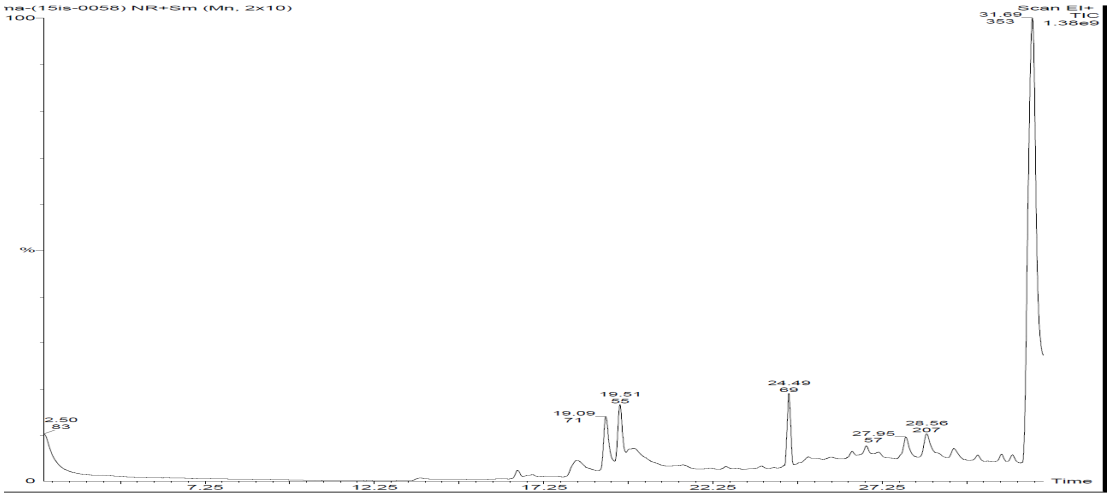
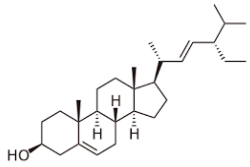
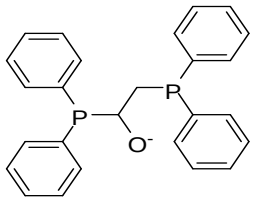


Table 5.GC-MS data for *Andrographis echiodies* PET ether Extract

NO	Compound Name	Rt (min)	MW	Molecular formula	structure
1	STIGMASTEROL	24.49	412	C29H48O	
2	PHOSPHINE OXIDE,1,2 ETHANEDIYLBIS[DIPHENYL]	31.70	430	C26H24O2P2	
3	2R-ACETOXYMETHYL-1,3,3-TRIMETHYL-4T-(3-METHYL-2-BUTEN-1-YL)-1T-CYCLOHEXANOL	28.56	282	C17H30O3	_

4.2.2 GC-MS analysis of ethyl acetate crude extract

The GC-MS chromatogram ofethyl acetate*A.echioides* leaf extractgave some major peaks are shown in fig 2 and the compound name retention time and molecular formula are given table 6.

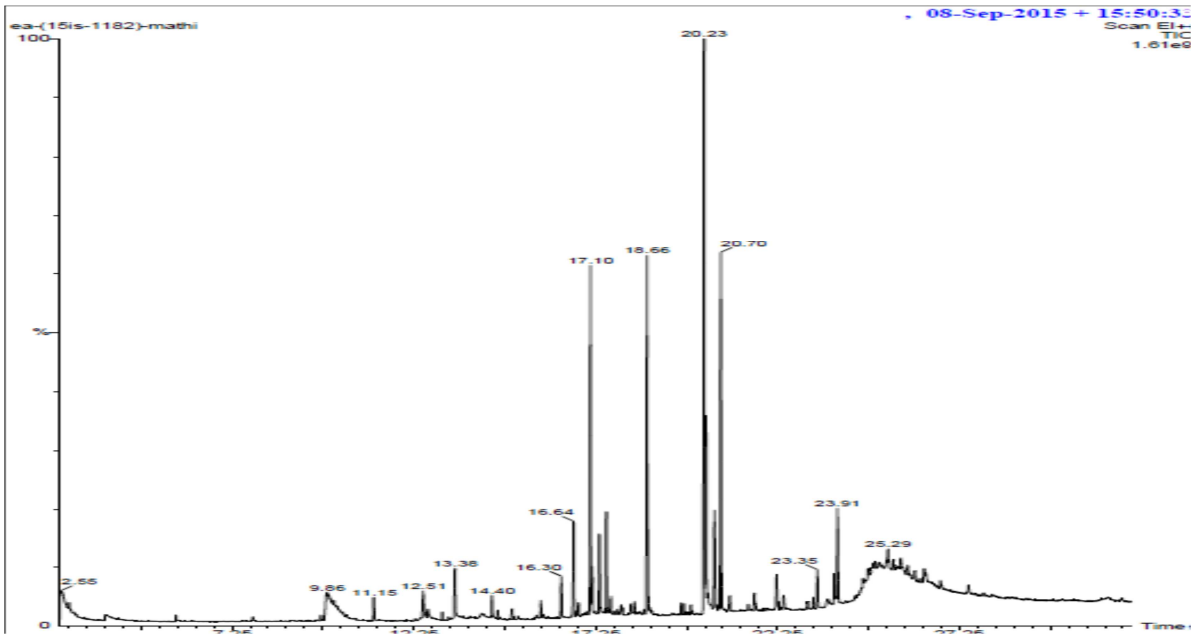
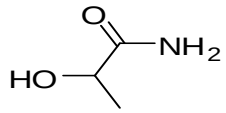
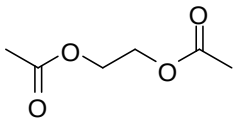
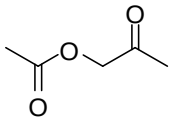
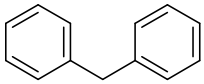
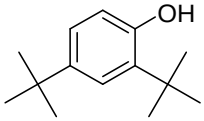
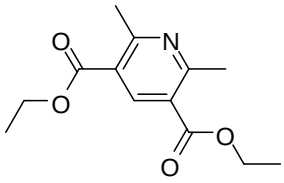
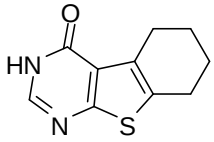
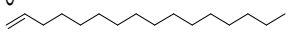
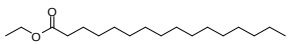
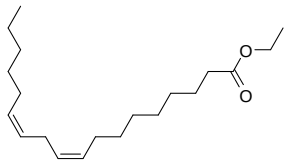
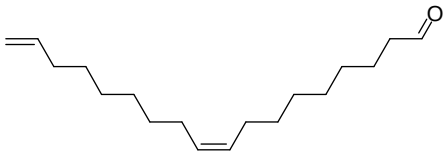
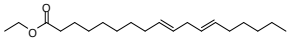
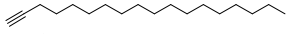
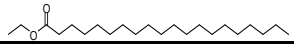
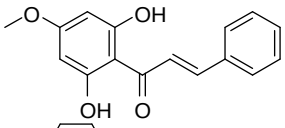
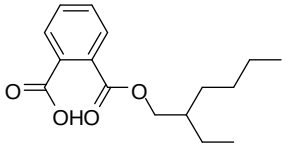
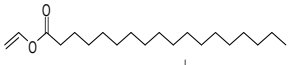
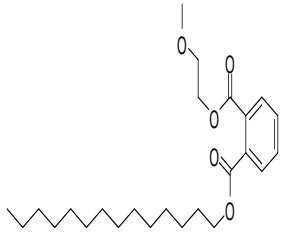
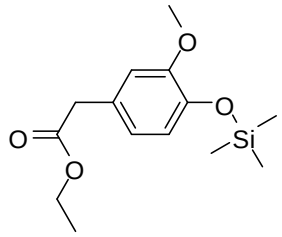
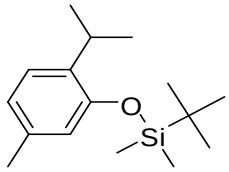
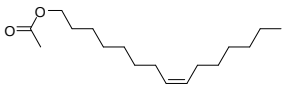


Figure 2.GC-MS analysis of Ethyl acetate extract

Table 6.GC-MS data for *Andrographis echiodies* using ethyl acetate extract

S.No	Compound Name	RT (min)	MW	Molecular formula	structure
1	PROPANAMIDE, 2-HYDROXY-	2.55	89	C3H7O2N	
2	1,2-ETHANEDIOL, DIACETATE	9.86	146	C6H10O4	
3	2-PROPANONE, 1-(ACETYLOXY)-	11.15	116	C5H8O3	
4	DIPHENYLMETHANE	12.50	168	C13H12	
5	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	13.37	206	C14H22O	
6	3(4H)-DIBENZOFURANONE, 4A,9B-DIHYDRO-6-(6-HYDROXY-M-TOLYL)-8,9B-DIME	15.74	320	C21H20O3	–
7	3,5-DIETHOXYCARBONYL-2,6-DIMETHYLPYRIDINE 894 941	16.30	251	C13H17O4N	
8	BENZOTHIENO[2,3-D]PYRIMIDIN-4(3H)-ONE, 5,6,7,8-TETRAHYDRO-	16.30	206	C10H10ON2S	
9	E-15-HEPTADECENAL	16.64	252	C17H32O	
10	1-HEXADECENE	16.64	224	C16H32	
11	1-EICOSYNE	17.10	278	C20H38	
12	HEXADECANOIC ACID, ETHYL ESTER	18.66	284	C18H36O2	
13	LINOLEIC ACID ETHYL ESTER	20.23	308	C20H36O2	
14	9,17-OCTADECADIENAL, (Z)-	20.23	264	C18H32O	
15	9,12-OCTADECADIENOIC ACID, ETHYL ESTER	20.23	308	C20H36O2	
16	1-OCTADECYNE	20.69	250	C18H34	
17	EICOSANOIC ACID, ETHYL ESTER	22.22	340	C22H44O2	

18	2-PROPEN-1-ONE, 1-(2,6-DIHYDROXY-4-METHOXYPHENYL)-3-PHENYL-, (E)-	22.42	270	C16H14O4	
19	1,2-BENZENEDICARBOXYLIC ACID, MONO(2-ETHYLHEXYL) ESTER	23.34	278	C16H22O4	
20	OCTADECANOIC ACID, ETHENYL ESTER	25.29	310	C20H38O2	
21	PHTHALIC ACID, 2-METHOXYETHYL TETRADECYL ESTER	25.83	420	C25H40O5	
22	BENZENEACETIC ACID, 3-METHOXY-4-[(TRIMETHYLSILYL)OXY]-, ETHYL ESTER	26.11	282	C14H22O4Si	
23	BENZENE, 2-[(TERT-BUTYLDIMETHYLSILYL)OXY]-1-ISOPROPYL-4-METHYL	26.24	264	C16H28OSi	
24	E-11(13-METHYL)TETRADECEN-1-OL	26.28	226	C15H30O	–
25	Z-8-PENTADECEN-1-OL ACETATE	26.28	268	C17H32O2	
26	3-OXA-4-(TRIFLUOROMETHYL)BORNANE	26.28	208	C10H15OF3	–

GC-MS analysis of methanolic extract crude extract

The GC-MS chromatogram ofmethanolic extract*A.echioides* leaves extractgave some major peaks are shown in fig 3 and the compound name retention time and molecular formula are given table 7.

Figure 3.GC-MS analysis of Methanolic extract

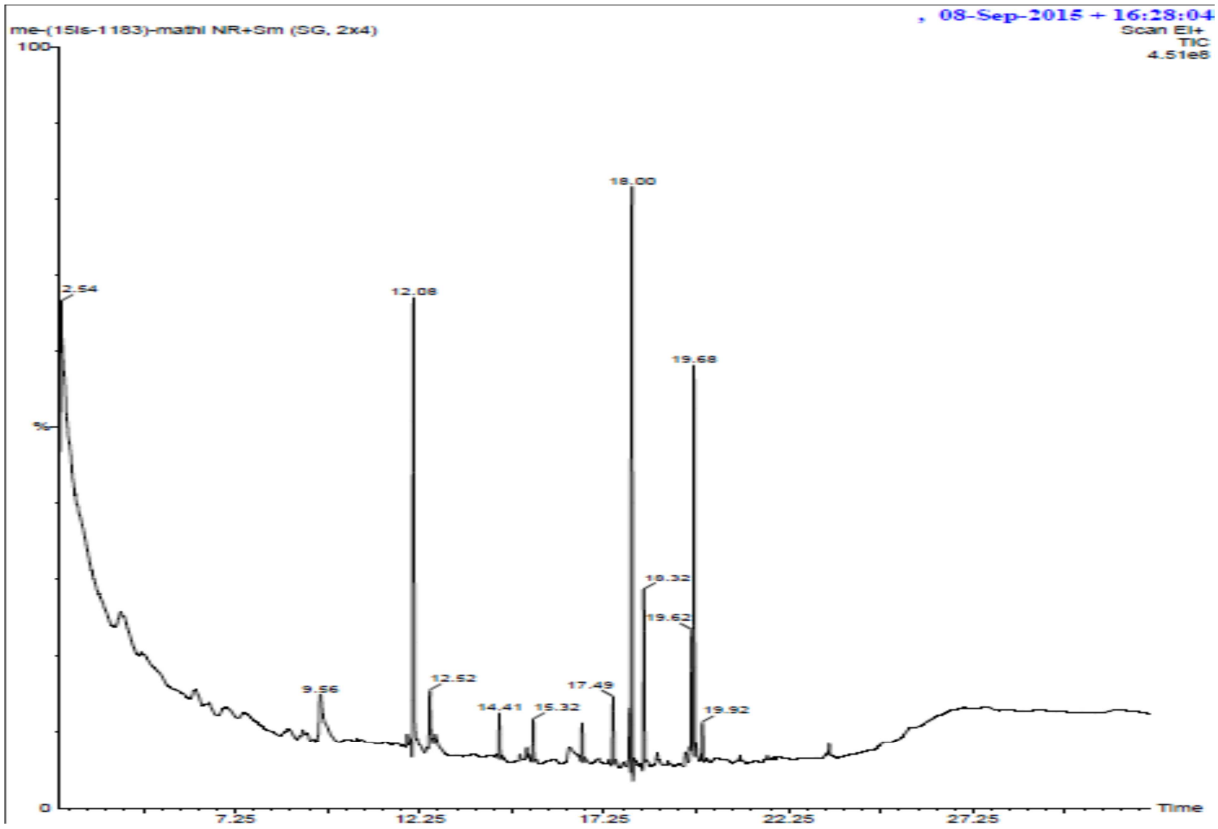
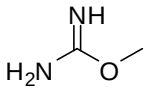
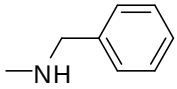
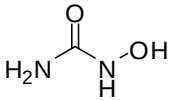
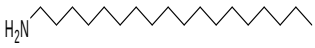
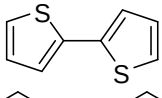
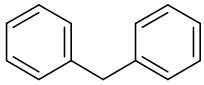
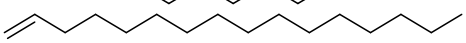
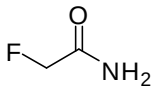
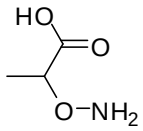
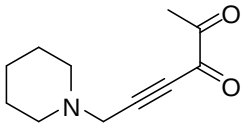
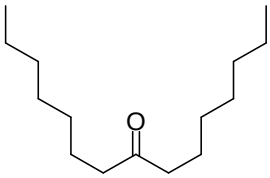
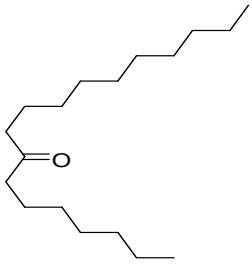
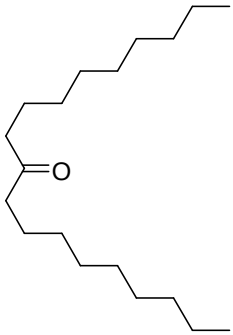
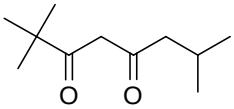
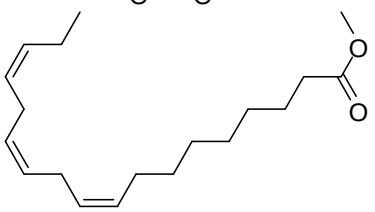
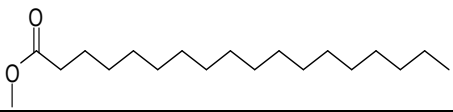


Table 7.GC-MS data for *Andrographis echiodies*using Methanolic extract

S.No	Compound name	RT (min)	MW	Molecular formula	structure
1	O-METHYLISOUREA	3.08	74	C2H6ON2	
2	SILANE, DIMETHYL-	3.08	60	C2H8Si	
3	BENZENEMETHANAMINE, N-METHYL-	9.56	121	C8H11N	
4	HYDROXYUREA	11.78	76	CH4O2N2	
5	1-OCTADECANAMINE	11.90	269	C18H39N	
6	2,2'-BITHIOPHENE	12.07	166	C8H6S2	
7	DIPHENYLMETHANE	12.52	168	C13H12	
8	1-HEXADECENE	14.40	224	C16H32	

9	ACETAMIDE, 2-FLUORO-	14.96	77	C2H4ONF	
10	PROPANOIC ACID, 2-(AMINOOXY)-	14.96	105	C3H7O3N	
11	2-BUTYNONE, 1-ACETYL-4-[1-PIPERIDYL]-	15.15	179	C11H17ON	
12	8-PENTADECANONE	15.32	226	C15H30O	
13	8-OCTADECANONE	15.32	268	C18H36O	
14	1,6;3,4-DIANHYDRO-2-DEOXY-.BETA.-D-LYXO-HEXOPYRANOSE	15.32	128	C6H8O3	
15	10-NONADECANONE	17.49	282	C19H38O	
16	3,5-OCTANEDIONE, 2,2,7-TRIMETHYL-	17.49	184	C11H20O2	
17	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, (Z,Z,Z)-	19.68	292	C19H32O2	
18	OCTADECANOIC ACID, METHYL ESTER	19.91	298	C19H38O2	

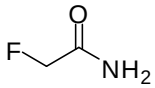
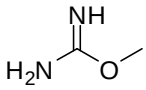
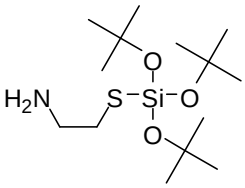
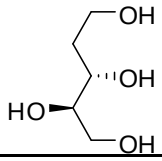
19	ACETAMIDE, 2-FLUORO-	20.92	77	C2H4ONF	
20	O-METHYLISOUREA	21.67	74	C2H6ON2	
21	S-[TRI-T-BUTOXYSILYL]-2-MERCAPTOETHYLAMINE	23.30	323	C14H33O3NSSi	
22	D-ERYTHRO-PENTOSE, 2-DEOXY-	23.30	134	C5H10O4	

Table.8 identified compounds and their reported uses

S.No	Compound	Uses
1	STIGMASTEROL	antipyretic, antineoplastic, inhibition of tumour growth and control of cholesterol[12]
2	2R-ACETOXYMETHYL-1,3,3-TRIMETHYL-4T-(3-METHYL-2-BUTEN-1-YL)-1T CYCLOHEXANOL	This compound responsible for various pharmocoligical actions like antibacterial, anti-inflamatory activites[13]
3	1,2-ETHANEDIOL, DIACETATE	Fragrances,cleaners and detergents[14]
4	PHENOL, 2,4-BIS(1,1 DIMETHYLETHYL)-	Antifungal activity, Antimicrobial, Antioxidant,and antimalarial activity[15]
5	DIPHENYLMETHANE	oxidizing agents[16]
6	E-15-HEPTADECENAL	Antimicrobial[17]
7	1-HEXADECENE	Antimicrobial and Antioxidant Activities[18]
8	HEXADECANOIC ACID, ETHYL ESTER	Antioxidant, Hypocholesterolemic Nematicide, Pesticide,Lubricant,Antiandrogenic,Flavor. [19]
9	LINOLEIC ACID ETHYL ESTER	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective Antiandrogenic, Antihistaminic, Anticoronary[20]
10	9,17-OCTADECADIENAL, (Z)-	antibacterial and antifungal [21]
11	9,12-OCTADECADIENOIC ACID, ETHYL ESTER	Antihistaminic, Anticoronary, Insectifuge, Antieczemic,Antiacne[20]
12	1-OCTADECYNE 857 877	Antimicrobial activity[22]
13	EICOSANOIC ACID, ETHYL ESTER	cosmetic and topical medicinal preparations[23]
14	1,2-BENZENEDICARBOXYLIC ACID, MONO(2-ETHYLHEXYL) ESTER	Plasticizer for PVC and other resins.[24]
15	OCTADECANOIC ACID, ETHENYL ESTER 413 722	antimicrobial activity[25]
16	O-METHYLISOUREA	enzymic activity.[26]
17	HYDROXYUREA	therapy for sickle cell disease.[27]
18	1-HEXADECENE	Antimicrobial.[28]
19	PROPANOIC ACID, 2-(AMINOOXY)-	antimicrobial, antiviral, antioxidant and antiinflammatory agents.[29]
20	D-ERYTHRO PENTOSE,2-DEOXY	Preservative[30]

CONCLUSION

The pharmacognostic investigations on physicochemical characteristics and flourescence analysishas a crucial role in standardization shows that*A.echioides* crude drugs prevents the adulteration and substitution and also it. The preliminary phytochemical screening of this leaves shows the presence of priliminary important secondary metabolites. In addition the GC-MS analysis shows the identfication of medicinally active compounds in the leaf extracts of *A.echioides* identified bioactive compounds indicates that*A.echioides* for various treatments in future. Hence the future research is focused on this plant to isolate the the identified bioactive compounds from the leaf extracts.

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