Pharmacogn J. 2019; 11(1): 129-140
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Comparative GC–MS Analysis of Bioactive Phytochemicals from Different Plant Parts and Callus of *Leptadenia reticulata* Wight and Arn.

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ABSTRACT

Aim: The aim of this study is identification and comparative analysis of bioactive phytochemicals present in methanol and ethyl acetate extracts of different plant organs and callus of *Leptadenia reticulata* by GC–MS technique. **Methodology:** The shade dried plant samples (leaves stem and root) and callus produced from leaf of *L. reticulata* were powdered and then sequentially extracted in methanol and ethyl acetate solvents. Total eight extracts were prepared which were Methanolic Leaf Extract (MLE), Methanolic Stem Extract (MSE), Methanolic Root Extract (MRE), Methanolic Callus Extract (MCE), Ethyl Acetate Leaf Extract (EALE), Ethyl Acetate Root Extract (EARE), Ethyl Acetate Stem Extract (EASE) and Ethyl Acetate Callus Extract (EACE). Then, each of the extracts was further subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis. **Results:** The GC–MS analysis of the eight extracts revealed the presence of 77 different types of high and low molecular weight phytochemicals and bioactive compounds in varying quantities. Some of the phytochemicals detected for first time in *L. reticulata* are γ-sitosterol**,** Campesterol, Pristane, Hexahydrofarnesol, Stearic acid, Arachidic acid**,** Coniferyl alcohol, n-Tetracosanol-1, Ascorbic acid 2,6-dihexadecanoate, (2S,3S)-3,7,4′-Trihydroxy-5-methoxy-6-methylflavanone etc. These chemical compounds are considered biologically active and pharmacologically important. **Conclusion:** This study gives a detailed comparison of detection and identification of various bioactive phytochemicals from different plant parts (leaves, stem and root) and callus of *L. reticulata*. This provides a basis for the biological and biochemical characterization of some newly detected biologically and pharmacologically important phytochemical components from this plant.

Key words: Callus, Fatty Acid, Root, Stem, Steroids, Terpenoids.

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History

- Submission Date: 20-08-2018;
- Review completed: 15-10-2018;
- Accepted Date: 15-11-2018

DOI : 10.5530/pj.2019.1.22

Article Available online
http://www.phcogj.com/v11/i1

INTRODUCTION

Phytochemicals present in medicinal plants have been part of phytomedicines since ancient times. Medicinal plants play a vital role in the prevention and treatment of various diseases and largely contributing in all existing prevention strategies. Various plant-based medicines have already proved their potential efficacy and safety.[1] Now a days, synthetic drugs are broadly used but their extreme use may cause severe side effects in body which are sometimes more serious than that of disease itself. Hence, pharmaceutical companies are spending a lot of money and time on the plants with potential medicinal properties for the formulation of the natural drugs which are safe and effective.[2] In developing countries all over the world, large number of people do not have access to synthetic drugs or they do not have potential to buy them, they still depend on traditional medicinal plants as they are cheaper and easily accessible. These reasons might account for the worldwide attention and use of medicinal plants. In 2013, WHO prepared and lunched 'WHO Traditional Medicine Strategy 2014–2023' which emphasized to join together traditional and complementary medicine to promote universal healthcare and to ensure the safety, quality and effectiveness of such medicines.[3]

Approximately 20% of the plants found in the world have undergone pharmaceutical or biological tests.[4] Plants have the ability to produce a number of compounds in the form of secondary metabolites that have diverse biological properties and they serve as active drugs against various diseases.[2,5] Amount of these secondary metabolites varies from species to species and plant to plant depending on the age and variations in climates and ecological factors.[2] The phytochemical accumulation in plants has generated understanding for the production of desired bioactive compounds with the prospective to produce desired component. In many

Cite this article: Godara P, Dulara BK, Barwer N, Chaudhary NS. Comparative GC–MS Analysis of Bioactive Phytochemicals from Different Plant Parts and Callus of *Leptadenia reticulata* Wight and Arn. Pharmacog J. 2019;11(1):129-40.

cases, crude extracts from medicinal plants are more biologically active than isolated compounds due to their synergistic effects.[6] Extraction and characterization of numerous bioactive compounds from these green plant factories have given birth to various high activity profile drugs. A number of different solvent systems like water, methanol, ethanol, chloroform, methanol, ethyl acetate, petroleum ether etc. have been reported to play important role for extraction of secondary metabolites, such as tannins, terpenoids, steroids, alkaloids, flavanoids, phenols and quinines.[2]

Leptadenia reticulata Wight and Arn. (*Asclepiadaceae*) is a well-known tonic and life giving drug with rejuvenating, restorative, antiabortificent and lactogenic properties. It is a perennial woody climber called as '*Jeevanti*' in Ayurveda which means 'life-giver' because it works as a stimulant and claimed to prevent miscarriage.[7,8] *Jeevanti* is used in Ayurveda since 4500 BC as a general body vigor provider. According to *Atharva-Veda* this plant promotes vitality and life.[7] *L. reticulata* grows well in tropical and subtropical climate with moderate rainfall and relative humidity. This plant is also found in arid regions with sandy soil, low organic matter and very low rainfall.[9] In India, *L. reticulata* is found in Sikkim, Karnataka, Rajasthan, Gujarat, Khasia hills, Nilgiris, Tamil Nadu, Laccadive Islands, Andhra Pradesh, Goa, Haryana, Kerala, Madhya Pradesh, Maharashtra, Orissa, Bihar, the sub-Himalayan tracts of Punjab, U.P. and Deccan Peninsula up to an altitude of 900 meters.[8,10] Apart from India, it is distributed in the tropical and subtropical parts of Africa, Cambodia, Mauritius, Sri Lanka, Burma, Nepal, Madagascar, Malay Peninsula, the Philippines etc.[10,11] *L. reticulata* has many biological activities like antiabortifacient,[12] galactagogue/lactogenic/milk increasing effect,[13] anti-implantation,[14] antimicrobial, antioxidant,[15] antitumour,[16] immunomodulatory activity,[17] antiepileptic potential,[18] analgesic,[9] antipyretic, anti-inflammatory,[19] hepatoprotective,[20] antiulcer,[21] anxiolytic,[22] diuretic,[23] cardioprotective,[24] antianaphylactic, antiasthmatic, antidepressant, vasodialator and hypotensive effects.[9] It is also used in the treatment of oligospermia, aphrodisiac, infertility and benign prostatic hyperplasia. The leaves and roots are used in tuberculosis, cough and against skin infections such as ringworm and wounds.[8] The therapeutic potential of this herb is because of the presence of organic acids, flavonoids, triterpenes, steroids, volatiles, amino acids, glucosides, tannins, inorganic ions and lignanoids.[9] Thus, *L. reticulata* is a widely distributed plant with immense therapeutic potential. These properties demand a thorough phytochemical analysis, evaluation and validation of this herbal drug for natural product development.

In the current literature available, it is observed that most of the studies are focused on either aerial part or in the whole plant of *L. reticulata*, there are no reports found on the complete phytochemical analysis of different plant parts (leaf, stem and root) separately of *L. reticulata* using methanol and ethyl acetate solvents. Besides, there is also lacking reports on the comprehensive phytochemical analysis on tissue culture *(in vitro)* callus samples generated from this plant. Therefore, this study is focused to evaluate and analyze plant parts and callus extracts of *L. reticulata* for the presence of phytoconstituents in order to identify and characterize bioactive compounds in the crude extracts prepared in both solvents (methanol and ethyl acetate) for chemical profiling by Gas Chromatography-Mass Spectrometric (GC–MS) technique. The GC–MS is normally used for direct analysis of unknown components existing in traditional medicines and medicinal plants. The results pertaining to GC-MS analysis have led to identification of number of bioactive compounds from *L. reticulata* sample extracts. They were identified through mass spectrometry attached with GC. This study revealed the accumulation pattern of many known bioactive compounds in different plant parts of *L. reticulata* and detected many new bioactive compounds which were not earlier reported in this plant.

MATERIALS AND METHODS

Instrument and Chemicals

GC–MS System (Thermo scientific GC 1300 and TSQ 8000 Triple quadrupole), Soxhlet apparatus (3840, Borosil Glass works Ltd., Mumbai, India), Murashige and Skoog Medium PT-100, 1-Napthalene acetic acid (NAA) (Himedia), 2,4-Dichlorophenoxyacetic acid (2,4-D) (Duchefa Biochemie), Methanol (Rankem) and Ethyl Acetate (Loba Chemie). All other chemicals and reagent were used of Analytical and Biological grade.

Collection of Plant Sample

The fresh plant parts of *L. reticulata* were collected (Priyanka Godara; March 20, 2015) from nearby area of Jaipur, Rajasthan, India. The identity of collected plant specimen was confirmed by depositing the voucher specimen number RUBL211619 in the herbarium of Department of Botany, University of Rajasthan, Jaipur. The plant parts were washed thoroughly with water to remove dust and dried under the shade at room temperature for approximately 15 days till constant dry weight. The dried plant parts were separately ground in liquid nitrogen to obtain the fine powder and kept in an air tight container till further use.

Callus Preparation

The desired amount of callus was produced by sub-culturing of primarily produced callus from leaf explants of *L. reticulata* on MS media supplemented with standardized amount of growth regulators i.e. 2,4-D (1.25 mg/l) + NAA (0.25 mg/l).

Preparation of Extracts

The dried and fine powdered samples (leaves, roots, stem and callus) of *L. reticulata* exhaustively extracted with solvents methanol and ethyl acetate. 10 g of each powdered plant materials (leaves, stem and roots) and 4 g of callus were packed in thimbles (each in duplicate). Each sample was extracted in methanol and ethyl acetate (100 ml each) separately using Soxhlet apparatus as per standard method. The Soxhlet extracted plant samples were vacuum evaporated using Rotary Vacuum Evaporator (BR Biochem- Scilogex RE100-Pro) to obtain eight dried solid extracts. These extracts were named as Methanolic Leaf Extract (MLE), Methanolic Stem Extract (MSE), Methanolic Root Extract (MRE), Methanolic Callus Extract (MCE), Ethyl Acetate Leaf Extract (EALE), Ethyl Acetate Stem Extract (EASE), Ethyl Acetate Root Extract (EARE) and Ethyl Acetate Callus Extract (EACE). The percent yield of extracts was 9.6% for MLE, 8.7% for MSE, 5.8% for MRE, 4.3% for MCE, 5.1% for EALE, 4.7% for EASE, 2.8% for EARE and 2.6% for EACE. All extracts were stored in vacuum tight container at 4°C in the refrigerator for further use.

GC–MS Analysis

The GC–MS analysis of *L. reticulata* dried and filtered extracts were carried out on a Thermo GC 1300 and TSQ 8000 Triple Quadrupole GC–MS system installed with auto sampler Al 1310. The program was set using capillary column TG-5MS AMINE (30 mm × 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 up to 270°C with holding time of 1 min. The injector temperature was set at 280°C and the carrier gas used was helium at a flow rate of 1.0 ml/min. GC–MS analysis was conducted using TSQ8000 with transfer line temperature 280°C and ion source temperature 230°C in EI mode. The MS scan parameters included electron impact ionization voltage of 70 eV and a mass range of 50–500 m/z. TSQ 8000 Triple Quadrupole MS detector was used for analysis and data was evaluated using total ion count (TIC) for compound identification and quantification.

Identification of Bioactive Phytochemicals

Phytochemical compounds extracted in different extracts of *L. reticulata* were identified by comparing the mass spectra of the detected components 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

The GC–MS chromatograms spectra obtained for all eight extracts revealed that *L. reticulata* is plenteously rich in bioactive compounds in all parts of plant as well as in callus (Figure 1). Each spectrum of extract shows the retention time in the column and the detected peaks correspond to the relative abundance of bioactive compounds detected in the particular extract. A total of 77 major phytochemical compounds were detected in leaves, stem, root and callus of *L. reticulata* extracted both in methanol and ethyl acetate. The name, molecular formula and the abundance of these bioactive compounds are presented in Table 1. The GC-MS chromatogram of leaf extract in methanol (MLE) and ethyl acetate (EALE) indicated the presence of 31 and 22 compounds respectively. Based on the abundance, in MLE and EALE major compounds were1-Tridecene (23.73%), Phytol acetate (13.90%), [6-hydroxy-2,2,6-trimethyl-3-(3-methylbut-2-enyl)cyclohexyl]methyl acetate (13.36%), β-sitosterol (9.27%), Palmitic acid (8.44%), 3,7,11-trimethyl-1-dodecanol (7.76%), γ-Sitosterol (6.86%), Stigmasterol (4.79% and 3.65%) and Campesterol (3.12% and 2.80%). Moreover, methanolic root extract (MRE) and ethyl acetate root extract (EARE) detected for the presence of 26 and 29 major bioactive compounds respectively. The most abundant were l-(+)-Ascorbic acid 2,6-dihexadecanoate (18.66%), Dibutyl phthalate (15.42%), γ-Sitosterol (12.54%), Stearic acid (10.50%), 10-Heneicosene (8.71%), β-amyrin (6.65%), Stigmasterol (6.04%), E-15-Heptadecenal (5.28%), n-Tetracosanol-1 (5.08%) and Campesterol (4.20%). In addition, 31 and 25 major phytochemicals were identified in stem extracts of both methanol (MSE) and ethyl acetate extracts (EASE) respectively. The most abundant compounds detected in both extracts were l-(+)-Ascorbic acid 2,6-dihexadecanoate (15.95% and 17. 55%) followed by β-sitosterol (13.40%), Methyl 8-oxo-17-octadecene-9,11-diynoate (7.51%), Stigmasterol (6.71%), γ-Sitosterol (6.35%), Hexahydrofarnesyl acetone (5.77%) and Campesterol (4.32%).

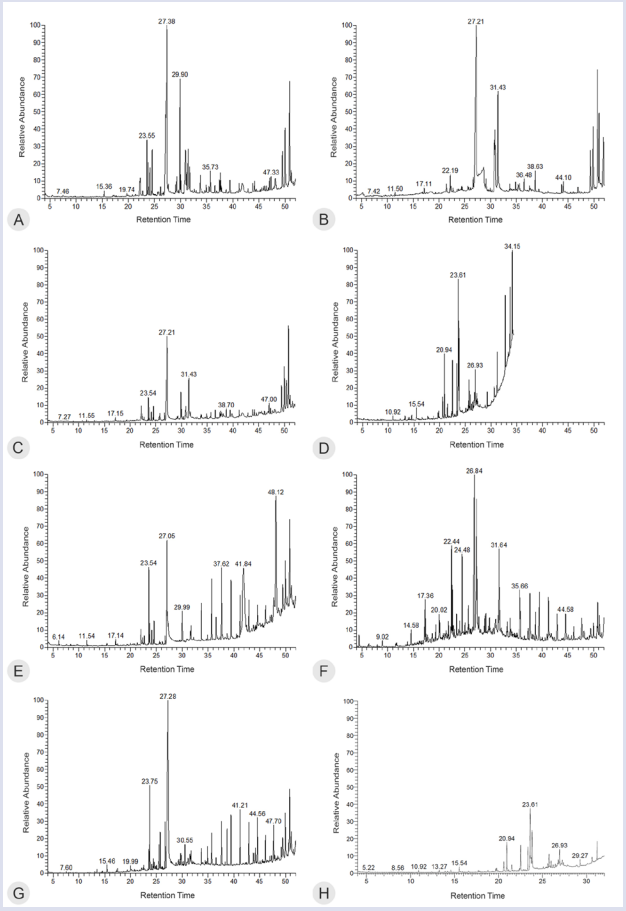

Figure 1: The GC–MS chromatograms of all eight extracts of *Leptadenia reticulata* showing relative abundance and retention time of phytochemicals. A: Methanolic Leaf Extract (MLE), B: Methanolic Root Extract (MRE), C: Methanolic Stem Extract (MSE), D: Methanolic Callus Extract (MCE), E: Ethyl Acetate Leaf Extract (EALE), F: Ethyl Acetate Root Extract (EARE), G: Ethyl Acetate Stem Extract (EASE), H: Ethyl Acetate Callus Extract (EACE).

The callus obtained from leaf explant of *L. reticulata* and its subsequent extraction in methanol (MCE) and ethyl acetate (EACE) solvents followed by GC-MS analysis, brought out that callus cells also expresses the bioactive phytochemicals in good amount. The GC-MS chromatogram of both MCE and EACE revealed the presence of 23 and 19 major bioactive compounds respectively. The major compound detected in both MCE and EACE extracts were l-(+)-Ascorbic acid 2,6-dihexadecanoate (16.18% and 20.78%) followed by Palmitic acid ethyl ester (6.70% and 8.60%), Eicosane (8.04%), Hexahydrofarnesyl acetone (7.24%), Palmitic acid methyl ester (5.14% and 6.61%) and Phthalic acid, di(2-propylpentyl) ester (5.88%).

DISCUSSION

Leptadenia reticulata is one of the major ingredients in many commercial herbal formulations including Confido (Speman forte), Galactin Vet (bolus), Safe herbs, Speman, Envirocare, Calshakti, 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 *L. reticulata*.[9] This plant has multiple therapeutic activities due to presence of various bioactive phytochemicals like stigmasterol, ß-sitosterol, α-amyrin, ß-amyrin, rutin, simiarenol, apigenin, reticulin, deniculatin, leptaculatin, l-α–tocopherol, p-coumaric acid, quercetin, phytol etc.[8] Present study gives a comparative account of various phytochemicals identified by GC-MS analysis in root, stem, leaves and callus. The comparative GC-MS analysis of plant parts and callus identified various classes of bioactive compounds that include steroids, terpenes, terpenoids, fatty acids and their esters, alcoholic and phenolic compounds, esters, hydrocarbons, coumarins, flavanoids, vitamin C and ketones (Table 1).

The bioactive compounds reported for the first time in *L. reticulata* through the present study are from group of Steroids (γ-sitosterol, Campesterol, Campesterol acetate, Stigmastan-3,5-diene, Cholesteryl Myristate, etc.), Terpenes (Pristane, Hexahydrofarnesol, Hexahydrofarnesyl acetone, Phytol acetate etc.), Fatty acids and their esters (Stearic acid, Arachidic acid, Isopropyl myristate, Linolenic acid ethyl ester, Hexadecanoic acid methyl ester, Linolenic acid methyl ester), Esters (Dibutyl phthalate, Phthalic acid Diisobutyl ester), Phenolic compounds (Coniferyl alcohol and 2-(2-hydroxypropan-2-yl)-4-methoxy-5-methyl phenol), Alcoholic compounds (n-Tetracosanol-1, Montanol, Quercitol, 2-Methyl-Z,Z-3,13-octadecadienol and Trans-9-hexadecen-1-ol), a Vitamin C fatty acid compound (Ascorbic acid 2,6-dihexadecanoate), a Coumarin compound (2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate), a flavanoidal

Table 1: List of compounds identified from various classes of bioactive phytochemicals by GC-MS analysis of methanolic and ethyl acetate extracts of leaves, root, stem and callus of *Leptadenia reticulata*.

Compound type	Compound name	Molecular formula	Retention Time (RT[1])	Methanolic extracts (% composition)				Ethyl Acetate extracts (% composition)			
				MLE	MRE	MSE	MCE	EALE	EARE	EASE	EACE
Steroids	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	25.7		0.39%	0.36%			1.76%‡		
	Stigmastan-3,5-diene	C29H48	39.78			0.92%‡					
	4,4-dimethyl-Cholesta-6,22,24-triene	C29H46	46.43	0.42%‡							
	(3á)-Cholesta-4,6-dien-3-ol	C27H44O	47	0.94%	0.54%	1.53%‡				0.55%	
	Cholesterol Myristate, Cholest-5-en-3-ol (3beta)-tetradecanoate	C41H72O2	47.31							0.61%‡	
	Campesterol acetate, (3á,24R)-Ergost-5-en-3-ol acetate	C30H50O2	47.33	1.05%‡	0.18%	0.80%					
	Campesterol	C28H48O	49.45	3.12%	4.20%	4.32%‡		2.80%	0.69%	2.36%	
	Stigmasterol	C29H48O	49.95	4.79%	6.04%	6.71%‡		3.65%	0.99%		
	γ-sitosterol, Clionasterol	C29H50O	50.71		12.54%‡			6.86%	2.59%	6.35%	
	β-sitosterol	C29H50O	50.82	9.27%		13.4%‡					
	Total % Composition			19.59%	23.89%	28.04%	0.00%	13.31%	6.03%	9.87%	0.00%
Terpenes/ Terpenoids	Dihydroactinidiolide, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-Benzofuranone	C11H16O2	15.46							0.53%‡	
	Pristane, 2,6,10,14-tetramethyl-Pentadecane	C19H40	20.13						1.08%‡		
	Phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	23.54	3.56%		2.70%	2.16%	4.38%‡		1.33%	2.78%
	Hexahydrofarnesyl acetone, 6,10,14-trimethyl-2-Pentadecanone	C18H36O	23.75	2.00%	0.17%	1.48%	5.64%	1.33%		5.77%‡	7.24%
	Hexahydrofarnesol, 3,7,11-Trimethyl-1-dodecanol	C15H32O	29.9	7.76%‡		3.22%	0.27%		1.00%		
	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	30.63	0.51%	0.69%	0.59%	1.77%	0.32%		1.28%	2.28%‡
	β-Simiarenol, (3á)-D:B-Friedo-B':A'-neogammacer-5-en-3-ol	C30H50O	41.79	2.06%‡		1.80%			1.80%		
	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	C17H30O3	41.84		0.46%			13.36%‡			
	Phytol acetate	C22H42O2	48.13	1.10%		1.50%		13.9%‡			
	β-amyrin	C30H50O	51.01	0.86%	6.65%‡	1.69%		1.39%	1.44%	1.94%	
	Total % Composition			17.85%	7.97%	12.98%	9.84%	34.68%	5.32%	10.85%	12.30%

Continued...

Table 1: Cont'd.

Compound type	Compound name	Molecular formula	Retention Time (RT[i])	Methanolic extracts (% composition)				Ethyl Acetate extracts (% composition)			
				MLE	MRE	MSE	MCE	EALE	EARE	EASE	EACE
Fatty acids and their Esters	Myristic acid, Tetradecanoic acid	C14H28O2	22.23	1.36%	1.70%	2.07%[‡]					
	Palmitic acid methyl ester, Hexadecanoic acid methyl ester	C17H34O2	22.52				5.14%			2.47%	6.61%[‡]
	Isopropyl myristate	C17H34O2	23.37						1.34%[‡]		
	Palmitic acid ethyl ester, Hexadecanoic acid ethyl ester	C18H36O2	23.81				6.70%			3.17%	8.6%[‡]
	Linolenic acid methyl ester, (Z,Z,Z)9,12,15-Octadecatrienoic acid methyl ester	C19H32O2	25.74				3.22%				4.14%[‡]
	Linolenic acid ethyl ester, Ethyl-9,12,15-octadecatrienoate	C20H34O2	26.93				3.89%				5%[‡]
	Palmitic acid, n-Hexadecanoic acid	C16H32O2	27.05					8.44%[‡]			
	Ascorbic acid dipalmitate, Ascorbic acid 2,6-dihexadecanoate	C38H68O8	27.28	3.16%	18.66%	15.95%	16.18%	1.03%		17.55%[‡]	20.78%
	Stearic acid, Octadecanoic acid	C18H36O2	31.43	3.63%	10.5%[‡]	6.56%					
	Oxalic acid allyl octadecyl ester	C23H42O4	34.25				0.86%[‡]				
	Arachidic acid, Eicosanoic acid	C20H40O2	35.32	0.58%	0.87%[‡]						
	2-mono Palmitin, Hexadecanoic acid, 2hydroxy1(hydroxymethyl)ethyl ester	C19H38O4	37.47	0.93%[‡]		0.59%					
	Hexatriacontyl pentafluoropropionate	C39H73F5O2	47.71						1.49%[‡]		
	Tetratriacontyl heptafluorobutyrate	C38H69F7O2	50.64						1.04%[‡]		
	1-Methyl-4-isopropyl-cyclohexyl 2-hydroperfluorobutanoate	C14H20F6O2	51.84		7.48%[‡]						
	Total % Composition			9.66%	39.21%	25.17%	35.99%	9.47%	3.87%	23.19%	45.13%
Phenolic & Alcoholic compounds	Hexadeca-9-en-1-ol	C16H32O	17.16					0.30%	1.85%[‡]		
	Coniferyl alcohol, 4-(1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C10H12O3	21.45	0.82%[‡]							
	Benzenemethanol, 2-(2-hydroxypropan-2-yl)-4-methoxy-5-methyl phenol	C11H16O3	22.03			0.42%		1.03%[‡]			
	Montanol	C21H36O4	22.14	1.04%[‡]							
	2-Hexyl-1-octanol	C14H30O	23.71		0.31%		1.54%	0.53%			1.98%[‡]
	14-Methyl-8-hexadecyn-1-ol	C17H32O	26.8				1.62%				2.09%[‡]
	Quercitol, 1-deoxy-Inositol	C6H12O5	28.65		2.26%[‡]						
	2-Methyl-Z,Z-3,13-octadecadienol	C19H36O	30.86		6.69%[‡]	2.20%					
	n-Tetracosanol-1	C24H50O	31.64						5.08%[‡]		
	5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl)-3-methylene-1,2-Pentanediol	C20H35BrO3	51.95					1.74%[‡]			
	Total % Composition			1.86%	9.26%	2.62%	3.16%	3.60%	6.93%	0.00%	4.07%

Continued...

Table 1: Cont'd.

Compound type	Compound name	Molecular formula	Retention Time (RT[1])	Methanolic extracts (% composition)				Ethyl Acetate extracts (% composition)			
				MLE	MRE	MSE	MCE	EALE	EARE	EASE	EACE
Hydrocarbons	Pentadecane	C15H32	14.58				0.34%		0.83%‡		0.44%
	Hexadecane	C16H34	17.36						2.14%‡	0.37%	
	Heptadecane	C17H36	20.02						1.38%‡		
	2-Methyl-7-octadecyne	C19H36	25.61				0.84%				1.08%‡
	10-Methylnonadecane	C20H42	27.24				1.77%		0.61%		2.28%‡
	10-Heneicosene (c,t)	C21H42	27.27		0.36%	0.34%	0.38%	0.50%	8.71%‡		0.49%
	1-Tridecene	C13H26	27.38	23.73%‡							
	2-methyl-Nonadecane, Isoeicosane	C20H42	27.39						2%‡		
	1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-Cyclohexane	C20H40	30.55				0.39%	0.68%		1.45%‡	
	Eicosane	C20H42	34.15	1.65%	0.51%	1.06%	8.04%‡	4.51%	3.14%	4.24%	5.68%
	1-Isopropyl-1,4,5-trimethylcyclohexane	C12H24	43.82			1.07%‡					
	2-methylhexacosane	C27H56	46.15					1.41%	1.96%	2.23%‡	
	Total % Composition			25.38%	0.87%	2.47%	11.76%	7.10%	20.77%	8.29%	9.97%
Esters	Phthalic acid di-isobutyl ester, 1,2-Benzenedicarboxylic acid bis-(2-methylpropyl) ester	C16H22O4	21.51	0.24%			1.77%			0.74%	2.28%‡
	Benzyl Benzoate	C14H12O2	21.82						1.1%‡		
	Phthalic acid, hept-3-yl isobutyl ester	C19H28O4	24.48			0.32%			4.34%‡		
	Dibutyl pthalate	C16H22O4	26.85	0.30%	0.78%	0.97%	4.79%	0.49%	15.42%‡	3.28%	6.15%
	2,8-Dimethylundecanoic acid methyl ester	C14H28O2	31.74	1.67%‡							
	Phthalic acid di(2-propylpentyl) ester	C24H38O4	33.67		1.65%	1.09%	5.88%‡	0.49%	1.56%	2.70%	
	Methyl 8-oxo-17-octadecene-9,11-diynoate	C19H26O3	50.37			7.51%‡					
	6-Tetradecanesulfonic acid butyl ester	C18H38O3S	51.96							1.76%‡	
	Total % Composition			2.21%	2.43%	9.89%	12.44%	0.98%	22.42%	8.48%	8.43%

Continued...

Table 1: Cont'd.

Compound type	Compound name	Molecular formula	Retention Time (RT[i])	Methanolic extracts (% composition)					Ethyl Acetate extracts (% composition)			
				MLE	MRE	MSE	MCE	EALE	EARE	EASE	EACE	
Others	3-(Dioxolan-2-yl)-1,1-dimethyl-2-phenylpropylhydroxylamine	C14H21NO3	4.44						0.94%[‡]			
	E-15-Heptadecenal	C17H32O	22.44			0.47%			5.28%[‡]			
	(2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone	C17H16O6	25.79			1.11%[‡]						
	S-(2-Benzothiazolyl) cysteine	C10H10N2O2S2	25.84	0.52%[‡]								
	3-Heptadecenal	$C_{17}H_{32}O$	25.55							1.72%[‡]		
	10-Nonadecanone	C19H38O	29.11						1.06%[‡]			
	1-Hexyl-2-nitrocyclohexane	C12H23NO2	29.7	0.31%			0.45%			0.87%[‡]	0.57%	
	cis,cis,cis-7,10,13-Hexadecatrienal	C16H26O	30.98	3.9%[‡]			0.69%				0.88%	
	(Z)-14-methyl-8-Hexadecenal	C17H32O	30.99						1.33%[‡]			
	4-Benzyloxybenzophenone	C20H16O2	36.55	0.52%	0.98%	1.11%		1.76%[‡]		0.65%		
	4-Propyl-2-hydroxycyclopent-2-en-1-one	C5H6O2	44.1	0.69%	1%[‡]					0.87%		
	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate	C30H33ClO6	49.95	0.42%	0.21%	0.69%				3.89%[‡]		
	Total % Composition			6.36%	2.19%	3.38%	1.14%	1.76%	8.61%	8.00%	1.45%	

[‡]indicates the retention time of the compound present in higher concentration among methanolic and ethyl acetate extracts of leaves, root, stem and callus.

compound (2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone) and some other compounds like Heptadecane, Pentadecane, Hexadecane, E-15-Heptadecenal and S-(2-Benzothiazolyl) cysteine. The details of biological activity and uses of some important first time reported phytochemical compounds have been summarized in Table 2.

Steroids

Steroids were present in different plant part extracts of *L. reticulata* in abundance and a total of 10 steroidal compounds were detected. Amongst all eight extracts highest amount of total steroidal content was detected in MSE (28.04%) followed by MRE (23.89%), MLE (19.59%), EALE (13.31%), EASE (9.87%) and EARE (6.03%). Steroidal compounds were surprisingly absent in both the callus extracts. Phytosterols were extracted in higher amounts in methanolic extracts. Phytosterols are very well known for their blood cholesterol lowering activity.[25] Phytosterols have antifungal, antibacterial, antiulcerative, antitumoral, anti-inflammatory, anti-cancerous and anti-atherogenic activities.[25-28] Stigmasterol was present in all plant parts but it was found absent in ethyl acetate stem extract and both the callus extracts. It was found in MSE (6.71%), MRE (6.04%), MLE (4.79%), EALE (3.65%) and EARE (0.99%). Stigmasterol is an unsaturated phytosterol and has various medicinally important properties like cholesterol-lowering, anti-osteoarthritic,[29] thyroid inhibitory, antiperoxidative, hypoglycemic,[30] antihepatotoxic, anti-inflammatory, antinociceptive, antiviral, cancer-preventive activities and sedative effects.[31] It is a strong antioxidant and shows antibacterial activity against multidrug resistant mycobacteria.[32] β-sitosterol was found in good quantities in methanolic stem (13.40%) and root extracts (9.27%). It was absent in all ethyl acetate extracts. β-sitosterol shows various biological activities like immunomodulatory, antioxidative, hepatoprotective activity[33] and used in the treatment of benign prostatic hyperplasia.[34] β-sitosterol shows antiviral effect against Hepatitis B Virus, HIV virus and tobacco mosaic virus (TMV).[33]

First time reported from *L. reticulata* in this study, γ-sitosterol or Clionasterol was found in MSE (12.54%), EALE (6.86%), EASE (6.35%) and EARE (2.59%). γ-sitosterol shows anticancerous,[35] hepatoprotective, anti-hyperglycemic activity and may act as a potential antidiabetic drug.[36,37] The Presence of Campesterol is detected in *L. reticulata* for the first time in this study. It was found in MRE (12.54%), EALE (6.86%), EASE (6.35%) and EARE (2.59%). Campesterol is a cholesterol absorption reducing agent and shows anticancer and antioxidant activities.[38] β-sitosterol and Campesterol are also used as biomarkers for cancer prevention.[28] Cholesterol Myristate or Cholest-5-en-3-ol(3beta)-tetradecanoate is a cholesterol fatty acid ester and detected in ethyl acetate stem extract (0.61%). Cholesterol Myristate is helpful in brain disease therapies, stem-cell transplantation, bone-marrow transplantation, treatment of bone diseases such as osteoporosis and chemotherapy.[39,40] 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione is a steroidal anti-mineralocorticoid and it was found in ethyl acetate root extract (1.76%) and traces were found in MRE and MSE. Some other steroidal compounds detected in extracts of *L. reticulata* were Cholesta-4,6-dien-3-ol (0.54-1.53%), Campesterol acetate (Ergost-5-en-3-ol acetate) in MLE (1.05%) and MSE (0.80%), 4,4-dimethyl-Cholesta-6,22,24-triene in MSE (0.92%) and Stigmastan-3,5-diene in MLE (0.42%).

Terpenes and Terpenoids

Terpenes and terpenoids were abundantly present in the extracts of *L. reticulata* and 10 different terpene/terpenoid compounds were found. Amongst all eight extracts, the highest amount of total terpenes/ terpenoids was detected in EALE (34.68%), then in MLE (17.85%), MSE (12.98%), EACE (12.30%), EASE (10.85%), MCE (9.84%), MRE (7.97%) and EARE (5.32%). Overall, both the leaf extracts contained high amount of terpenes/terpenoids. Terpenes and terpenoids have attracted much

Table 2: Some of the newly identified compounds from different parts and callus of *L. reticulata*.

S.No.	Compound Name	Class of Compound	Plant part/ callus	Biological activity/ use
1	γ-sitosterol	Steroid	Lf, Rt, St	Anticancerous, hepatoprotective, antihyperglycemic activity, antidiabetic drug[35-37]
2	Campesterol	Steroid	Lf, Rt, St	Anticancer, antioxidant, hypocholesterolemic[38]
3	Stigmastan-3,5-diene	Steroid	St	No activity reported
4	Cholesterol Myristate, Cholest-5-en-3-ol (3beta)-tetradecanoate	Steroid	St	Brain and bone diseases (osteoporosis) therapies, stem-cell & bone-marrow transplantation, chemotherapy.[39,40]
5	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	Terpenoid	Lf, Rt	Anticancer, antiinflammatory[54,55]
6	Pristane, 2,6,10,14-tetramethyl-Pentadecane	Terpene	St	Antiinflamatory, anti-leishmanial activity, used in human uveitis, diffuse pulmonary hemorrhage (DPH)[56,57]
7	Hexahydrofarnesol	Terpenoid	Lf, Rt, St, Ca	Squalene synthatase inhibitor, anti-hyperlipidemic, anti-atherosclerotic[53]
8	Stearic acid, Octadecanoic acid	Fatty acid	Lf, Rt, St	Antibacterial and antifungal[65]
9	Ascorbic acid dipalmitate, Ascorbic acid 2,6-dihexadecanoate	Vitamin C Fatty acid compound	Lf, Rt, St, Ca	Antioxidant, cardio protective, cancer preventive, anti-infertility agent[71-72]
10	Linolenic acid methyl ester, (Z,Z,Z)9,12,15-Octadecatrienoic acid methyl ester	Fatty acid ester	Ca	Antibacterial, anticandidal, antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, 5-Alpha reductase inhibitor and antiandrogenic activities.[32]
11	Arachidic acid, Eicosanoic acid	Fatty acid	Lf, Rt	Improves lipid transport and lipid metabolism, anti-mutagenic properties[75]
12	Coniferyl alcohol	Alcohol	Lf	Antioxidant, antiprostaglandin, antiaggregant, fungicide, antiradicular and pesticidal activity[38]
13	Quercitol, 1-deoxy-Inositol	Alcohol	Root	Drought stress tolerance as osmolyte in plants, can be used in production of antidiabetic drugs[79,85]
14	n-Tetracosanol-1	Alcohol	Rt	Antimutagenic, antibacterial activity, lowers cholesterol, enhancing immune functions, platelet aggregation and endothelial cell damage.[68,75,76]
15	S-(2-Benzothiazolyl) cysteine	Cystiene compound	Lf	No activity reported
16	(2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone	Flavanoidal compound	St	No activity reported
17	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate	Coumarin compound	Lf, Rt, St	No activity reported

Lf: Leaf, Rt: Root, St: stem, Ca: Callus

attention because of their important physiological and ecological roles and as defense compounds.[41] They show biological activities like anti-oxidant,[42] hepato-protective,[43] and cholinesterase inhibitors.[44] β-amyrin was found in all plant part extracts (0.86-6.65%) except for both callus extracts and highest amount was present in MRE (6.65%). It is a penta-cyclic triterpenol and have been reported for antioxidant, anti-apoptic, anti-inflammatory and anti-fibrotic activities.[45,46] β-simiarenol was found in MLE (2.06%), MSE (1.80%), EARE (1.80%) and it was absent in callus extracts. It is a triterpenoid and showed *in vitro* leishmanicidal activity against *Leishmania donovani promastigotes*.[47] Phytol was found in all extracts of EALE (4.38%), MLE (3.56%), EACE (2.78%), MSE (2.70%), MCE (2.16%) and EASE (1.33%) except for both the root extracts. Phytol is an important acyclic monounsaturated diterpene alcohol with various biological properties like anti-inflammatory,[48] anti-cancer,[49] antioxidant,[50] diuretic, anti-allergic, immunostimulant, anti-trypanosomal, cholesterol lowering, antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* and used in antischistosomal therapy.[51] Phytol acetate was found in highest amount in EALE (13.90%), then MST (1.50%) and MLE (1.10%). It was found absent in root and callus extracts. Phytol acetate is also an acyclic diterpene alcohol and shows anti-inflammatory, anti-leishmanial activity.[52] Hexahydrofarnesol or 3,7,11-trimethyl-1-dodecanol was found in highest amount in MLE (7.76%), MSE (3.22%), EARE (1.00%) and traces were found in MCE. Hexahydrofarnesol is a sesquiterpenoid and acts as a squalene synthatase inhibitor which makes it an anti-hyperlipidemic and anti-atherosclerotic agent.[53] 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol was present in highest amount in EALE (13.36%) and traces in MRE. It is a sesquiterpenoid and acts as an anticancer[54] and potent anti-inflammatory agent.[55] Pristane or 2,6,10,14-tetramethyl-Pentadecane was found only in EARE (1.08%). Pristane shows anti-inflammatory activity and also has therapeutic uses in leishmania disese, human uveitis and diffuse pulmonary hemorrhage.[56,57] Dihydroactin-idiolide or 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone was present in EASE (0.53%). It is a monoterpenoid essential oil and it shows analgesic, antidiabetic, antibacterial and antifungal activities.[32] Hexahydrofarnesyl acetone or 6,10,14-trimethyl-2-Pentadecanone is an isoprenoid ketone essential oil and it was found in highest amount in EACE (7.24%), EASE (5.77%), MCE (5.64%), MLE (2.00%), MSE (1.48%) and EALE (1.33%). It was found in traces in both the root extracts. The 4,8,12,16-Tetramethylheptadecan-4-olide is an isoprenoid γ-lactone, it was found in all extracts except for EARE. The highest amount was present in both callus extracts EACE (2.28%), MCE (1.77%) and EASE (1.28%).

Fatty Acids and their Esters

Fatty acids and their esters were present in abundance in different extracts of *L. reticulata* and 15 major different fatty acid compounds were found. The highest amount of total fatty acid and their ester compounds was found in EACE (45.13%), MRE (39.21%), MCE (35.99%) followed by MSE (25.17%), EASE (23.19%), MLE (9.66%), EALE (9.47%) and EARE (3.87%). Fatty acids are widely used as inactive ingredients (excipients) in drug preparations, cosmetics, fat emulsions, liposomes and also show antibacterial activity.[58,59] Palmitic acid or n-Hexadecanoic acid was found in MLE (8.44%). It is a saturated fatty acid that has anti-androgenic, antifibrinolytic, hemolytic 5-alpha reductase inhibitor, antioxidant, hypo-cholesterolemic,[60] anti-inflammatory, phos-pholipase inhibitor,[61] anti-cancerous,[62] antimicrobial, nematicide and mosquito larvicidal properties.[63-65] Myristic acid and Stearic acid are saturated fatty acids and were detected in significant amounts only in methanolic leaf, stem and root extracts. Myristic acid or Tetradecanoic acid was found in MSE (2.07%), MRE (1.70%) and MLE (1.36%). Myristic acid shows positive effects on HDL cholesterol and hence improving HDL to total cholesterol ratio.[66] It shows antioxidant, cancer preventive,[63] antibacterial, antifungal,[65] larvicidal, nematicide and repellent activity.[63,67] Stearic acid or octadecanoic acid was found in MRE (10.50%) and MLE (3.63%) and it shows antimicrobial activity.[65] Palmitic acid methyl ester or Hexadecanoic acid methyl ester was found in EACE (6.61%), MCE (5.14%) and EASE (2.47%). It is a saturated fatty acid and shows antioxi-dant, antitumor, immunostimulant, chemopreventive and lipoxygenase inhibitory,[32,68] antimicrobial activity and it is a potent mosquito larvicide.[69,70] Similarly, Palmitic acid, ethyl ester or Hexadecanoic acid ethyl ester was found in EACE (8.60%), MCE (6.70%) and EASE (3.17%). It has antioxidant, hemolytic, anti-androgenic, nematicide and pesticidal activities and also acts as 5-alpha reductase inhibitor.[60] Vitamin C com-pound l-(+)-Ascorbic acid 2,6-dihexadecanoate was found in abundance in EACE (20.78%), MRE (18.66%), EASE (17.55%), MCE (16.18%). This shows potential antimicrobial, anti-cancerous[71] and antioxidant activity.[72] Linolenic acid methyl ester and Linolenic acid ethyl esters are polyun-saturated fatty acid esters and are present in good quantities only in both methanolic and ethyl acetate callus extracts. Linolenic acid ethyl ester or Ethyl-9,12,15-octadecatrienoate was found in EACE (5.00%) and MCE (3.89%). Linolenic acid ethyl ester act as acetylcholinesterase (AChE) inhibitor and can be used in neurodegenerative disorders treatment.[73,74] Linolenic acid methyl ester or (Z, Z, Z) 9,12,15-Octadecatrienoic acid methyl ester was found in EACE (4.14%) and MCE (3.22%). It shows antibacterial, anticandidal, antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, 5-Alpha reductase inhibitor and antiandrogenic activities.[32] Arachidic acid or Eicosanoic acid was found in MLE (0.58%) and MRE (0.87%). It is a saturated fatty acid involved in natural biological processes of transport and metabolism of lipids and shows anti-mutagenic properties.[75] Overall, Fatty acids were present in abundance in callus extracts.

Alcoholic and Phenolic Compounds

There were 10 significant alcoholic and phenolic compounds identified in the extracts of both solvents. The highest amount of alcoholic compounds was found in root extracts of MRE (9.26%) then in EARE (6.93%), EACE (4.07%), EALE (3.60%) and MCE (3.16%). Stem and leaf extracts contained lower amounts of alcoholic compounds. Phenolic compounds found in *L. reticulata* extracts were Coniferyl alcohol and 2-(2-hydroxypropan-2-yl)-4-methoxy-5-methyl phenol. Coniferyl alcohol was present in MLE (0.82%) and it has been reported to show antioxidant, antiprostaglandin, antiaggregant, fungicide, antiradicular and pesticidal activity.[38] The presence of another important alcoholic compound detected in EARE (5.08%) was n-Tetracosanol-1. It is a fatty acid derivative of lignoceric acid and it shows antimutagenic[75] and antibacterial activity.[68] n-Tetracosanol-1 is one of the constituents of policosanol which modifies several cardiovascular disease risk factors by reducing LDL oxidation, platelet aggregation and endothelial cell damage.[76] 2-Methyl-Z,Z-3,13-octadecadienol was present in MRE (6.69%) and MSE (2.20%), it shows anticancer activity and increases zinc bioavailability.[77] Trans-9-hexadecen-1-ol was found in EARE (1.85%) and traces in EALE (0.30%), it shows anti-inflammatory and anticancer activity.[78] Quercitol or 1-deoxy Inositol is a cyclic polyalcohol found in MRE (2.26%) which works as a powerful osmolyte and helps in drought stress tolerance in plants.[79]

Hydrocarbons

12 different hydrocarbon compounds were detected in different extracts of *L. reticulata*. The highest amount of hydrocarbons was present in MLE (25.38%), followed by EARE (20.77%), MCE (11.76%), EACE (9.97%), EASE (8.29%), EALE (7.10%), MSE (2.47%) and traces were found in MRE. Different hydrocarbons were present in varying amounts in different

extracts such as Pentadecane was found in traces in EARE (0.83%), EACE (0.44%) and MCE (0.34%). Pentadecane exhibited antimicrobial activity against *Leishmania infantum Promastigotes* and *Amastigotes* parasites, antifungal activity against *Fusarium oxysporum* and antioxidant activity.[68,80,81] In addition, Hexadecane was present in EARE (2.14%) and EASE (0.37%). Hexadecane shows antimicrobial and antioxidant activity.[68] Another hydrocarbon Eicosane was present in all eight extracts with highest amounts in MCE (8.04%) followed by EACE (5.68%), EALE (4.51%), EASE (4.24%) and EARE (3.14%). Eicosane shows antibacterial activity and also used in petrochemical industry.[67] 10-Heneicosene (c,t) was found in traces in all the extracts except MLE and EASE, with highest amount in EARE (8.71%). It can be used as aggregation pheromone for diptera.[82] Heptadecane was found in EARE (1.38%) that shows anti-inflamatory, antimicrobial activities.[68,83]

Esters

A total of 8 ester compounds other than fatty acid esters were also detected and identified in varying composition in different extracts of EARE (22.42%), MCE (12.44%), MSE (9.89%), EASE (8.48%), EACE (8.43%), MRE (2.43%), MLE (2.21%) and EALE (0.98%). Among the detected fatty acid esters, Linolenic acid ethyl ester is used in the treatment of neurodegenerative disorders,[73,74] and Linolenic acid methyl ester has shown properties like antimicrobial, nematicide, antihistaminic, anti-inflammatory, anticancer, antiarthritic, anticoronary, hepatoprotective, antieczemic, antiacne and antiandrogenic activities.[32]

Other Compounds

Some other compounds of interest were found in different extracts like E-15-Heptadecenal was present in significant amounts in EARE (5.28%) and it shows antibacterial[84] and antioxidant activity.[68] EARE contained Isopropyl myristate (1.34%) used in cosmetic and topical medicinal preparations and also shows antioxidant and antibacterial activity.[68] A coumarin compound 2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-ylcarbonate was found in EASE (3.89%). MSE contained a flavanoidal compound (2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone (1.11%). Another compound of interest found in MLE was S-(2-Benzothiazolyl) cysteine.

CONCLUSION

Leptadenia reticulata (*Jeevanti*) is a traditional medicinal plant and found to be a rich source of bioactive phytochemicals possessing various biological activities. This study presented a comprehensive analysis of leaf, stem, root and callus extracts in methanol and ethyl acetate. The outcome of GC-MS analysis of this study, presented a detailed comparison of various classes of bioactive phytochemicals identified from different plant parts and callus. A comparative analysis of total 77 different bioactive compounds have been done and further discussed in details on the basis of potential biological role as well as medicinal importance. The present study has first time reported some potential bioactive phytochemicals in *L. reticulata* that includes γ-sitosterol**,** Campesterol, Pristane, Hexahydrofarnesol, Stearic acid, Arachidic acid**,** Coniferyl alcohol, n-Tetracosanol-1, Ascorbic acid 2,6-dihexadecanoate, (2S,3S)-3,7,4'-Trihydroxy-5-methoxy-6-methylflavanone etc. As the medicinal value of similar bioactive components in other plant extracts are already proved, no wonder if these phytochemicals in *L. reticulata* may also have equal efficacy. It could be concluded that all eight extracts of *L. reticulata* contain significant abundance of various bioactive compounds from the various categories of bioactive compounds such as steroids, terpenes, fatty acids and their esters, alcoholic and phenolic compounds, hydrocarbons etc. The GC-MS analysis particularly of callus extracts showed high accumulation of fatty acids which was not generally seen in leaves that were used as explant. Further, this study may provide future prospects for isolation, biological and medicinal characterization of some more compounds from this plant.

ACKNOWLEDGEMENT

The authors express deepest sense of gratitude to Late Dr. D. V. Rao, Associate Professor, Department of Botany, University of Rajasthan, Jaipur whose blessings at every step have inspired to accomplish this work. Authors thank Arun Devana, USIC department, University of Rajasthan for providing access to GC-MS facility and helping in data acquisition. Authors acknowledge Central Instrumentation Facility at Department of Botany, University of Rajasthan, Jaipur.

FINANCIAL SUPPORT

This research was carried out using fellowship under scheme UGC-BSR [F.NO- 2009/219(BSR)] funded by UGC, India to P.G. This study was supported, in part, by contingency grant from DST-PURSE and UGC-DRS-II Botany, University of Rajasthan, Jaipur.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

MLE: Methanolic Leaf Extract; **MSE:** Methanolic Stem Extract; **MRE:** Methanolic Root Extract; **MCE:** Methanolic Callus Extract; **EALE:** Ethyl Acetate Leaf Extract; **EASE:** Ethyl Acetate Stem Extract; **EARE:** Ethyl Acetate Root Extract; **EACE:** Ethyl Acetate Callus Extract.

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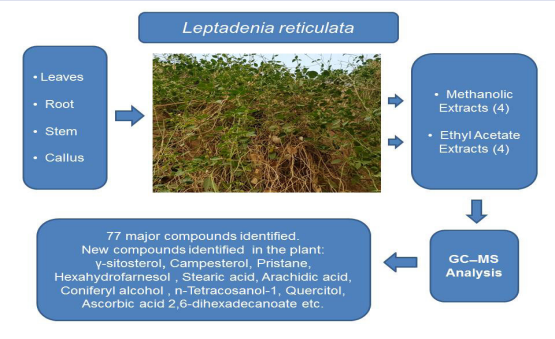
GRAPHICAL ABSTRACT



SUMMARY

- This study presented a comprehensive GC-MS analysis of Bioactive phytochemicals of leaves, stem, root and callus extracts in methanol and ethyl acetate from *Leptadenia reticulata*, locally known as Jeevanti. The outcome of this study presented a detailed comparison of 77 different bioactive phytochemicals belonging to various classes of compounds and further discussed in details on the basis of potential biological role as well as medicinal importance. The present study has first time reported some potential bioactive phytochemicals in *L. reticulata* that includes γ-sitosterol, Campesterol, Pristane, Hexahydrofarnesol, Stearic acid, Arachidic acid, Coniferyl alcohol, n-Tetracosanol-1, Ascorbic acid 2,6-dihexadecanoate, (2S,3S)-3,7,4′- Trihydroxy-5-methoxy-6-methylflavanone etc.

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Cite this article: Godara P, Dulara BK, Barwer N, Chaudhary NS. Comparative GC–MS Analysis of Bioactive Phytochemicals from Different Plant Parts and Callus of *Leptadenia reticulata* Wight and Arn. Pharmacog J. 2019;11(1):129-40.