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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(5): 255-261

www.thepharmajournal.com
Received: 15-03-2019
Accepted: 18-04-2019

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In-vivo and *In-vitro* phytochemical GC-MS analysis of volatile constituents of *Andrographis paniculata* (Burm.f.) Nees

Bunty Kumar Dulara, Priyanka Godara and Neelam Barwer

Abstract
Aim: The present study aims at identifying and comparative analysis of various active phytochemicals from plant parts (leaf and stem) and *in-vitro* callus sample of *Andrographis paniculata* using Methanolic and Petroleum Ether Extracts through GC-MS technique.
Methodology: *Andrographis paniculata* leaf, stem and callus (obtained from leaf explant) samples were dried at room temperature, powdered and then sequentially extracted in Methanol and Petroleum Ether as solvents using soxhlet apparatus. Total five extracts were prepared viz: Methanolic Leaf Extract (MLE), Methanolic Stem Extract (MSE), Methanolic Callus Extract (MCE), Petroleum Ether Leaf Extract (PELE) and Petroleum Ether Stem Extract (PESE). Further, these five extracts obtained were analyzed for the presence of various volatile phytochemical compounds using GC-MS technique.
Results: The GC–MS analysis of the five extracts revealed the presence of 34 different high and low molecular weight phytochemicals with varying quantities. Some of the phytochemicals detected in *A. paniculata* are 1-(+)-Ascorbic acid 2,6-dihexadeconoate, Eicosane, à-D-Glucose, 3-Nitrobenzyl iodide,2-Pentadecanone 6,10,14-trimethyl, 6-Tetradecanesulfonic acid, butyl ester, Phytol,1-Hexyl-2-nitrocyclohexane, N-'Methy-l-N-'phenyl-N-tosylformohydrazide etc. These phytochemicals are considered biologically active and pharmacologically important.
Conclusion: The results obtained from the comparative GC-MS analysis of different samples of *A. paniculata* shows the presence of various bioactive compounds with some newly identified compounds. Therefore, *A. paniculata* is recommended as a phytopharamaceutically important plant.

Keywords: *Andrographis paniculata*, phytochemicals, secondary metabolites, methanol, petroleum ether, GC-MS, callus

Introduction
Mother Nature has gifted us with variety of plants that are one among the most precious gifts which had been used as lifesaving medicines since ancient times when no other form of medicine was available. Since ancient times, a plant as a whole or their different parts has been used in one or the other form to treat several ailments [1]. The plant derived medicines work on the basic principle of maintaining the homeostasis of entire body along with disease to be cured. Thus the active constituents of the herbal medicine work in cohesion and not as separate entities like the modern day allopathic drugs [2]. Also, plant based medicines show minimal side effects as compared to the modern medicines [3]. In 2013, WHO launched "WHO Traditional Medicine Strategy 2014-2023". This WHO strategy aims at collaborative working of traditional herbal medicine and modern complementary medicine in order to ensure better health solutions which are cheaper with minimal or no side effects [4].
Owing to all these factors related to modern day medicine, the entire focus has now started shifting towards the use of herbal medicines. Among approximately 4,00,000 plants species, approximately 6-7% plants are studied for their biological activity and very few have been clinically and phytochemically investigated. Thus, recent medical research has started exploring the complete chemical characterization of plethora of medicinal plants available and analyzing the various classes of phytochemicals such as alkaloids, steroids, flavonoids, phenols, tannins and terpenoids etc. Every year approximately one lakh secondary metabolites are derived from around 50,000 plant species and ~4,000 new secondary metabolites are being isolated from a broad range of plant species [5]. These phytochemicals have been used in human healthcare (as antioxidants, drugs), used in several industries (as dyes, flavors, fragrances), and used for agricultural purposes (as insecticides and pheromones) etc. [6].

*Andrographis paniculata (*Burm.F) Nees (*Acanthaceae*), commonly known as green chireta, creat and Maha-tikta (king of bitters) has been tradionally used in Ayurveda as Kalmegha/Kalamegha, meaning "dark cloud" in the treatment of snake bite, bug bite, diabetes, dysentery, fever and malaria [7]. *A. paniculata* is commonly used as traditional medicine in India, Pakistan, Bangladesh, China, Malaysia, Hong Kong, Indonesia and Thailand [8, 9]. It is native to Taiwan, Mainland China and India. *A. paniculata* is also commonly found in the tropical and subtropical Asia, Southeast Asia, and some other countries including Cambodia, Caribbean islands, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand, and Vietnam [10-12], in different phytogeographical and edaphic zones of China, America, West Indies and Christmas Island [12]. In India, it is widely spread and is a common herb usually in plains and hilly areas.

A. paniculata has been reported to have a wide range of biological/pharmacological effects like antimicrobial, antimalarial [13, 14], anticancer [15, 16], anti-HIV [17], antidairrheal [18], antihyperglycemic [19], antihepatitis [20], antioxidant [21], anti-inflammatory [22], cytotoxic [23], cardiovascular [24], immunostimulatory [25] and sexual dysfunction [26]. Few other biological activities such as antiplatlet aggregation activity [27] and other myriad health benefits have also been reported [28, 29, 30]. The therapeutic potential of *A.paniculata* is mainly attribute due to the presence of diterpenoids along with other flavonoids, quinic acids and xanthones.

The current literature available focuses on either aerial part or in the whole plant of *A. paniculata*, there are no studies found based on complete and comparative phytochemical analysis of different plant parts (leaf, stem, and root) separately using methanol and petroleum ether as base solvents. Also, there is no data available on the comprehensive phytochemical analysis on callus culture (*in vitro*) samples obtained from this plant.

Thus, *A. paniculata* is a widely distributed therapeutic herb and requires a thorough and detailed comparative analysis of phytochemicals bioactive constituents and their bioevelaution, validation and efficacy to be used as a potent herbal drug. Therefore, the present study focuses on evaluation and analysis of plant parts (leaf and stem) and callus extracts of *A. paniculata* for the presence of phytoconstituents, further identification and characterization of bioactive compounds in the crude extracts prepared in methanol and petroleum ether for chemical profiling by Gas Chromatography-Mass Spectrometric (GC–MS) technique. The results obtained from GC–MS analysis have led to identification of number of bioactive compounds in *A. paniculata* extracts. They were identified through mass spectrometry attached with GC. The GC-MS analysis revealed the comparative pattern of the accumulated bio-active phytochemicals along with some new compounds which were identified for the first time and not reported earlier.

Materials and Methods

Instrument and Chemicals

Soxhlet apparatus (3840, Borosil Glass works Ltd., Mumbai, India), GC–MS System (Thermo scientific GC 1300 and TSQ 8000 Triple quadrupole), Murashige and Skoog (MS) Medium PT-100, 2, 4-Dichlorophenoxyacetic acid (2, 4-D) (DuchefaBiochemie), 1-Napthaleneacetic acid (NAA) (Himedia), Methanol (Rankem) and Petroleum Ether (-RANKEM). All other reagents and chemicals used were of analytical and biological grade.

Collection of Plant Sample

Sample collection for *A. paniculata* was carried out (Bunty Kumar Dulara) from the surrounding outer areas of Jaipur city, Rajasthan, India. The collected plant sample was assigned voucher specimen number RUBL611302 and deposited in the herbarium of Department of Botany, University of Rajasthan, Jaipur for confirmation of identity. The collected samples (leaf and stem) were thoroughly washed under running tap water for complete dust removal and then dried in shade at room temperature for 10-12 days until a constant dry weight of the sample was obtained. The dried leaf and stem samples were grounded to powder and stored in air-tight containers for further use.

Callus Preparation

The optimum amount of callus induction in *A. paniculata* was observed using leaf explants. The desired amount of callus sample for experiment was obtained from repeated sub-culturing of primary callus using MS media supplemented with 2,4-D (1.0mg/l).

Preparation of Extracts

The fine powdered samples of *A.paniculata* (leaf, stem and callus) were extracted in solvents methanol and petroleum ether using Soxhlet apparatus. 5gof each powdered sample (leaf, stem and callus) were packed in thimbles and extracted in methanol and petroleum ether (250ml each) separately as per standard laboratory protocols. The Soxhlet extracted plant samples were evaporated at room temperature for 72 hours by using aluminum foil to obtain five dried solid extracts. The extracts obtained were labeled as Methanolic Stem Extract (MSE), Methanolic Leaf Extract (MLE), Methanolic Callus Extract (MCE), Petroleum Ether Stem Extract (PESE) and Petroleum Ether Leaf Extract (PELE).All extracts were properly labeled and stored at 4 °C in vacuum tight container for further use.

Preliminary phytochemical analysis

The preliminary secondary metabolites analysis of *A. paniculata* Methanolic Stem Extract (MSE), Methanolic Leaf Extract (MLE), Methanolic Callus Extract (MCE), Petroleum Ether Stem Extract (PESE) and Petroleum Ether Leaf Extract (PELE) was done for detecting the presence of saponins, flavonoids, alkaloids, triterpenoids, steroids and phenolic compounds using standard biochemical estimation protocols [6].

GC-MS Analysis

The GC–MS analysis of *A.paniculata* extracts was carried out on a Thermo GC 1300 and TSQ 8000 Triple Quadrupole GC–MS system with auto sampler Al 1310 at USIC Department of University of Rajasthan, Jaipur. The program settings were capillary column TG-5MS AMINE (30 m × 0.25 mm; film thickness 0.25 µm) with initial temperature set to 70 °C for 1 min, then gradually increases at 4 °C/min upto 270 °C (holding time-1 min). The injector temperature was set at 280 °C with carrier gas as helium at a flow rate of 1.0 ml/min. GC–MS analysis was carried out using TSQ8000 with transfer line temperature 280 °C and ion source temperature 230 °C in EI mode. The MS scan parameters obtained included electron impact ionization voltage of 70 EV and a mass range of 50–500 m/z. Analysis was done using TSQ 8000 Triple Quadrupole MS detector and data evaluation using total ion count (TIC) for compound identification and quantification.

Identification of Bioactive Phytochemicals

The mass spectra of compounds obtained after GC-MS analysis different extracts of *A. paniculata* were identified by comparing it with the mass spectral data of known components available in the National Institute of Standards and Technology (NIST) library. Compound concentrations were calculated from the GC peak areas of the total ion current (TIC).

Results

Preliminary phytochemical analysis

The preliminary secondary metabolites analysis was done using standard biochemical protocols [6]. The table below shows the presence of various secondary metabolites in whole plant as compared to the stem and leaf Methanolic and Petroleum Ether extract.

Table 1: Preliminary phytochemical analysis in *A. paniculata* (Burm.F.) Nees

Secondary Metabolites	Methanol Extract			Petroleum Ether	
	Callus	Leaf	Stem	Leaf	Stem
Saponins	+	+	+	+	+
Flavanoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Phenolic compounds	-	+	+	+	+
Glycosides	+	+	+	+	+
Tannins	+	+	+	+	+

Present : (+) ; Absent : (-)

GC-MS Chromatogram analysis

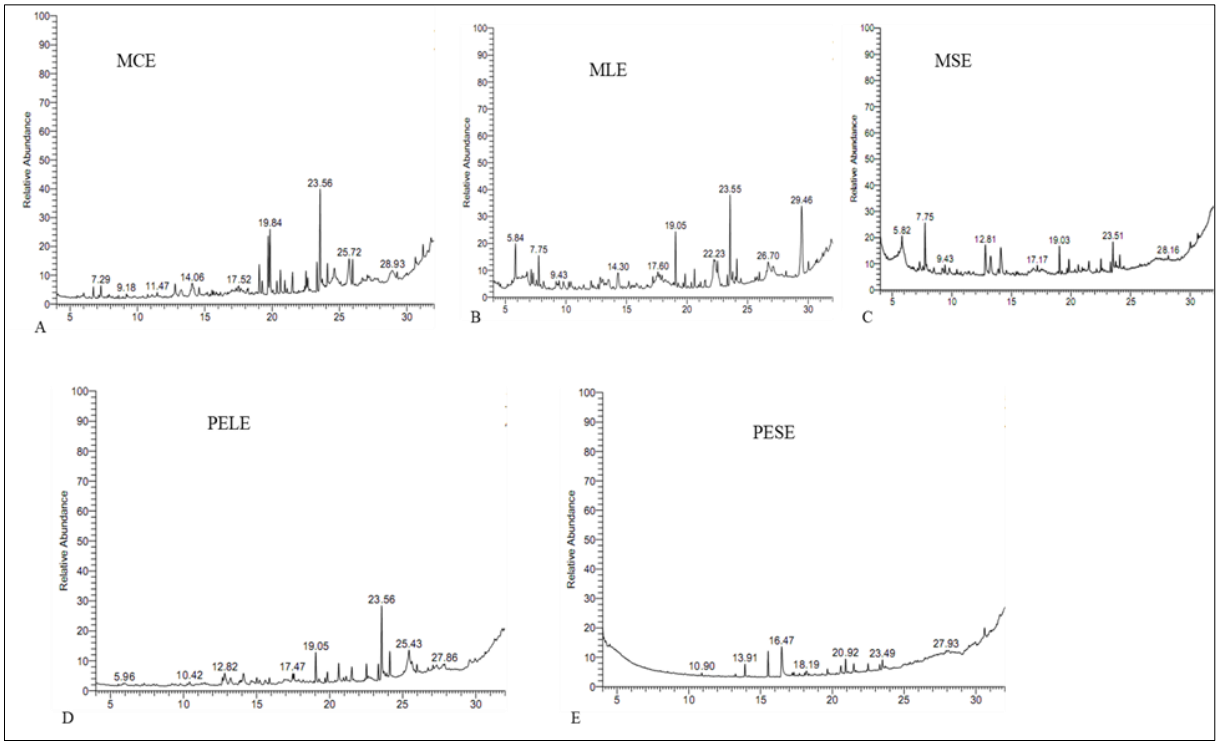

Fig 1: GC-MS chromatograms of all five extracts of *Andrographis paniculata* showing relative abundance and retention time of Phytochemicals. A: Methanolic Callus Extract (MCE); B: Methanolic Leaf Extract (MLE); C: Methanolic Stem Extract (MSE); D: Petroleum Ether Leaf Extract (PELE); E: Petroleum Ether Stem Extract (PESE)

Table 2: List of compounds identified from various classes of bioactive phytochemicals by GC-MS analysis of Methanolic and Petroleum Ether extracts of leaf, stem and callus of leaf explants of *Andrographis paniculata* (*Denotes the extract with highest composition of that compound)

S. No.	Compound Name	Molecular Formula	Retention Time (RT*)	Methanolic Extracts (% composition)			Petroleum Ether Extracts (% composition)		Biological activity
				MCE	MLE	MSE	PELE	PESE	
1	2-Propenamide,N-[2-(dimethylamino)ethyl]	C7H14N2O	6.78	-	2.37 #	0.61	-	-	Antimicrobial and Antifungal [46]
2	1-Benzylamino-2-benzyloxyethane	C16H19NO	7.29	1.79	1.62	1.90 #	-	-	Antimicrobial and Antifungal [31]
3	3-Selenetanol,3-(4-methoxyphenyl	C10H12O2Se	9.18	0.40	-	0.84 #	-	-	Not Reported
4	1,3-Dioxolane,2-(3-methoxypropyl)-2-methyl	C8H16O3	9.43	-	1.04	1.84 #	-	-	Antimicrobial and Antifungal [48]
5	7-Tridecanol	C13H28O	9.79	-	1.48	1.62 #	-	-	Antimicrobial and Antifungal [32]
6	Benzaldehyde, 3-(chloroacetoxy)-4-methox	C10H9ClO4	11.47	0.39 #	-	0.23	-	-	Antimicrobial and Antifungal [49]
7	N-Vinylpyridinium bromide	C7H8BrN	12.08	-	1.14 #	0.32	-	-	Antimicrobial and Antifungal [55]
8	2-Iodo-cinnamic acid	C9H7IO2	12.65		0.51	-	1.82	-	Not Reported
9	3-Nitrobenzyl iodide	C7H6INO2	12.80	2.35	2.37	9.60 #		-	Antimicrobial and Antifungal [32]
10	Sucrose	C12H22O11	13.25	1.65	1.87	7.14 #	2.12	-	Anti-arthritic [33]
11	á-D-Glucopyranose,1,6-anhydro (Cellulose)	C6H10O5	14.06	2.83	3.73 #	-	3.31	-	Antimicrobial and Antifungal [56, 57]
12	à-D-Glucose	C6H12O6	15.01			11.0 #	1.37	-	Antimicrobial and Antifungal [34]
13	Oxalic acid, allylnonyl ester	C14H24O4	15.54	0.60	1.86 #	-	-	-	Used in soaps/disinfectant [35]
14	3-Heptadecena	C17H32O	17.71	0.48	0.72 #	-	-	-	Antimicrobial and Antifungal [50]
15	Cyclohexane,1,1'[1,2bis(1,1-dimethylethyl)-1-,2-ethanediyl]bis, R*,R*)-(ñ)	C22H42	17.93	-	0.75	0.32	-	1.40 #	Not Reported

16	1(2H)-Naphthalenone, 6-(1,1-dimethylethyl) octahydro-2,8a-dimethyl	C16H28O	19.25	1.79 #	-	0.37	-	-	Precursor of vitamin E and vitamin K1 [35]
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	C20H40O	20.61	2.82	1.91	1.42	4.29	4.22 #	Antimicrobial and Antifungal [43]
18	2-Pentadecanone 6,10,14-trimethyl	C18H36O	20.94	1.40	-	0.61	0.60	6.89 #	Antimicrobial and Antifungal [36]
19	Phthalic acid, hex-3-yl isobutyl ester	C18H26O4	21.51	2.96	0.77	2.40	3.92	4.66 #	Antimicrobial and Antifungal [36]
20	Di-sec-butyl Phthalate	C16H22O4	22.42	0.51	1.11	1.99	3.23 #		Anti-oxidative/anti-inflammatory [44]
21	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	C17H34O2	22.51	2.42	-	2.71	3.59	3.71 #	Anti-oxidative/anti-inflammatory [37]
22	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C38H68O8	23.56	13.82	10.11	-	16.70 #		Anti-oxidative/anti-inflammatory [53]
23	Phthalic acid, hept-4-yl isobutyl ester	C19H28O4	23.71	1.57	-	-	0.34	4.66 #	Antimicrobial and Antifungal [51]
24	N-'Methy-l-N-'phenyl-N-tosylformohydrazide	C15H16N2O3S	24.11	-	2.57	2.80 #	-	-	Anti-tumor activity [38]
25	Eicosane	C20H42	24.62	2.43	14.49 #	-	-	-	Anti-oxidative/anti-inflammatory [54]
26	Phytol	C20H40O	25.97	2.96 #			2.04		Antimicrobial and Antifungal, anti-oxidative/anti-inflammatory [45]
27	1-Hexyl-2-nitrocyclohexane	C12H23NO2	26.70	0.98	-	-	-	2.32 #	Antimicrobial and Antifungal [39]
28	2-Nonadecanol	C19H40O	27.23	0.68 #	0.58	-	0.55	-	Disinfectant [40]
29	6-Tetradecanesulfonic acid, butyl ester	C18H38O3S	28.93	2.38	4.03 #	-	0.79	-	Antimicrobial and Antifungal [41]
30	2-Hexyl-1-octanol/2-Ethyl-1-dodecanol	C14H30O	29.27	0.81	0.45	0.82	1.89	2.34 #	Anti-Helminthic [52]
31	Sulfurous acid, cyclohexylmethyltetradecyl ester	C21H42O3S	30.02	-	1.29	1.50 #	-		Not Reported
32	9-Hexacosene	C26H52	30.73	0.49	-	0.74 #	-	0.55	Antimicrobial and Antifungal [32]
33	Sulfurous acid, cyclohexylmethyl isobutyl ester	C11H22O3S	31.79	2.30 #	0.86	-	-	1.15	Not Reported
34	Sulfurous acid, butyl nonyl ester	C13H28O35	31.90	-	1.14 #	-	-	0.73	Antimicrobial and Antifungal [46]

Table 3: List of compounds identified from various classes of bioactive phytochemicals by GC-MS analysis along with their reported Biological activities

S. No.	Compound Name	Molecular Formula	Retention Time (RT)	Biological Activity
1	2-Propenamide,N-[2-(dimethylamino)ethyl]	C7H14N2O	6.78	Antimicrobial and Antifungal [46]
2	1-Benzylamino-2-benzyloxyethane	C16H19NO	7.29	Antimicrobial and Antifungal [31]
3	3-Selenetanol,3-(4-methoxyphenyl	C10H12O2Se	9.18	Not Reported
4	1,3-Dioxolane,2-(3-methoxypropyl)-2-methyl	C8H16O3	9.43	Not Reported
5	7-Tridecanol	C13H28O	9.79	Antimicrobial and Antifungal [48]
6	Benzaldehyde, 3-(chloroacetoxy)-4-methox	C10H9ClO4	11.47	Antimicrobial and Antifungal [32]
7	N-Vinylpyridinium bromide	C7H8BrN	12.08	Antimicrobial and Antifungal [49]
8	2-Iodo-cinnamic acid	C9H7IO2	12.65	Antimicrobial and Antifungal [55]
9	3-Nitrobenzyl iodide	C7H6INO2	12.80	Not Reported
10	Sucrose	C12H22O11	13.25	Antimicrobial and Antifungal [32]
11	á-D-Glucopyranose,1,6-anhydro (Cellulose)	C6H10O5	14.06	Anti-arthritic [33]
12	à-D-Glucose	C6H12O6	15.01	Antimicrobial and Antifungal [56, 57]
13	Oxalic acid, allylnonyl ester	C14H24O4	15.54	Antimicrobial and Antifungal [34]
14	3-Heptadecena	C17H32O	17.71	Used in soaps/disinfectant [35]
15	Cyclohexane,1,1'[1,2bis(1,1-dimethylethyl)-1-,2-ethanediyl]bis, R*,R*)-(ñ)	C22H42	17.93	Antimicrobial and Antifungal [50]
16	1(2H)-Naphthalenone, 6-(1,1-dimethylethyl) octahydro-2,8a-dimethyl	C16H28O	19.25	Not Reported
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	C20H40O	20.61	Precursor of vitamin E and vitamin K1 [35]
18	2-Pentadecanone 6,10,14-trimethyl	C18H36O	20.94	Antimicrobial and Antifungal [43]
19	Phthalic acid, hex-3-yl isobutyl ester	C18H26O4	21.51	Antimicrobial and Antifungal [36]
20	Di-sec-butyl Phthalate	C16H22O4	22.42	Antimicrobial and Antifungal [36]
21	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	C17H34O2	22.51	Anti-oxidative/anti-inflammatory [44]
22	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C38H68O8	23.56	Anti-oxidative/anti-inflammatory [37]
23	Phthalic acid, hept-4-yl isobutyl ester	C19H28O4	23.71	Anti-oxidative/anti-inflammatory [53]
24	N-'Methy-l-N-'phenyl-N-tosylformohydrazide	C15H16N2O3S	24.11	Antimicrobial and Antifungal [51]
25	Eicosane	C20H42	24.62	Anti-tumor activity [38]
26	Phytol	C20H40O	25.97	Anti-oxidative/anti-inflammatory [54]
27	1-Hexyl-2-nitrocyclohexane	C12H23NO2	26.70	Antimicrobial and Antifungal, anti-oxidative/anti-inflammatory [45]
28	2-Nonadecanol	C19H40O	27.23	Antimicrobial and Antifungal [39]
29	6-Tetradecanesulfonic acid, butyl ester	C18H38O3S	28.93	Disinfectant [40]
30	2-Hexyl-1-octanol/2-Ethyl-1-dodecanol	C14H30O	29.27	Antimicrobial and Antifungal [41]
31	Sulfurous acid, cyclohexylmethyltetradecyl ester	C21H42O3S	30.02	Anti-Helminthic [52]
32	9-Hexacosene	C26H52	30.73	Not Reported
33	Sulfurous acid, cyclohexylmethyl isobutyl ester	C11H22O3S	31.79	Antimicrobial and Antifungal [32]
34	Sulfurous acid, butyl nonyl ester	C13H28O35	31.90	Not Reported

Results

Preliminary phytochemical analysis for the presence of secondary metabolites (Saponins, flavanoids, alkaloids, triterpenoids, steroids, phenolics, glycosides and tannins) was done using whole plant and methanolic and petroleum ether extract of stem and leaf (Table 1). The preliminary data shows the presence of these secondary metabolites in callus as well as in different plant parts (leaf and stem).The GC-MS chromatogram spectra obtained for all five extracts revealed that *A. paniculata* is plenteously rich in bioactive compounds in leaf, stem and callus (Figure 1). A total of 34 effective compounds were identified from the chromatogram. The bioactive compounds were predicted by their retention time (RT), peak area percentage (%) and molecular weight with the help of NIST Library (Table 2). Of all the five extracts, callus extracts showed the presence of most of the bioactive compounds (Table 2) as compared to the MSE, MLE, MCE, PESE and PELE. The retention rate too was high in compounds of callus extract.

On the basis of the highest % composition of the identified compounds were the five extracts were compared(as shown in Table 2) and it was found that PELE contained higher

amounts of 1-(+)-Ascorbic acid 2,6-dihexadeconoate (16.70%), Di-sec-butyl Phthalate (3.23%) and Tetradecanoic acid, 10,13 dimethyl, methyl ester (3.59%). PESE contained higher amounts of 2-Pentadecanone 6,10,14-trimethyl (6.89%), Phthalic acid, hex-3-yl isobutyl ester (4.66%), Phthalic acid, hept-4-yl isobutyl ester (4.66%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.22%), 2-Hexyl-1-octanol/2-Ethyl-1-dodecanol (2.34%), 1-Hexyl-2-nitrocyclohexane (2.32%) and Tetradecanoic acid, 10,13-dimethyl-, methyl ester (3.71%). MSE contained highest amounts of Eicosane (14.49%), à-D-Glucose (11.08%), Sucrose (7.14%), 3-Nitrobenzyl iodide (9.60%) and N-'Methy-l-N-'phenyl-N-tosylformohydrazide (2.80%). MLE contained high amounts of 6-Tetradecanesulfonic acid, butyl ester (4.03%), á-D-Glucopyranose, 1,6-anhydro/Cellulose (3.73%) and 2-Propenamide,N-[2-(dimethylamino) ethyl (2.37%). MCE contained highest amount of Phytol (2.96%).Among the 35 compounds, the compounds with high % composition have been reported to show significant biological activityas listed in Table 3.

Discussion

The herb *Andrographis panniculata* is one of the major ingredients in many commercial herbal formulations including Sbl *Andrographis paniculata* Dilution 1000ch, Planatary Herbals, Full Spectrum *Andrographis*, Garlico Herbal Kalmegha Powder, Antisept and Chyawanprash. Presence of flavonoids, phytosterols, terpenes and terpenoids, fatty acids, tannins, carbohydrates, glycosides, saponins, free catechols, starches, and phenolic compounds was identified by various researchers in different solvent extracts of *A.paniculata* [58]. This plant has multiple therapeutic activities due to presence of various bioactive phytochemicals like 1-(+)-Ascorbic acid 2,6-dihexadeconoate, Eicosane, à-D-Glucose, 3-Nitrobenzyl iodide, 2-Pentadecanone 6,10,14-trimethyl, 6-Tetradecanesulfonic acid, butyl ester, Phytol,1-Hexyl-2-nitrocyclohexane and N-'Methy-l-N-'phenyl-N-tosylformohydrazide etc.

Among the 34 compounds, the compounds with high % composition have been reported to show significant biological activity such as (+)-Ascorbic acid 2, 6-dihexadeconoate was present in shows anti-oxidant activity [37]. Eicosane shows anti-tumorous activity [38], à-D-Glucose shows antimicrobial and antifungal activity [56, 57], 2-Pentadecanone 6,10,14-trimethyl shows anti-microbial activity [43], Phthalic acid, hex-3-yl isobutyl ester shows anti-microbial activity [41], Phthalic acid, hept-4-yl isobutyl ester shows antimicrobial and antifungal activity [36], 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is a precursor of vitamin E and K1 [35], 6-Tetradecanesulfonic acid, butyl ester have disinfectant activity [40], á-D-Glucopyranose,1,6-anhydro/Cellulose shows anti-arthritic [33], Di-sec-butyl Phthalate shows anti-fungal activity [36], Phytol have antimutagenic activity and wound healing property [54], 2-Hexyl-1-octanol/2-Ethyl-1-dodecanol shows antimicrobial and anti-fungal [41], 1-Hexyl-2-nitrocyclohexane shows anti-inflammatory activity [45], 2-Propenamide,N-[2-(dimethylamino)ethyl shows anti-microbial and anti-fungal activity [46] and N-'Methy-l-N-'phenyl-N-tosylformohydrazide also shows anti-microbial and anti-fungal activity [51].

Present study gives a comparative account of various phytochemicals identified by GC-MS analysis in stem, leaves and callus. The comparative GC-MS analysis of plant parts and callus identified various classes of bioactive compounds that include saponins, flavanoids, alkaloids, triterpenoids, steroids, phenolics, glycosides and tannins (Table 1 and Table 2). The details of biological activity of the identified phytochemical compounds have been summarized in Table 3.

Conclusion

A. paniculata has overwhelming therapeutic potentials which has increased in the past few years. The data obtained from the preliminary phytochemical analysis and the GC-MS analysis has clearly revealed the presence of large spectrum of pharmacological properties of *A. paniculata*. Presence of these extensive pharmacologically important bio-activities, *A. paniculata* can be safely regarded as one of the modern catholicons. However, the compounds identified through the GC-MS analysis needs further validation based on the clinical studies. Clinical studies based on the efficacy of the identified phytochemicals through the GC-MS analysis for conditions like (anti-oxidant, anti-diabetic, anti-microbial, anti-fungal, anti-cancerous, hepatoprotective) using human subjects would bring an in-depth knowledge of the benefits of *A. paniculata* as a potent herbal plant. Thus, in order to explore the medical/therapeutic benefits of *A. paniculata*, further research should focus on clinical studies and also on multiplication of the herb through tissue culture based methods in order to meet the surplus demand for the pharmacological studies. The chemical profiling of the callus tissue samples has clearly indicated that it is good alternative to procure *A. paniculata* as and when required as callus tissue too is a store house of all the active phytoconstituents.

Acknowledgments The authors are thankful to Head, Department of Botany, University of Rajasthan for providing necessary facilities in the department and also to the USIC Department, University of Rajasthan for providing GC-MS analysis facility.

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