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GC-MS Characterization of Antioxidative Compounds from the Stem Bark and Flower Extracts of *Schefflera* Species, from Western Ghats

Deepa Raghavendra Hebbar[1], Monnanda Somaiah Nalini[2*]

[1]*Department of Botany, Maharani's Science College for Women, JLB road, Mysore, Karnataka, India*

[2]*Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India*

[*]***Corresponding author:*** *Dr. M. S. Nalini, Associate Professor, Department of Studies in Botany, University of Mysore. Manasagangotri, Mysore, Karnataka, India, Tel: +91 821 2419765; Fax: +91 821 2419759; E-mail: nmsomaiah@gmail.com*

ABSTRACT

Objective: *Investigation and identification of antioxidative compounds of methanolic flower extract of S. venulosa and ethyl acetate stem bark extract of S. wallichiana (Araliaceae) from Western Ghats.*

Methods: *Powdered stem bark and flower materials were subjected to Soxhlet extraction using various solvents according to their polarity. The methanolic flower extract of S. venulosa and the ethyl acetate stem bark extract of S. wallichiana were reconstituted in the respective solvents. One µl of the extracts was injected for GC-MS analysis. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral (NIST-MS) database.*

Results: *The methanolic flower extract of S. venulosa revealed the presence of 120 compounds whereas ethyl acetate stem bark extract of S. wallichiana showed the presence of 115 compounds. Among those, 23 compounds from methanol flower extract of S. venulosa and 25 compounds from the ethyl acetate stem bark extract of S. wallichiana have been identified as the probable antioxidant compounds based on the functional groups they possess.*

Conclusion: *Many bioactive compounds were identified from two Schefflera species. Thus, the identification of different biologically active compounds in these extracts warrants further biological and pharmacological studies.*

Keywords: Antioxidative compounds, Characterization, Gas chromatography, *Schefflera* species.

INTRODUCTION

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in medicine [1]. Plants used in traditional medicine contain a wide range of substances that can be used to cure chronic as well as infectious diseases [2]. An essential part in the investigation of plant is the identification of the biologically active compounds present in the plant leading to further biological and pharmacological studies [3].

The genus *Schefflera* is an epiphytic and lianoids, with about 1100 species widely distributed in the tropics and subtropics. The leaves and stem bark of several *Schefflera* species are used as a remedy for cough and as a diuretic. The ethnomedicinal uses of *Schefflera* include the treatment of asthma, liver diseases, rheumatism, arthritis, sprains, fracture, stomach pain, antipyretic, anti-inflammatory, analgesic, migraine and general tonic [4]. *S. venulosa* extract contains mainly caffeoyl acids, quercetin glycoside and oleanolic acid glycoside which helps in blood circulation and prevents cardiac and cerebral vascular diseases [5]. Phytochemical studies on *S. venulosa* and *S. wallichiana* have identified the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides steroids, terpenoids and reducing sugars. The estimation of total phenolic content and free radical assays such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), Ferric reducing antioxidant power (FRAP) and Reducing power assay revealed that the methanolic flower extracts of *S. venulosa* and ethyl acetate stem bark extracts of *S. wallichiana* showed potent antioxidant activity when compared to standard antioxidants such as ascorbic acid and butylatedhydroxytoulene (BHT) [6]. The identification of bioactive compounds present in methanolic flower extracts of *S. venulosa* and ethyl acetate stem bark extracts of *S. wallichiana* is conducted for further studies. There are no published literatures that determine the bioactive compounds present in the different extracts of *Schefflera* species studied. Therefore this study is aimed to investigate the bioactive compounds in the methanolic flower extracts of *S. venulosa* and the ethyl acetate stem bark extracts of *S. wallichiana*.

MATERIALS AND METHODS

Collection of plant materials

S. wallichiana and *S. venulosa* were collected from the natural forests of Kodagu (N12°20′14.97″and E75°48′24.86″), in the Western Ghats during May 2014. The stem bark was separated with a machete and flowers were detached from the plant, excised with plier and placed in zip lock polythene bags, labeled, brought to the laboratory and processed for further use. They were dried under shade, powdered and stored for further use.

Preparation of the extract

Around, 500 g of the powdered stem bark and flower material was subjected to Soxhlet extraction (Borosilicate glass, Padmashree Scientific®) using various solvents according to their polarity. After extraction,the filtrate was collected and the solvent was evaporated using rotary evaporator.

Chemicals

Analytical grade solvents such as hexane, chloroform, ethyl acetate, ethanol, methanol, silica gel of mesh size (60-120), acetonitrile, acetic acid, acetone and EDTA were purchased from SRL and Merck limited, India. All other chemicals used were of analytical grade.

GC-MS analysis

The methanolic flower extract of *S. venulosa* (5 mg/ml) and the ethyl acetate stem bark extract of *S. wallichiana* (5 mg/ml) were reconstituted in the respective solvents. One µl of the extracts was injected for GC-MS analysis. GC-MS analysis was carried out using a Clarus 500 PerkinElmer Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold–PerkinElmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), capillary column (30 m x 0.25 mm) at The South India Textile Research Association (SITRA), Coimbatore, Tamilnadu, India. The instrument was set to an initial temperature of 40°C, and maintained at this temperature for 5 min. After completion, the oven temperature was raised up to 280°C, at 6°C per min raise and maintained for 15 min. Helium (1 ml/min) was used as a carrier gas. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral database (NIST-MS; http://www.sisweb.com/software/ms/nist.htm#gc).

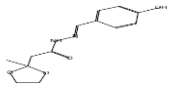
Identification of bioactive compounds

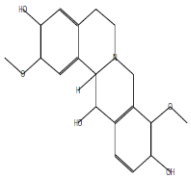
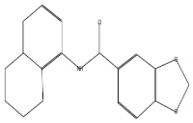
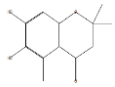
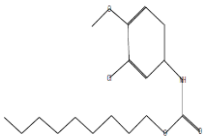
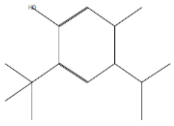
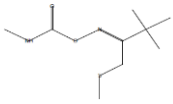
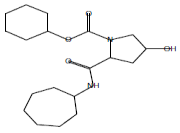
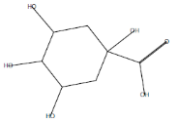
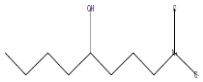
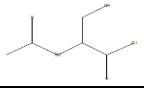
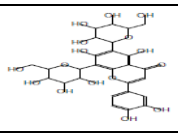
The GC-MS data were interpreted with the aid of the National Institute of Standards and Technology (NIST) database. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The percentage of each component for both the extracts was calculated separately for the relative peak area of each component in the chromatogram. The retention time, peak area, structure and molecular weight of the compounds were identified from both plant extracts and represented.

RESULTS

The probable antioxidant compounds identified from the methanolic flower extract of *S. venulosa* and ethyl acetate stem bark extract of *S. wallichiana* are summarized in Tables 1 and 2 respectively. The methanolic flower extract of *S. venulosa* revealed the presence of 120 compounds whereas, the ethyl acetate stem bark extract of *S. wallichiana* showed the presence of 115 compounds. Among those, 23 compounds from methanolic flower extract of *S. venulosa* and 25 compounds from the ethyl acetate stem bark extract of *S. wallichiana* have been identified as the probable antioxidant compounds based on the functional groups they possess.

Table 1: Probable antioxidant compounds from methanol flower extract of *S. venulosa* by GC-MS.

Sl. no.	Compound name	Molecular formula	Molecular weight	Retention Time	Peak area %	Structure
1	1,3-Dioxolane-2-acetic acid, 2-methyl-hydrazide	$C_{13}H_{16}N_2O_4$	264	3.05	1.93	

2	Corydalidzine	$C_{19}H_{21}NO_5$	343	6.76	2.42	
3	N-[1-(5,6,7,8-Tetrahydronaphthyl)]-3,4-methylenedioxybenzamide	$C_{18}H_{17}NO_3$	295	6.76	2.42	
4	6,7-Dihydroxy-2,2,5-trimethyl-4-chromanone	$C_{12}H_{14}O_4$	222	8.89	0.73	
5	Carbamic acid	$C_{17}H_{26}ClNO_3$	327	11.82	0.55	
6	2-ter-Butyl-4-isopropyl-5-methylphenol	$C_{14}H_{22}O$	206	13.87	1.54	
7	Thiofanox	$C_9H_{18}N_2O_2S$	218	16.3	6.86	
8	Pyrrolidine-1-carboxylicacid,2-cycloheptylaminocarbonyl-4-hydroxy-,phenyl ester	$C_{19}H_{26}N_2O_4$	346	19.26	0.54	
9	Quinic acid	$C_7H_{12}O_6$	192	20.71	21.28	
10	(S)-(+)-1-Nitro-4-octanol	$C_8H_{17}NO_3$	175	20.71	21.28	
11	2-Acetylamino-3-hydroxy-propionic acid	$C_5H_9NO_4$	147	20.71	21.28	
12	Lucenin 2	$C_{27}H_{30}O_{16}$	610	22.11	2.97	

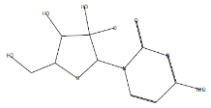
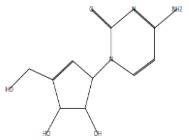
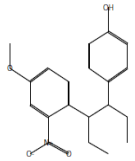
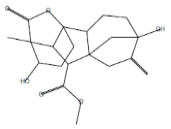
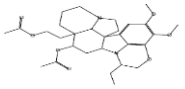
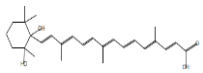
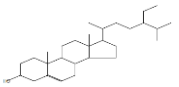
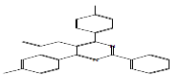
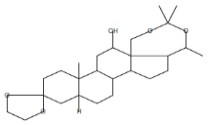
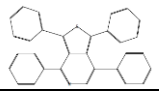
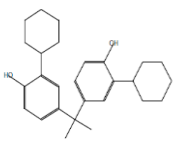
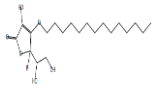
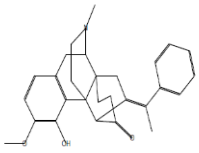
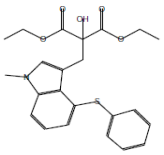
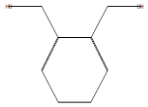
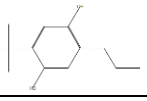
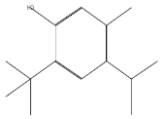
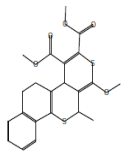
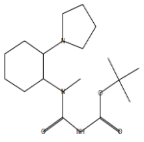
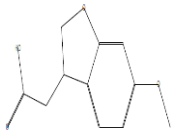
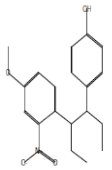
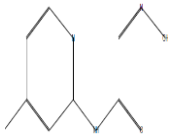
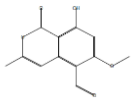
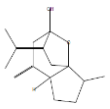
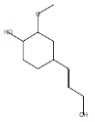
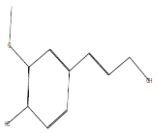
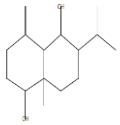
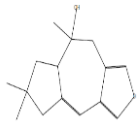
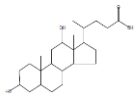
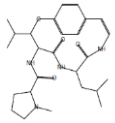
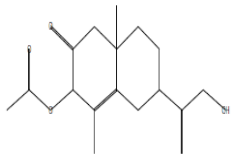
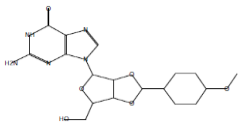
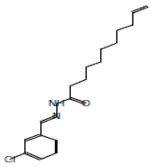
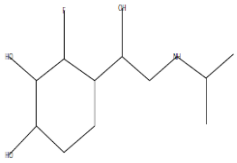
13	Cytidine-2'-D	$C_9H_{12}DN_3O_5$	243	22.11	2.97	
14	2(1H)-Pyrimidinone, 4-amino-1-(4,5-dihydroxy-3-(hydroxymethyl)-2-cyclopen	$C_{10}H_{13}N_3O_4$	239	22.11	2.97	
15	(3R*, 4S*)-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydroxyphenyl) hexane	$C_{19}H_{23}NO_4$	329	22.82	0.59	
16	Gibberellin A1 methyl ester	$C_{20}H_{26}O_6$	362	31.37	0.71	
17	Dihydroobscurinervinedioldiacetate	$C_{29}H_{40}N_2O_7$	528	31.37	0.71	
18	Azafrin	$C_{27}H_{38}O_4$	426	32.4	4.18	
19	Stigmast-5-en-3-ol,(3a,24S)-(CAS)	$C_{29}H_{50}O$	414	32.4	4.18	
20	5-Allyl-2-phenyl-4,6-bis(4-tolyl)pyrimidine	$C_{27}H_{24}N_2$	376	36.21	0.96	
21	Pregnane-12,18,20-triol,18,20-isopropylidene-3,3-ethylenedioxy-	$C_{26}H_{42}O_5$	434	37.56	1.68	
22	1,3,4,7-Tetraphenylthienol[3,4-c]pyridine	$C_{31}H_{21}NS$	439	40.02	0.51	
23	4,4'-Isopropylidene-bis-(2-cyclohexylphenol)	$C_{27}H_{36}O_2$	392	40.02	0.51	

Table 2: Probable antioxidant compounds from ethyl acetate stem bark extract of *S. wallichiana* by GC-MS.

S. No.	Compound name	Molecular formula	Molecular weight	Retention Time	Peak area %	Structure
1	3-O-Tetradecyl-L-ascorbic acid	$C_{20}H_{36}O_6$	373	3.1	41.71	
2	3-Methoxy-4-hydroxy-17-methyl-18-[(E)-a-methylbenzylidene]-3-(triohenylmethoxy)-5,14-ethanomorphinan-6-one	$C_{28}H_{31}NO_3$	429	5.34	1.46	
3	Diethyl 2-hydroxy-2-(1-methyl-4-phenylthio-1H-indol-3-ylmethyl)propanedioate	$C_{23}H_{25}NO_5S$	427	5.34	1.46	
4	2-hydroxymethylbenzenemethanol	$C_8H_{10}O_2$	138	9.87	5.19	
5	2-Allyl-5-t-butylhydroquinone	$C_{13}H_{18}O_2$	206	13.9	2.8	
6	2-tert-Butyl-4-isopropyl-5-methylphenol	$C_{14}H_{22}O$	206	13.9	2.8	
7	5,6Dihydro 1-methoxycarbonyl)-12-methyl-4aH,12H-2,11-dithiachrysene	$C_{22}H_{22}O_5S_2$	430	14.54	0.65	
8	Urea, N-t-butoxycarbonyl-N-[2(trans)-(1-tetrahyropyrrolyl)-1(E)-cyclohexyl]-	$C_{17}H_{31}N_3O_3$	325	14.54	0.65	

9	3-[(3-methoxy-propylamino)-methyl]-5,8a-dimethyl-3a,4,6,7,8,8a,9,9a-octahydro-3H-naphto[2,3-b]furan-2-one	$C_{19}H_{31}NO_3$	321	16.5	0.51	
10	6-methoxy-2,3-dihydro-1-benzofuran-3-acetic acid	$C_{11}H_{12}O_4$	208	18.97	0.48	
11	$(3R^*,4R^*)$-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydroxyphenyl)hexane	$C_{19}H_{23}NO_4$	329	18.97	0.48	
12	2-(Hydroxyiminoacetamido)-4-methypyridine	$C_8H_9N_3O_2$	179	19.78	0.93	
13	5-formyl-8-hydroxy-6-methoxy-3-meethylisocoumarin	$C_{12}H_{10}O_5$	234	21.29	1.95	
14	Isocurcumenol	$C_{15}H_{22}O_2$	234	21.29	1.95	
15	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	22.08	5.44	
16	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	$C_{10}H_{12}O_3$	180	22.08	5.44	

17	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-(CAS)	$C_{10}H_{12}O_3$	180	22.08	5.44	
18	Platambin	$C_{15}H_{26}O_2$	238	23.25	1.45	
19	Furoscrobiculin	$C_{15}H_{20}O_2$	232	23.84	1.95	
20	Deoxycholic acid	$C_{24}H_{40}O_4$	392	31.15	1.42	
21	Ceanothine C (CAS)	$C_{26}H_{38}N_4O_4$	470	33.72	0.55	
22	Acetic acid 7-(1-Hydroxymethyl-vinyl)-1,4A-Dimethyl-3-oxo-2,3,4,4A,5,6,7,8-octah	$C_{17}H_{24}O_4$	292	33.72	0.55	
23	2',3'-O-p-Anisylideneguanosine	$C_{18}H_{19}N_5O_6$	401	33.72	0.55	
24	N'-(3-Chlorobenzylidene)-10-Unedecenoic acid Hydrazide	$C_{18}H_{25}ClN_2O$	320	34.2	1.21	
25	Benzeneethanamine, 2-fluoro-a,3,4-trihydroxy-N-isopropyl-	$C_{11}H_{16}FNO_3$	229	34.2	1.21	

DISCUSSION

The isolation of bioactive compounds from the crude plant extracts is practically more complex task. Due to the enormous diversity of compounds from plants, it offers a considerable challenge for their isolation and identification. In the present study, two extracts showing potent antioxidant activity, *viz*., methanolic flower extract of *S. venulosa* and ethyl acetate stem bark of *S. wallichiana* with an IC_{50} value of 16.26±0.17 and 18.36±0.20 respectively were analyzed for the first time by GC-

MS to determine the type(s) of probable antioxidant compounds present [6]. The antioxidant activity being one of the important biological activities is much concentrated these days. The antioxidant activity species has not been much documented from the species of *Schfflera*. Only two species *S. leucantha* [7] and *S. actinophylla* [8] have been studied for their antioxidant activity by DPPH assay. Till date, no reports exist on the isolation and identification of antioxidant compounds from *Schefflera* species based on GC-MS or any other spectral studies.

Based on the studies, some of the constituents revealed by GC-MS are biologically active compounds. They were proven to possess pharmacologic activities which may contribute to the healing potential of the plant. Azafrin, a proven natural carotenoid antioxidant identified from methanol flower extract of *S. venulosa*, is said to exhibit cardioprotective effects against myocardial injury [9]. Some of the compounds like carbamic acid are used as muscle relaxants [10], Corydalizine, an alkaloid, is used as a nematicide [11] and Quinic acid is a cyclitol used as an astringent. It is a building block in the preparation of the treatment of Influenza A and B strains called Tamiflu [12] Similarly Isocurcumenol, identified from ethyl acetate stem bark of *S. wallichiana* is said to be an antitumour agent [13]. Deoxycholic acid facilitates fat absorption and cholesterol excretion [14]. Because of the diversity and complexity of the natural mixtures of bioactive compounds in the crude plant extracts, it is rather difficult to characterize every compound present and elucidate its structure in a single study.

CONCLUSION

Identification of these compounds in the plants studied, serves as the basis in determining the possible health benefits of the plants leading to further biological and pharmacological studies. The isolation, identification and characterization of compounds from two extracts showing potent antioxidant activity viz. methanol flower extract of *S. venulosa* and ethyl acetate stem bark extract of *S. wallichiana* were analyzed by GC-MS to determine the type(s) of probable antioxidant compounds present. Nearly twenty-three probable antioxidant compounds from the methanol flower extract of *S. venulosa* and twenty five probable antioxidant compounds from the stem bark ethyl acetate extract of *S. wallichiana* were identified.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest was involved in this study.

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