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Research Article

PHYTOCHEMICAL EVALUATION OF *TILIACORA RACEMOSA* COLEBR. USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: The objective of this study is to characterize the phytoconstituents of *Tiliacora racemosa* Colebr. using gas chromatography mass spectrometry (GC-MS).

Methods: Preliminary phytochemical and physicochemical analysis was carried out using standard procedures. GC-MS analysis of methanolic extract was carried out using Thermo GC-Trace Ultra version: 5.0, Thermo MS DSQ with a DB 35MS capillary standard non-polar column and gas chromatograph interfaced to a mass selective detector (MS DSQ II) with Xcalibur software.

Results: Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, triterpenoids, steroids, proteins and amino acids, carbohydrates, saponins and coumarin. Quinones, anthraquinones, glycosides and fixed oil were absent. GC-MS analysis revealed the presence of 28 compounds of which quinic acid (retention times [RT]: 15.65) and inositol, 1-deoxy-(CAS) (RT: 19.24) was observed as abundant compounds.

Conclusion: The presence of various bioactive compounds confirms the medicinal importance and it's application for curing various diseases by traditional practitioners. However, isolation and characterization of potential bioactive compounds would lead to drug formulation.

Keywords: *Tiliacora racemosa,* Gas chromatography-mass spectrometry, Phytochemicals.

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INTRODUCTION

In India, from ancient time, different parts of medicinal plants have been used to cure specific ailments. Today, there is a widespread interest in drugs derived from plants. The shortcomings of the drugs available today propel the discovery of new pharmacotherapeutic agents in medicinal plants [1]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway [2]. The medicinal actions of plants unique to a particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [3]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [4].

Tiliacora racemosa is a climbing shrub belonging to the Family Menispermaceae. This plant is utilized in many Ayurvedic preparations [5]. Paste of leaves and roots are applied externally for cuts and wounds [6], and decoction of root and leaf paste is applied for treating snakebite [7,8]. Leaves have anti-dandruff properties and kill the lice and nits [9]. The decoction of the leaf along with the paste of long peppers is given for strangury [10]. Taking into consideration of it's medicinal importance, the present study was aimed at identifying the phytoconstituents of methanolic extract of *T. racemosa* using gas chromatography-mass spectrometry (GC-MS).

METHODS

Chemicals

The chemicals used in this study were all purchased from Avantor Performance Materials India Ltd., Thane, Maharashtra, India.

Collection and authentication of plant materials

Fresh plants of *T. racemosa* were collected from Guruvareddiyur section, Chennampatti range, Western Ghats, Tamil Nadu, India. The collected plant was identified with the help of Flora [11] and authenticated by the Botanical Survey of India (Southern circle), Coimbatore. The voucher number of the specimen is BSI/SRC/5/23/2016/Tech./1521.

Preparation of plant extract

The collected fresh leaves were washed thoroughly with running tap water, shade dried at room temperature and ground into powder using a blender. Powdered plant material was successively extracted with petroleum ether, chloroform, methanol and water as solvents (50 g/250 ml) using Soxhlet apparatus for 6–10 h. Powdered plant material was air-dried before each extraction. The extract obtained using each solvent was evaporated to remove excess solvent and then refrigerated at 4°C for further use.

Physicochemical analysis

Physicochemical characteristics of powdered sample such as moisture content, total ash, acid insoluble ash, sulfated ash and water soluble ash were determined by following standard procedures [12,13].

Preliminary phytochemical screening

Preliminary qualitative phytochemical screening of different successive solvent extracts was carried out according to standard procedures [12–14] to identify the secondary metabolites and other phytochemicals.

GC-MS analysis

GC-MS analysis of a methanolic extract of leaves of *T. racemosa* was carried out at the South India Textile Research Association (SITRA) to analyze the composition of different volatile compounds. The analysis

was performed on a Thermo GC-Trace Ultra version: 5.0, Thermo MS DSQ with a DB 35 MS capillary standard non-polar column (30 m×0.25 mm, 0.25 µm), and gas chromatograph interfaced to a mass selective detector (MS DSQ II) with Xcalibur software. 1 µl of sample was injected in the splitless mode and the injector temperature was 250°C. Helium was used as a carrier gas with a flow rate of 1 ml/min. The oven temperature was programmed initially at 70°C and then was increased to 260°C at the rate of 6°C/min. The total runtime was 37.52 min. Electron ionization mass spectra were measured at 70eV over mass range (m/z) of 50–650 atomic mass units (amu).

Identification of bioactive components

The bioactive components were identified by comparing the mass spectrum of unknown components with the data available in the NIST and WILEY library sources. Biological activities of identified compounds were retrieved by the literature review and from Dr. Duke's Phytochemical and Ethnobotanical database.

RESULTS

The extractive yield was found to be 4.34%, 2.19%, 7.19%, and 1.78% for the solvents namely, petroleum ether, chloroform, methanol and water respectively. The results of the physicochemical evaluation of *T. racemosa* are presented in Table 1. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, triterpenoids, steroids, proteins and amino acids, carbohydrates, saponins and coumarin in different solvent extracts. Quinones, anthraquinones, glycosides and fixed oil were absent in all solvent extracts (Table 2).

GC-MS was carried out in methanolic extract of leaves of *T. racemosa* and the total ion chromatogram showing the GC-MS profile of the identified compounds is shown in Fig. 1. The peaks in the chromatogram were compared and identified with the database of NIST and WILEY library. GC-MS analysis revealed the presence of 28 compounds (Table 3) which include different fatty acids and heterocyclic compounds. The most abundant compound observed was quinic acid (retention time [RT]: 15.65) and inositol, 1-deoxy- (CAS) (RT: 19.24) with peak area percentage 27.12 and 19.88 respectively, and totally representing 47% of the total peak area. This was followed by erythritol, à-D-Glucopyranoside, á-D-fructofuranosyl, 1-Nitro-1-deoxy-d-glycero-l-mannoheptitol, DL-Arabinose and other compounds. The compound with the lowest peak area percentage was quercetin 7,3',4'-Trimethoxy and 9,12-Octadecadienoyl chloride with peak area percentage of 0.65 and 0.66 respectively. The compound 9,12,15-Octadecatrienoic acid and inositol, 1-deoxy- (CAS) occurred at two retention time each with the highest peak area percentage of 2.21 and 19.88 at retention time 25.72 and 19.24 respectively.

Table 1: Physicochemical values of leaf powder of *Tiliacora racemosa*

Physicochemical properties	Values (%)
Moisture content	3.75
Total ash	7.086
Acid insoluble ash	38.7
Sulfated ash	4.67
Water-soluble ash	67.9

DISCUSSION

The extraction yield calculated for petroleum ether, chloroform, methanol and water extracts of *T. racemosa* showed that methanol extract registered a higher percentage of yield. It is explained that the polarity level and species nature are playing a major role in extracting the secondary metabolites [15]. Qualitative phytochemical screening is a preliminary analysis done before the detailed phytochemical and pharmacological investigation. These phytochemicals possess a wide range of medicinal properties.

Alkaloids possess antibacterial and antidiabetic properties [16,17]. Flavonoids are known to possess anticancer, antiviral, anti-inflammatory and antioxidant properties [18,19]. Phenolic compounds are well known to possess biological activities such as antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial and anticancer [20,21]. Glycosides also have immense therapeutic efficacy as they are found in almost every medicinal plant and steroids are responsible for cholesterol-reducing properties and regulating the immune response [22]. Triterpenoids have diverse biological activities that include immunostimulation, antimicrobial, anti-inflammatory, anti-cancer and antiviral properties [23,24].

GC is one of the most widely used techniques and has become one of the most important tools for the separation of volatile compounds. GC-MS analysis of *T. racemosa* revealed the presence of 28 compounds with many biological properties which may contribute to the medicinal properties of the plant. For instance, 9,12,15-Octadecatrienoic acid (Z,Z,Z) (Linolenic acid, RT 25.72) possesses anti-inflammatory, anticancer, nematicide, hepatoprotective, antihistaminic, antieczemic, 5-alpha reductase inhibitor and antiarthritic properties [25]. N-Hexadecanoic acid (palmitic acid, RT 22.42) can be an antioxidant, hypercholesterolemic, antiandrogenic, antifibrinolytic and 5-alpha reductase inhibitors. Vitamin E is known to possess anti-aging, analgesic, antidiabetic, anti-inflammatory, antioxidant, antileukemic and hepatoprotective properties [26,27].

Table 2: Qualitative phytochemical analysis of leaf powder of *Tiliacora racemosa*

Phytoconstituents	Inference			
	Petroleum ether	Chloroform	Methanol	Water
Alkaloids	+	+	+	+
Flavonoids	+	+	+	−
Phenols	+	+	+	+
Tannins	+	−	+	−
Triterpenoids	−	+	+	−
Steroids	−	+	+	−
Carbohydrates	−	−	+	−
Glycosides	−	−	−	−
Proteins and Amino acids	−	−	−	+
Quinones	−	−	−	−
Anthraquinones	−	−	−	−
Saponins	−	−	+	+
Fixed oil	−	−	−	−
Coumarin	−	−	+	+

(+) : Present, (−) : Absent

Table 3: Phytochemical constituents of methanolic extract of *Tiliacora racemosa*

Compound Name	Retention Time (min)	Peak Area (%)	Molecular formula	Molecular weight	Nature of the compound
DL-Arabinose	3.51	3.17	C_5H10O_5	150	Monosaccharide
Desulfosinigrin	4.84	0.98	$C_{10}H17NO_6S$	279	Substituted carbohydrate
Methyl 6-oxoheptanoate	6.16	0.83	C_8H14O_3	158	Ester
Erythritol	7.38	5.61	C_4H10O_4	122	Sugar alcohol
Dodecane	7.85	2.98	$C_{12}H_{26}$	170	Alkane
2,3-Dihydro-benzofuran	8.16	1.88	C_8H_8O	120	Terpenoid
3-(Diethoxymethyl)-1,5-bis (trimethylsilyl)-1,4-pentadiyn-3-ol	8.56	1.54	$C_{16}H_30O_3Si_2$	326	Heterocyclic compound
1-Nitro-1-deoxy-d-glycero-l-mannoheptitol	9.24	3.43	$C7H_{15}NO_8$	241	Sugar alcohol
9,12,15-Octadecatrienoic acid	10.27 25.72	1.81 2.21	$C_{27}H_52O_4Si_2$	496	Unsaturated fatty acid
Benzeneacetic acid	11.84	0.76	$C_8H_8O_2$	136	Carboxylic acid
à-D-Glucopyranoside, á-D-fructofuranosyl	12.19	5.53	$C_{12}H_23O_{11}$	343	Carbohydrate
4-Methylmannose	13.76	2.95	$C_7H_{14}O_6$	194	Carbohydrate
Quinic acid	15.65	27.12	$C_7H_{12}O_6$	192	Monocarboxylic acid
Inositol, 1-deoxy- (CAS)	16.41 19.24	1.96 19.88	$C_6H_{12}O_5$	164	Sugar alcohol
Hexadecanoic acid, methyl ester (CAS)	21.77	0.88	$C_{17}H_{34}O_2$	270	Fatty acid ester
Hexadecanoic acid (CAS)	22.42	1.77	$C_{16}H_{32}O_2$	256	Saturated fatty acid
1-Methyl-8-phenyl-3,4-dihydropyrrolo[1,2-a] pyrazine	22.95	0.78	$C_{14}H_{14}N_2$	210	Heterocyclic compound
á-D-Mannofuranoside, 1-O-(10-undecenyl)-	23.64	1.01	$C_{17}H_{32}O_6$	332	Carbohydrate
9,12-Octadecadienoyl chloride, (Z, Z)-	25.07	0.66	$C_{18}H_{31}C_{10}$	298	Unsaturated hydrocarbon
Quercetin 7,3',4'-Trimethoxy	27.80	0.65	$C_{18}H_{16}O_7$	344	Flavonoid
9,12,15-Octadecatrienoic acid, 2[(trimethylsilyl) oxy]-1[[(trimethylsilyl) oxy] methyl] ethyl ester, (Z, Z, Z)-	28.33	1.10	$C_{27}H_{52}O_4Si_2$	496	Fatty acid ester
Pregnan-3-one, (5à)-	30.54	1.44	$C_{21}H_{34}O$	302	Ketone
1,2 Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS)	31.44	2.59	$C_{24}H_{38}O_4$	390	Carboxylic acid ester
Vitamin E	32.60	0.76	$C_{29}H_50O_2$	430	Vitamin compound
Stigmasta-3,5-dien-7-one (CAS	33.30	0.78	$C_{29}H_{46}O$	410	Ketone
9-Octadecenamide, (Z)- (CAS)	35.43	0.81	$C_{18}H_{35}NO$	281	Amide
Campesterol	36.18	1.72	$C_{28}H48O$	400	Sterol
Stigmasterol CAS	37.61	2.43	$C_{29}H_{48}O$	412	Sterol

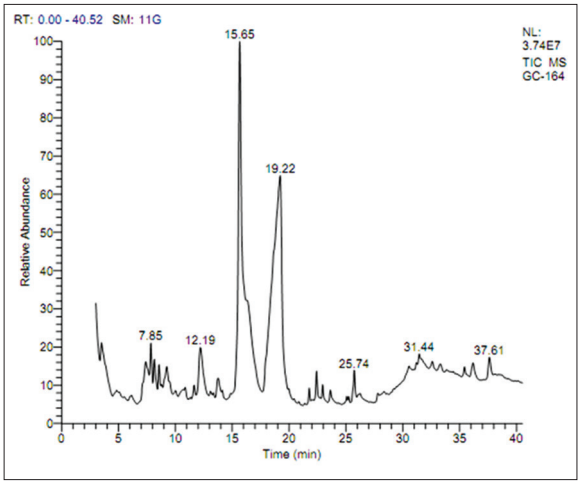

Fig. 1: Gas chromatography-mass spectrometry chromatogram of methanolic extract of *Tiliacora racemosa*

In the present study, mostly all compounds are fatty acids, esters, and carbohydrates. Similar kind of results has also been observed by Gupta and Kumar in GC-MS analysis of *Camellia sinensis* and *Terminalia arjuna* [28]. Chaveerach *et al.* carried out GC-MS analysis of *Tiliacora triandra* and reported the presence of various bioactive compounds [29]. Similar results have been obtained in the present study, but this is probably the first report indicating a higher percentage of quinic acid in methanolic extract of leaves of *T. racemosa* which is known to possess choleretic [26], antioxidant [30] and anticancer [31] properties. The results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant [3].

CONCLUSION

The presence of various bioactive compounds confirms the medicinal importance and its application for curing various diseases by traditional practitioners. However, isolation and characterization of potential bioactive compounds would lead to drug formulation.

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AUTHORS CONTRIBUTIONS

Yogeshwari C collected the ethnomedicinal plant and carried out experimental work such as extract preparation and phytochemical analysis. Kumudha P is the principal investigator who supervised the work and corrected the manuscript for publication. Both authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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