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Research Article

Phytochemical, Fluorescence Screening and GC-MS Analysis of Various Crude Extracts of *Anastatica hierochuntica*

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ABSTRACT
The objective of the study is to perform phytochemical screening, quantification of primary metabolites, and characterize the chemical composition of the different solvent extracts of *Anastatica hierochuntica* plant parts viz. stem, seed and leaf by GC-MS analysis. The dried powdered plant parts of *A. hierochuntica* were tested for the presence of phytochemicals and quantitatively evaluated for Carbohydrates, protein, lipids content and fluorescence characteristics as well as sequentially extracted with Hexane, Ethyl acetate, methanol and extracts were analyzed by gas chromatography-mass spectrometry to identify and characterize the chemical compounds present in the crude extracts. Phytochemical analysis of methanol and water extracts showed presence of major classes of phytochemicals. Carbohydrate and lipid content were abundant in seed and stem. Gas chromatography-mass spectrometry results revealed presence of different phytoconstituents in methanolic extract of stem, seed and leaf. All the three solvent extracts of the plant parts possess major bioactive compounds that are responsible for many pharmacological activities being reported.

KEYWORDS: *Anastatica hierochuntica*; Phytochemical; primary metabolites; Fluorescence; GC-MS analysis

INTRODUCTION

Anastatica hierochuntica is a monotypic species of Anastatica genus belonging to Brassicaeae family commonly called in Arabic as KaffMaryam (Mary's hand), Rose of Jericho, Genggam Fatimah and predominately grown in Middle East and North Africa [1]. Folklore consume as a herbal tea before childbirth to ease delivery and reduce uterine hemorrhage, treat asthma, respiratory diseases, dysentery, colds, fevers, headaches,conjunctivitis and combat sterility [2].

It has also been reported to exhibit antioxidant [2,3], antimicrobial[4], anti-melanogenesis [5], nitric oxide inhibitor[6], hepatoprotective [7], gastroprotective [8], anti-inflammatory [3] and immunostimulatory properties [9] .Two novel benzofuranoflavanones compounds Anastatin A and B [7] and three neolignans hierochins A, B and C [6] have been identified in the plant. Various phytochemical like naringenin,

eriodictyol, aromadendrin, taxifolin,3-O-methyl taxifolin, epitaxifolin, [7] lariciresinol, kaempferol, luteolin, rutin, ß-sitosterol, -3-O-ß-D-glucopyranoside, dehydrodi coniferyl alcohol, balanophonin and evofolinB,silychristin, silychristin, silybin A, silybin B, isosilybin A, isosilybin B, luteolin, kaempferol, quercetin, rutin ,dehydrodiconiferyl alcohol, p-hydroxybenzoic acid, p-methoxy benzoic acid, 3,4- dihydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid, p-hydroxybenzaldehyde, 3,4-dihydroxybenzal dehyde, vanillin, acetova nillone, 2,40-dihydroxy-30-methoxy aceto phenone, hydroxylpropioguaiacone, 2,3-dihy-droxy-1-(4-hydroxy-3-methoxyphenyl-1-pro panone), trans-cinnamic acid, trans-ferulic acid and coniferaldehyde[5] has been identified in the whole plant. Luteolin-6-C-hexosyl-8-C-pentoside, luteolin-6-C-pentosyl -8-C-hexoside, isovitexin-7-O glucoside, orientin, isoorientin, isovitexin, luteolin-O-glucoside, diosmetin-8-C-gluco-side, luteolin-O-glucuro-nide, isoorientin-2"-O-glucoside, luteolin-O-glucu-ronide, diosmetin-8-C-glucoside, dihydroxy-benzoic acid hexoside, 3, 4-dihydroxybenzoic acid, 5- O-caffeoylquinic acid, 3, 4-O-dicaffeoylquinic acid, 4, 5- O-dicaffeoylquinic acid, taxifolin- O-hexose and kaempferol-3-O-glucoside have been identified in the plant seed [2].

Even though many researchers have been postulated the primary metabolite and phytochemical constituent of the plant [2,10,11] , there is a lack of knowledge about these constituents specific to the plant parts. The present work is to evaluate the different parts of the plant viz. stem, seed and leaf to explore the prevailing bioactive compounds in *A.hierochuntica* organic extracts by Gas Chromatography- Mass Spectrophotometry (GC-MS) analysis and to determine its primary metabolites and fluorescence character of the powdered plant material.

MATERIALS AND METHODS

Plant collection and Preparation of solvent extracts:

Anastatica hierochuntica was collected in dried condition from Zubara in area of Al Magdah farms in northern Qatar and authenticated by local herbalist. The whole plant material was parted as stem, seeds and leaf and triturated to fine particles using a mechanical grinder. About 100 g of each powder plant material were continuously extracted with 300 ml of analysis for Hexane, Ethyl acetate, methanol and water at ambient temperature with intermittent shaking for 3 days [12]. Finally from each filtrate the solvent was removed using rotary evaporator under reduced pressure and low temperature. The yield of each extract was weighed and stored at 4°C until use.

Qualitative phytochemical analysis:

Phytochemical analysis was performed for Hexane, Ethyl acetate, methanol and water extracts of *A.hierochuntica* Stem, Seed and leaf to determine the presence of different phytochemicals covering Alkaloids, Carbohydrates, Aminoacids, Phenols, Coumarins, Tannins, Quinones, Glycosides, Flavanoids, Betacyanin, terpenoids, Triterpenoids, Cardic glycosides and Saponins as method described [13,14,15].

Quantitative determinations of primary metabolites

Estimation of total soluble carbohydrates, protein and Lipid were individually assessed in their dry and powdered form of *A.hierochuntica's* stem, seed and leaf. All the analysis was done in triplicates.

Determination of total soluble carbohydrates:

The total soluble carbohydrate content was calculated as mentioned [16] using Glucose as a standard. Concisely, 100 mg of sample was hydrolyzed with 5 ml of 2.5 N HCl in a boiling water bath for 3 hours and cooled to room temperature, solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was made up the volume to 1 ml with distilled water. Then 1.0ml of phenol reagent and 5.0 ml of sulphuric acid was added and incubated for20 min at room temperature. The absorbance was read at 490 nm against a reagent blank. The results are expressed as mean ± SD.

Determination of proteins:

Protein content was determined according to the method [17] by using Bovine Serum Albumin as standard. Initially to extract protein, 100mg of sample was mixed with 50 ml of 50% methanol

at 25°C for overnight and centrifuged at 7,000 rpm for 10 min. Then 0.2 ml of the supernatant was made up to 1ml with distilled water and mixed with 5 ml of alkaline copper reagent then incubated at room temperature for 30 minutes. Later 0.5 ml of Folin–Ciocalteau reagent was added and again incubated for 10 minutes at room temperature. Absorbance was read at 660 nm against a reagent blank. The results are expressed as mean±SD.

Determination of total lipid content:

Lipid content was determined according to the AACC Approved Method 30-25.01 with minor modifications [18]; 10gm sample was extracted with 150 ml of petroleum ether in Soxhlet for 16 hours, at a solvent condensation rate of 2–3 drops/sec. The obtained extract was concentrated and evaporated to dryness at room temperature. The weight of extract gives the total lipid content. The results are expressed as mean±SD.

Fluorescence Analysis:

The fluorescence characteristics of the powdered plant material were performed [19]. Briefly, a small quantity of dried and finely powdered of *A. hierochuntica's* stem, seed and leaf sample was placed on microscopic slide and 1-2 drops of petroleum ether, Ethyl Acetate, equal volume of Ethyl Acetate: Hydrochloric acid, Methanol, Chloroform, Acetone, Sulphuric Acid, Nitric Acid, Hydrochloric acid, Sodium Hydroxide were added separately and mixed by gently tilting the slide, after 1-2 minutes the slides were observed in day light and ultraviolet light at 365 nm wave length by placing the slide inside the UV viewer chamber.

Gas Chromatography/ Mass Spectrum Analysis:

For GC–MS study, each extract filtrate (Hexane, Ethyl acetate and methanol) of the plant material were prepared by mixing a small portion of the extract with sodium sulfate to remove the sediments and traces of water in the extract and then filtered through 0.22µ membrane filter, resultant filtrate was concentrated by nitrogen evaporator.

The GC–MS analysis of the bioactive compounds was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.999%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the sample were diluted with respective solvents was injected in an 1:5 split mode, Data was evaluated on GC retention time on HP-5MS column and matching of the spectra using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS/NIST library. Measurement of peak areas and data processing were carried out by Mass hunter Qualitative software. Relative quantity of the chemical compounds present in each of the extracts of *Anastatica hierochuntica* was expressed as percentage based on peak area produced in the chromatogram.

RESULTS

Preliminary Phytochemical analysis:

Preliminary phytochemical analysis of all the extract showed absence of Glycosides, Triterpenoids and Saponins. Hexane extracts of Stem showed the incidence of carbohydrates and leaf for alkaloids, while seed accounts for both. Similarly, presence of carbohydrates, alkaloids and coumarins was observed in Ethyl acetate fraction of Seed, whereas stem shows alkaloids and leaf fraction contains carbohydrates and quinones as presented in Table 1.

All major classes of secondary metabolites evaluated were present except Alkaloid and Amino acid in stem's methanol fraction. Presence of Coumarin, Quinones, Flavanoids, Betacyanin, Terpenoids, Cardiac glycosides were observed in methanol fraction Seed and Leaf, in addition tannins and Carbohydrates, Aminoacids were also present in seed and leaf respectively. In the same way aqueous extract contained all secondary metabolites analyzed except phenols while quinones and Cardiac glycosideswere absent in stem and leaf extract correspondingly.

Table 1: Preliminary Phytochemical Analysis of *A.hierochuntica* plant parts

Extract		Alkaloids	Carbohydrates	Amino acids	Phenols	Coumarins	Tannins	Quinones	Glycosides	Flavanoids	Beta cyanin	Terpenoids	Triterpenoids	Cardic glycosides	Saponin
stem	H	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	EA	+	-	-	-	-	+	+	-	-	+	+	-	-	-
	M	-	+	-	+	+	+	+	-	+	+	+	-	+	-
	W	+	+	+	-	+	+	-	-	+	+	+	-	+	-
seed	H	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	EA	+	+		-	+	-	-	-	-	-	+	-	+	-
	M	-	-	-	-	+	+	+	-	+	+	+	-		-
	W	+	+	+	-	+	+	+	-	+	+	+	-	+	-
leaf	H	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	EA	-	+	-	-	-		+	-	-	-	-	-	-	-
	M	-	+	+	-	+	-	+	-	+	+	-	-	-	-
	W	+	+	+	+	+	+	+	-	+	+	+	-	-	-
Test		Dragendorff reagent	Molisch reagents	Ninhydrin	Ferric chloride	Sodium hyroxide	Ferric chloride	Sulphuric acid	Ammonia solution	Lead acetate	Alkaline test	Salkowski test	Libermann-reagent	Killer killiani test	Frothing test

H-Hexane, EA- Ethyl acetate, M-Methanol, W-Water, +present,-absent.

Quantitative determinations of primary metabolites

Quantification of primary metabolites was studied for *A. hierochuntica* Stem, Seed and leaf has been charted in Table2. Higher amount of Carbohydrates were found in seed (174±2.36 mg/g DW) followed by Stem (162.57±1.08mg/g DW) and leaf (112.56±1.43mg/g DW). Protein content were reported to be higher in Stem (149.18±1.59mg/g DW) compared to Seed

(132.52±1.14mg/g DW) and leaf (97.04±1.77mg/g DW). Seeds (2.92±0.15mg/g DW) possessed greater amount of lipid content relative to stem (1.64±0.08mg/g DW) and leaf (0.79±0.21 mg/g DW)

Table 2: Quantitation of Primary metabolites in *A.hierochuntica* plant parts

Plant Parts	Carbohydrates mg/g dry weight	Protein mg/g dry weight	Lipids mg/g dry weight
Stem	162.57±1.08	149.18±1.59	1.64±0.08
Seed	174±2.36	132.52±1.14	2.92±0.15
Leaf	112.56±1.43	97.04±1.77	0.79±0.21

Values are mean±SD

Fluorescence Analysis

Fluorescence were exhibited by various chemical constituents present in the *A. hierochuntica* Stem, Seed and leaf upon reacting with acids and organic solvents and are tabulated in Table 3. Basic color including red, green, yellow and brown and its derivatives were observed.

Table 3: Fluorescence Analysis of *A.hierochuntica* plant parts

Solvents	Stem		Seed		Leaf	
	Visible	UV	Visible	UV	Visible	UV
Petroleum ether	colorless	yellow	colorless	yellow	colorless	yellow
Ethyl Acetate	colorless	yellow	colorless	yellow	Pale green	yellow
Ethyl Acetate: Hydrochloric Acid	greenish brown	green	brown	green	Pale green	green
Methanol	red	yellow	Red	yellow	green	yellow
Chloroform	reddish green	yellow	reddish green	yellow	olive green	yellow
Acetone	reddish green	yellow	reddish green	yellow	green	yellow
Sulphuric Acid	black	black	brown	green	green	yellow
Nitric Acid	Pale red	green	red	green	yellow	yellow
Hydrochloric acid	brown	green	pale red	olive green	green	yellow
Sodium Hydroxide	Pale red	green	pale red	dark green	green	yellow

Gas Chromatography/ Mass Spectrum Analysis:

The GC-MS profiles of Hexane, ethyl acetate and methanol extracts of Stem, Seed and Leaf were studied extensively. A total of 30 compounds were identified in Stem extracts (Table 4), hentriacontane (27.04%), 13-Docosenamide (15.143%) and 1-butanol 2-methylacetate(39.86%)were present in abundance in hexane, ethyl acetate and methanol extracts of Stem correspondingly. Dotriacontane peak percentage was 9.085%in hexane as well as 12.614% in Ethyl acetate extracts of stem. n-Hexadecanoic acid occurrence were higher in Ethyl acetate(9.397%)than methanol(1.993%) extracts of stem. Apart from this, merge quantity of 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione and Palmitic Acid, TMS derivative presence were common in hexane and Ethyl acetate extracts of stem. Other notable quantity of Hentriacontane (27.497%),Iberin nitrile (22.639%) and Dotriacontane (12.614%) was present in hexane, methanol and ethyl acetate extracts of Stem. 13-Docosenamide(Z) was found to be higher in ethyl acetate(15.143%) fraction compared to Hexane(7.197%) and methanol(2.616%).

Table 4: Chemical Constituents in *A.hierochuntica* Stem

Sl. No	Compounds In Stem	Retention Time	Peak Area%		
			Hexane	Ethyl acetate	Methanol
1	Oxalic acid, allylnonyl ester	6.997		0.065	
2	1-Butanol, 2-methyl-, acetate	7.8727			39.862
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.0766			2.965
4	5-Hydroxymethylfurfural	8.9414			3.706
5	1-Iodo-2-methylnonane	9.3106		0.083	
6	Iberin nitrile	10.4618			22.639
7	Undecane, 2-methyl-	11.3068	0.192		
8	Decane, 2,3,5,8-tetramethyl-	12.1515	0.354		
9	Iberin	12.5			7.604
10	1-Octanol, 2-butyl-	13.1472	0.203		
11	2-Pentadecanone, 6,10,14-trimethyl-	13.5948	0.588		
12	Phthalic acid, butyl oct-3-yl ester	14.979		0.253	
13	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	15.6235	0.793	0.366	
14	n-Hexadecanoic acid	16.4057		9.397	1.993
15	Palmitic Acid, TMS derivative	17.557	0.226	0.41	
16	Dodecyl acrylate	18.1427	2.674		
17	9-Octadecynoic acid	19.0389		1.501	
18	Tricosane, 2-methyl-	21.5773	2.762		
19	9-Octadecenamide, (Z)-	22.5203		10.281	
20	Octadecanamide	22.9224		0.945	
21	Hexatriacontane	25.219	2.291		
22	Hentriacontane	27.0483	27.497		
23	Octadecane, 6-methyl-	27.6489		1.157	
24	13-Docosenamide, (Z)-	28.7396	7.197	15.143	2.616
25	Campesterol	29.6047	9.298		
26	Cholesta-4,6-dien-3-ol, (3ß.)-	29.9294		0.584	
27	Heptacosane	30.4583		3.102	
28	7-Dehydrodiosgenin	32.9482		1.976	
29	Dotriacontane	33.8901	9.085	12.614	
30	Stigmasterol	36.9308			0.749

Nevertheless, Seeds extracts showed the presence of 50 compounds (Table 5).Heptacosane and 9-Octadecenamide, (Z) were found to be common in Hexane extracts with 22.045% and 4.439% besides ethyl acetate extracts with 1.451% and 22.811%.n-Hexadecanoic acid, 9,12-Octadecadienoic acid, methyl ester, (E,E)- and 13-Docosenamide were mutually present with relative percentage in Ethyl acetate (1.442%,1.807%,32.577%) and methanol (4.061%, 0.448%,1.07%)fraction. Remarkable quantity of Dotriacontane (21.936%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.585%), and 2',4'-Dimethoxyaceto phenone(10.281%) were found in hexane, ethyl acetate and methanol extracts of Seed correspondingly.

Table 5: Chemical Constituents in *A.hierochuntica* Seed

Sl. No	Compounds In Seed	Retention Time	Peak Area%		
			Hexane	Ethyl acetate	Methanol
1	Isobutyl acetate	3.7717	0.194		
2	Hydroperoxide, heptyl	4.1577	0.364		
3	Furfural	4.3418			0.338
4	Dimethyl Sulfoxide	4.6668			0.762
5	Cyclopropane, isothiocyanato-	4.9092			1.519
6	Dimethyl sulfone	5.4986			0.112
7	6-Oxa-bicyclo[3.1.0]hexan-3-one	5.6418			0.106
8	2-Furancarboxaldehyde, 5-methyl-	5.9558			0.545
9	Dimethyl trisulfide	6.0825			1.858
10	Octane, 2-methyl-	6.997		0.145	
11	Butanenitrile, 4-(methylthio)-	7.4046			1.129
12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.2198			8.585
13	Tetrasulfide, dimethyl	8.8974			0.359
14	5-Hydroxymethylfurfural	9.0792			3.719
15	Manganese, tricarbonyl[(1,2,3,4,5-.eta.)-1-methyl-2,4-cyclopentadien-1-yl]-	9.1674		0.058	
16	Decane, 2,9-dimethyl-	9.3106		0.106	
17	2-Methoxy-4-vinylphenol	9.7457			1.362
18	DL-Proline, 5-oxo-, methyl ester	10.572			1.04
19	benzaldehyde, 4-hydroxy-3,5-bis(1-methylethyl)-	10.6914	0.143		
20	Oxalic acid, allylnonyl ester	11.3068	0.093	0.153	
21	2',4'-Dimethoxyacetophenone	11.9492			10.281
22	Decane, 2,3,5,8-tetramethyl-	12.1514	0.172	0.128	
23	2-Allyl-3-thioxo-hexahydro-pyrrolo[1,2-c]imidazol-1-one	13.4641			0.333
24	7-Methyl-Z-tetradecen-1-ol acetate	14.72			0.688
25	Pentadecanoic acid	14.9569			0.136
26	1,2-Benzenedicarboxylic acid, butyl octyl ester	14.9735		0.299	
27	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	15.629		0.408	
28	Hexadecanoic acid, methyl ester	15.8328			0.524
29	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	15.943			0.289
30	n-Hexadecanoic acid	16.3947		1.442	4.061
31	Palmitic Acid, TMS derivative	17.557	0.246	0.126	
32	Sinapic acid methyl ester	18.2786			0.511
33	9,12-Octadecadienoic acid, methyl ester, (E,E)-	18.3447		1.807	0.448
34	11-Octadecenoic acid, methyl ester	18.532			0.989
35	9,12-Octadecadienoic acid (Z,Z)-	19.0278			1.732
36	cis-Vaccenic acid	19.1435			2.581
37	Octadecanoic acid	19.474			0.839
38	Hexadecanamide	19.6668		0.967	
39	9-Octadecenamide, (Z)-	22.3716	4.439	22.811	

40	Tricosane, 2-methyl-	22.5786	1.352		
41	Glycerol 1-palmitate	24.4648			0.206
42	1-Octadecanesulphonyl chloride	24.6907		0.245	
43	Dotriacontane	27.0202	21.936		
44	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	27.5497			1.285
45	13-Docosenamide, (Z)-	28.7616		32.577	1.07
46	Heptacosane	30.4583	22.045	10.451	
47	γ-Tocopherol	32.7443			0.138
48	Campesterol	36.2698			0.508
49	Stigmasterol	36.9254			0.466
50	γ-Sitosterol	38.6			1.662

Total of 49 compounds were identified in the Leaf extracts (Table 6).Hentriacontane was reported with the highest peak percentage in Hexane (63.844%) and Ethyl acetate(23.352%) while Iberin nitrile (22.444%) in methanol fraction of Leaf. n-Hexadecanoic acid and 9,12-Octadecadienoic acid, methyl ester, (E,E) were equally present in hexane (2.766% and 0.2%) and methanol (2.916% and 0.453%) fraction. Likewise 2-Pentadecanone, 6, 10, 14-trimethyl-, Heptacosane, Dotriacontanewas conjointly present in Hexane and ethyl acetate fraction.13-Docosenamide (Z) was found to be higher in ethyl acetate (14.308%) compared to Hexane (1.974%) and methanol (1.218%) fraction. Significant amount of Heptacosane (15.002%), 3',5'-Dimethoxyacetophenone (12.262%) and Dotriacontane (23.352%), 9-Octadecenamide (9.053%) were present in hexane, methanol and ethyl acetate extracts of Leaf accordingly.

Table 6: Chemical Constituents in *A.hierochuntica* Leaf

Sl. No	Compounds In Leaf	Retention Time	Peak Area%		
			Hexane	Ethyl acetate	Methanol
1	Pentane, 2-bromo-	3.9285	0.128		
2	2-Hexanol	4.169	0.106		
3	Cyclopropane, isothiocyanato-	4.8761			0.951
4	Dimethyl trisulfide	6.0604			0.441
5	S-Methyl methanethiosulfinate	6.1265			2.394
6	Hydroperoxide, 1-methylhexyl	6.1605	0.279		
7	Ethanone, 1-(2-methylcyclopropyl)-	6.3395	1.508		
8	Butanenitrile, 4-(methylthio)-	7.355			0.665
9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.0821			3.123
10	5-Hydroxymethylfurfural	8.9415			5.01
11	Dihydrocarvyl acetate	9.6399	0.029		
12	2-Methoxy-4-vinylphenol	9.7402			0.544
13	Iberin nitrile	10.5224			22.444
14	3,6-Octadecadiynoic acid, methyl ester	11.2497		0.132	
15	3',5'-Dimethoxyacetophenone	11.9161			12.262
16	Iberin	12.4945			4.251
17	2-Pentadecanone, 6,10,14-trimethyl-	13.6004	0.361	0.777	
18	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	13.8242	0.152		
19	9-Eicosyne	14.632		0.8	
20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.1994		0.637	
21	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	15.618		0.337	

22	Hexadecanoic acid, methyl ester	15.8217			0.62
23	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	15.9319			0.277
24	n-Hexadecanoic acid	16.3947		2.766	2.916
25	Palmitic Acid, TMS derivative	17.5571		0.386	
26	Sinapic acid methyl ester	18.229			0.496
27	9,12-Octadecadienoyl chloride, (Z,Z)-	18.4328			0.887
28	4,8,12-Trimethyltridecan-4-olide	18.635	0.538		
29	9,12-Octadecadienoic acid (Z,Z)-	18.9396			1.224
30	Oleic Acid	19.0498			2.166
31	Octadecanoic acid	19.4024			0.686
32	4,8,12,16-Tetramethylheptadecan-4-olide	22.311		0.592	
33	9,12-Octadecadienoic acid, methyl ester, (E,E)-	22.4102		0.72	0.453
34	9-Octadecenamide, (Z)-	22.5203		9.053	
35	α-Tocospiro A	23.3563	0.811		
36	Glycerol 1-palmitate	24.8449			0.775
37	Dotriacontane	25.2303	1.776	11.423	
38	Hentriacontane	27.1602	63.844	23.352	
39	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	27.5441			2.682
40	Heptacosane	27.6489	15.002	2.808	
41	13-Docosenamide, (Z)-	28.7341	1.974	14.308	1.218
42	α-Tocospiro B	29.6155		0.796	
43	Octacosane	30.4583		7.627	
44	Stigmastan-3,5-diene	31.1524		2.853	
45	Stigmastan-6,22-dien, 3,5-dedihydro-	32.2596		0.796	
46	γ-Tocopherol	32.7388			0.25
47	Campesterol	36.2588			0.872
48	Stigmasterol	36.9088			0.831
49	γ-Sitosterol	38.5614			2.502

DISCUSSION

Phytochemical analysis revealed the presence of most secondary metabolites in methanol and Aqueous extracts of the plant material. However it was obvious to note that there was rich incidence of carbohydrates and alkaloids in the studied extracts of the plant materials. Photosynthesis result in the formation of carbohydrates acting as a major of energy which are directly or indirectly involved in the modification of the physico-chemical characters of other compounds [20].

Occurrences of primary metabolites were consistent with phytochemical analysis, as carbohydrates predominant protein and lipids quantitatively. Carbohydrates and lipids content were predominately higher in seeds. This high lipid content would have facilitated *A.hierochuntica* to acclimatize drought conditions by altering its membrane lipid composition [21].

The powdered plant material reacted with different chemical reagents to produce color by converting them to fluorescent end product which helped us to determine the fluorescence characteristics of the plant. It is measured to be one of the key factors to evaluate the quality of the drug as it will benefit during drug formulation [22].

In our study, Oleamide and Erucamide were predominately present in Ethyl acetate fraction of the plant material compared to hexane and methanol. Specifically, Oleamide(9-Octadecenamide) act as a potent sleep inducer by interacting with neurotransmitters[23] and inhibits lymphocyte proliferation[24]. Erucamide (13-Docosenamide) are important neuro-signaling molecules as they modulate physiological functions in a receptor-mediated

manner and act as endogenous bio regulators to treat tumor growth, circulatory disease, inflammation, nociception, nervousness, and depression [25].

Glucosinate derivativs such as Iberin and Iberin nitrile were present in Stem and Seed of methanol fraction. Iberin, an isothiocyanate with sulforaphane group which induces apoptosis with multiple pathways of carcinogenesis (p21, caspase and cyclin-dependent kinases)[26]. Glucosinates are popularly found in Cruciferae family are produced by biosynthesis of amino acid, and are known to exhibit anticarcinogenic properties with different toxicological effects[27] and found to be a promising micronutrient for therapeutic application.

2-Methylbutyl acetate, a carboxylic ester was identified as a major component in the methanol extracts of Stem and it is considered to be one of the key volatile aromatic compounds that contribute to the characteristic aroma in all spice and fruits [28] and establish its application as a flavoring agent.

4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methylhas been classified as fragment of flavanoids which when synthesized by plants termed as phytoestrogens was present in methanol extract of the plant material and substantially higher in seeds. It has displayed numerous biological activities including radical scavenging [29], anti-proliferation and pro-apoptotic effects in Human Colon Cancer Cells [30] and induced apoptosis in hepato-carcinoma cells [31].

3', 5'-Dimethoxyacetophenone and 2', 4'-Dimethoxyacetophenone belonging to aromatic ketone are present methanol extracts of Seed and leaf of the plant. Plants encompassing 3', 5'-Dimethoxyacetophenone are found to exhibit larvicidal [32], antioxidant and cytotoxic property against human brain and cervical cancer cells[33]. Further expansions of research on these compounds are welcomed in future as acetophenone groups possess many pharmacological important activities like sedative and anticonvulsant.

Hentriacontane, Dotricontane and Heptacosane are acyclic hydrocarbon, the former was recognized to be present in large quantity among the studied phyto constituents in hexane and ethyl acetate fraction of leaf and considerably in hexane extracts of Stem, while Dotricontane was concentrated in hexane extracts of seeds compared to hexane and ethyl actate fraction of stem and leaf, whereas Heptacosane occurrence was high in hexane and ethyl acetate fraction of seed than leaf. Hentriacontane's biological activity includes anti-inflammatory[34], antioxidant[35] and anti-tumor[36]. Similarly Dotricontane and Heptacosane found to exhibit Antitubercular[37] , anti-edematous [38] and antidermatophytic [39], pesticidal [40] properties respectively.

Campesterol profoundly identified as phytosterol was predominately high in Hexane fraction of Stem compared to methanol fraction of seed and leaf and, its pharmacologically important properties are anti-inflammatory [41], immune-modulatory [42] and cytotoxicity effect against cancer cell lines[43] . Campesterol can be regarded as lipid lowering natural metabolites [44] because of its low absorption effects in small intestine which has facilitated many researchers to study its distinguishing nature.

The GC–MS analysis of hexane, ethyl acetate and methanol extracts of *A. hierochuntica's* Stem, Seed and leaf crude extracts publicized the presence of various bioactive compounds including Phenols, Glucosinate, terpenes, esters, thiols, fatty acid amines, fatty acid alcohol, sterols, hydrocarbons.

CONCLUSION

The major compounds including Oleamide, Erucamide, Iberin, Iberin nitrile, 2-Methylbutyl acetate, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 3',5'-Dimethoxyaceto phenone, 2',4'-Dimethoxyacetophenone, Hentri-acontane , Dotricontane, Heptacosane and Campesterol were identified and characterized in *A. hierochuntica* by GC-MS which partakes its use in therapeutic application. Further comprehensive *in vitro* and *in vivo* correlation studies along with isolation of active constituents are required to unravel novel treatment strategies of the plant.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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