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GC-MS determination of bioactive components of *Wedelia chinensis* (Osbeck) Merrill

***H. Rehana Banu[a] and N. Nagarajan[b]**

[a] *Department of Botany, Nirmala College for Women, Red fields, Coimbatore*
[b] *PG and Research Department of Botany, Kongunadu Arts and Science College, G. N. Mills Post, Coimbatore*

ABSTRACT

Medicinal plants are sources of important therapeutic aids for alleviating human ailments. The traditional use of medicinal plants leaf extract for diseases is quite common in developing countries like India. In a view to understand the scientific reason behind its medicinal value, an attempt is made in this study, to analyze major bioactive compounds present in the leaf extract from Wedelia chinensis (Osbeck) Merrill (Family Asteraceae) by GC-MS. The major chemical constituents are 2-Tridecanone (CAS) (4.51%), n-(methoxyphenylmethylene) carbamic acid ethyl ester (1.65%), and 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (13.68%). The presence of some of these constituents in the plant extract provides the scientific evidences for the antimicrobial, anti-tumor, and antioxidant properties of the plant. Further study is required to find out the specific phytochemical which is responsible for its medicinal value.

Keywords: *Wedelia chinensis*, Asteraceae, GC–MS, 2-Tridecanone and phytochemical.

INTRODUCTION

Wedelia chinensis (Osbeck) Merrill (Syn. *Solidago chinensis*) (Family - Asteraceae) is a small much branched annual herb with camphor like odour. *Wedelia chinensis* is a tender, spreading, and hairy herb, with the branches usually less than 50 cm long. The leaves are oblong to oblong-lanceolate, 2-4.5 cm in length, and narrowed at both ends. The margins are entire or obscurely toothed; and both surfaces are covered with sharp-pointed, appressed, straight, and stiff hairs. The heads are stalked, about 1 cm in diameter, and yellow. The involucral bracts are oblong-ovate. The ray flowers are 8-12, spreading, about equal to the bracts, and broad; the disk flowers number about 20, and are short, narrow, and pointed. The achenes are nearly cylindric, and hairy [1].

The herb contains wedelolactone and demethylwedelolactone (Coumestans derivatives) possessing potent anti-hepatotoxic effect and is incorporated as a major ingredient in a number of developed potent anti-hepatotoxic phytopharmaceuticals formulations. It is useful in the treatment of osteoporosis of knee and also possesses anti-inflammatory activity [2,3,4]. The plant is used in the Indian System of medicine for tonic properties [5].

There is an increased quest to obtain natural antioxidants with broad-spectrum action. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. The aim of this research effort was to identify the phytochemical constituents present in the leaves in order to understand the nature of the bioactive component responsible for its therapeutic value.

EXPERIMENTAL SECTION

Plant Material

Wedelia chinensis (Osbeck) Merrill leaves were collected from Attakatti Hills, India. The plant material was taxonomically identified by Dr. V. Balasubramanian, Associate Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India and was deposited at the college herbarium for future reference.

Preparation of plant extracts

For extraction about 50g of the shade dried and powdered leaf material was taken. The powdered material was transferred into 250 ml quick fit flask and extracted in the soxhlet extractor for 48 hours [6,7] using of organic solvents namely petroleum ether, chloroform, ethyl acetate and methanol separately according to the increasing polarity of the solvents. The extracts were filtered over Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass [8] for further studies.

Gas chromatography-MS analysis

GC-MS analysis was performed in Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India. Five ml of methanol extract was evaporated to dryness and reconstituted in 2 ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35 mr column (10 m x 0.5 mm, 0.25 mm film thickness). Heating programs were executed from 100-250ºC at 3 minutes by using helium as a carrier gas with a flow rate of 1ml/min in the split mode (1:50). An aliquot (2 ml) of oil was injected into the column with the injector heater at 250ºC.

Analytical conditions

Injection temperature at 250ºC, interface temperature at 200ºC, quadruple temperature at 150ºC and ion source temperature at 230ºC were maintained. Injection was performed in split less mode.

Identification of components (Data analysis)

The mass spectra of compounds in samples were obtained by electron ionization (El) at 70 eV, and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST [9,10].

Identification of components:

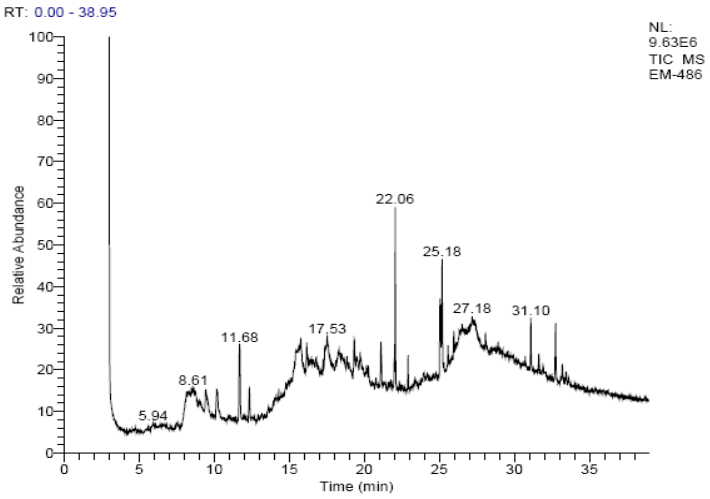
Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

RESULTS

Gas chromatography mass spectrometry (GC-MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. GC-MS methods proved to be very effective and sensitive for the separation and detection of complex mixtures of phytochemicals [11,12,13].

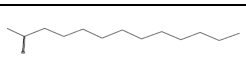
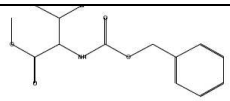
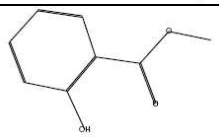
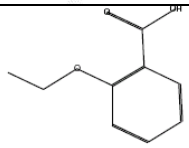
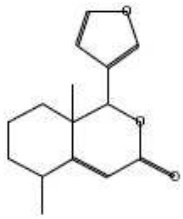
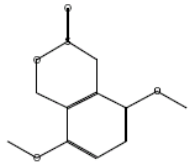
The GC-MS analysis of *Wedelia chinensis* leaf extract revealed the presence of 25 compounds (phytochemical constituents) (Figure 1) that could contribute to the medicinal property of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage are presented in (Table 1).

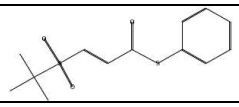
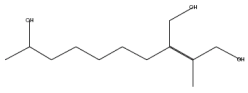
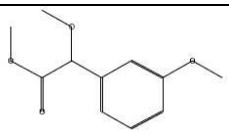
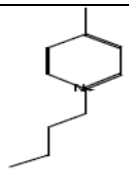
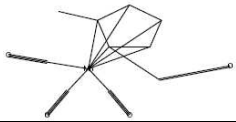
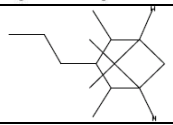
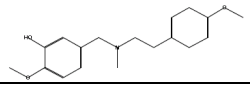
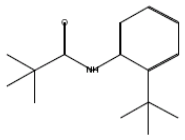
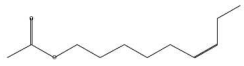
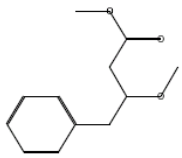
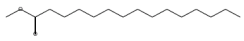
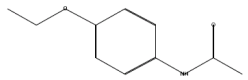
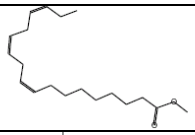
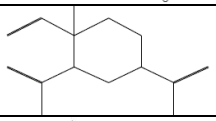
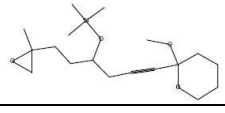
Figure 1. GC-MS Analysis of *Wedelia chinensis* leaves



Four compounds of ester group, 3 compounds of amino group, two compounds of acetate, aromatic, benzoic acid and sulfur groups and one compound each in ketone, alkene, alkaloid, aldehyde, hydrocarbon, amide, fatty acid ester, linolenic acid ester, sesquiterpene and silica group were identified. The first compound identified with less retention time (8.15min) was 2-Tridecanone (CAS), while n-(methoxyphenylmethylene) carbamic acid, ethyl ester had long retention time (33.19min). The phytochemicals identified through GC-MS analysis showed many biological activities that are listed in Table 2.

Table 1. Components detected in *Wedelia chinensis* leaf

No	RT	Peak Area %	Name of Compound	Molecular Formula	MW	Structures
1	8.15	4.51	2-Tridecanone (CAS)	$C_{13}H_{26}O$	198	
2	8.63	2.54	methyl (2R,3S)-(N-benzyloxycarbonyl)-2-amino-3--bromobutyrate	$C_{13}H_{16}BrNO_4$	329	
3	9.44	3.71	Benzoic acid, 2-hydroxy-, methyl ester	$C_8H_8O_3$	152	
4	10.18	3.24	Benzoic acid, 2-ethoxy-	$C_9H_{10}O_3$	166	
5	11.68	7.98	(1RS,5SR,8aRS)-1-(3-furyl)-5,8a-dimethyl-1,5,6,7,8,8a-hexahydro-3H-2-benzopyran-3-one	$C_{15}H18O3$	246	
6	12.33	2.58	4,5-(1,4-Dimethoxybenzo)-3,6-dihydro-1,2-oxathiin-2-oxide	$C_{10}H_{12}O_4S$	228	

7	14.76	1.43	2-Propenethioic acid, 3-[(1,1-dimethylethyl)sulfonyl]-, S-phenyl ester, (E)- (CAS)	$C_{13}H_{16}O_3S_2$	284	
8	15.74	8.65	1,9-Dihydroxy-3-(hydroxymethyl)-2-methyldec-2-ene	$C_{12}H_{24}O3$	216	
9	16.17	1.94	Methyl 2-Methoxy-2-(3-metoxyphenyl)acetate	$C_{11}H_{14}O_4$	210	
10	17.53	5.05	N-Butyl-4-methylpyridinium	$C_{10}H_{16}N$	150	
11	18.29	1.44	(-)-(1R)-2-Methylcymantrenecarboxaldehyde	$C_{10}H_7MnO_4$	246	
12	18.85	1.88	trans-3-Propyl-cis-4-methyl-cis-pinane	$C_{14}H_{26}$	194	
13	19.32	5.22	N-(3-Hydroxy-4-methoxybenzyl)-N-[2-(4-methoxycyclohexa-1,4-dien-1-yl)ethyl]-N-methylamine	$C_{18}H_{25}NO_3$	303	
14	19.74	2.44	N-(2-tert-Butylphenyl)pivalamide	$C_{15}H_{23}NO$	233	
15	20.26	2.09	(E)-6-Nonenyl acetate	$C_{11}H_{20}O_2$	184	
16	21.11	3.53	2-(3,4-Dimethoxyphenyl)tetrahydropyran	$C_{12}H_{16}O_3$	208	
17	22.06	10.04	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	
18	22.91	1.55	Phenacetin	$C_{10}H_{13}NO_2$	179	
19	25.18	13.68	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292	
20	25.96	1.27	á-elemene	$C_{15}H_{24}$	204	
21	26.50	2.55	7-methyl-1-(tetrahydro-2-methoxypyran-2-yl)-4-teimethylsiloxy-7,8-epoxyoct-1-yne	$C_{18}H_{32}O_4Si$	340	

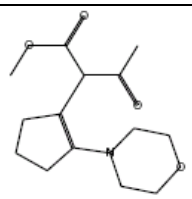
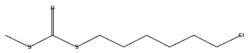
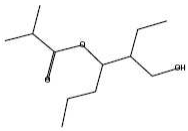
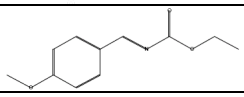
22	27.32	3.44	Methyl ester of à-acetyl-2-(4-morpholinyl) cyclopenteneacetic acid	$C_{14}H_{21}NO_4$	267	
23	31.10	3.28	S-(6-chloro-1-hexyl) S'-methyl dithiocarbonate	$C_8H_{15}ClOS_2$	226	
24	32.74	4.30	Propanoic acid, 2-methyl-, 2-(hydroxymethyl)-1-propylbutyl ester	$C_{12}H_{24}O_3$	216	
25	33.19	1.65	n-(methoxyphenylmethylene) carbamic acid, ethyl ester	$C_{11}H_{13}NO_3$	207	

Table 2. Nature and biological activities of the phytochemical compounds of methanolic leaf extract of *Wedelia chinensis* by GC-MS analysis

No	Name of the compound	Nature of compound	**Activity
1	2-Tridecanone (CAS)	Ketone compound	No activity reported
2	methyl (2R,3S)-(N-benzyloxycarbonyl)-2-amino-3--bromobutyrate	Amino compound	Antimicrobial
3	Benzoic acid, 2-hydroxy-, methyl ester	Benzoic acid compound	Antimicrobial
4	Benzoic acid, 2-ethoxy-	Benzoic acid compound	Antimicrobial, Preservative
5	(1RS,5SR,8aRS)-1-(3-furyl)-5,8a-dimethyl-1,5,6,7,8,8a-hexahydro-3H-2-benzopyran-3-one	Aromatic compound	No activity reported
6	4,5-(1,4-Dimethoxybenzo)-3,6-dihydro-1,2-oxathiin-2-oxide	Sulfur compound	Antimicrobial
7	2-Propenethioic acid, 3-[(1,1-dimethylethyl)sulfonyl]-, S-phenyl ester, (E)- (CAS)	Ester compound	No activity reported
8	1,9-Dihydroxy-3-(hydroxymethyl)-2-methyldec-2-ene	Alkene compound	No activity reported
9	Methyl 2-Methoxy-2-(3-metoxyphenyl)acetate	Acetate compound	No activity reported
10	N-Butyl-4-methylpyridinium	Alkaloid	Antimicrobial, Antiinflammatory
11	(-)-(1R)-2-Methylcymantrenecarboxaldehyde	Aldehyde compound	Antimicrobial
12	trans-3-Propyl-cis-4-methyl-cis-pinane	Hydrocarbon	No activity reported
13	N-(3-Hydroxy-4-methoxybenzyl)-N-[2-(4-methoxycyclohexa-1,4-dien-1-yl)ethyl]-N-methylamine	Amino compound	Antimicrobial
14	N-(2-tert-Butylphenyl)pivalamide	Amide compound	Antimicrobial
15	(E)-6-Nonenyl acetate	Acetate compound	No activity reported
16	2-(3,4-Dimethoxyphenyl)tetrahydropyran	Aromatic compound	No activity reported
17	Pentadecanoic acid, methyl ester	Fatty acid ester	No activity reported
18	Phenacetin	Amino compound	Antimicrobial
19	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	Linolenic acid ester	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
20	á-elemene	Sesquiterpene	Antimicrobial, Anticancer
21	7-methyl-1-(tetrahydro-2-methoxypyran-2-yl)-4-teimethylsiloxy-7,8-epoxyoct-1-yne	Silica compound	No activity reported
22	Methyl ester of à-acetyl-2-(4-morpholinyl)cyclopenteneacetic acid	Ester compound	Antimicrobial
23	S-(6-chloro-1-hexyl) S'-methyl dithiocarbonate	Sulfur compound	Antimicrobial
24	Propanoic acid, 2-methyl-, 2-(hydroxymethyl)-1-propylbutyl ester	Ester compound	Antimicrobial
25	n-(methoxyphenylmethylene) carbamic acid, ethyl ester	Ester compound	Antimicrobial

DISCUSSION

In the last few years gas-chromatography mass-spectrometry has become firmly established as a key technological platform for metabolite profiling in both plant and non-plant species [14,15,16,17,18]. Until relatively recently only a limited number of plant research laboratories had access to gas-chromatography mass-spectrometry instrumentation, however, such machines are increasingly becoming more commonplace.

The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (RT 25.18) possesses anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic antieczemic, antiacne, antiandrogenic, antiarthritic, anticoronary and insectifuge properties. Previously, 9(Z),12(Z),15(Z)-octadecatrienoic acid, methyl ester, also known as α- linolenic acid methyl ester known to inhibit proliferation of ER-positive and ER- negative breast cancer cells [19]. It is a potent antiangiogenic agent in colorectal cancer and in HUVEC cells [20]. The other compounds such as N-(3-Hydroxy-4-methoxybenzyl)-N-[2-(4-methoxycyclohexa-1,4-dien-1-yl)ethyl]-N-methylamine (RT 19.32), (-)-(1R)-2-Methyl cymantrenecarboxaldehyde (RT 18.29) and N-(2-tert-Butylphenyl) pivalamide (RT 19.74) are reported to be antimicrobial.

The GC-MS analysis of the methanolic extract of *W. chinensis* leaves revealed the presence of sulphur compounds that included thiophenes. Thiophenes are sulphur-containing plant metabolites occurring in the Asteraceae plant family [21,22]. Due to their remarkable long wavelength ultraviolet light-dependent biocidal activities, thiophenes have potent phototoxic effects on animal viruses with membranes, bacteria, fungi or nematodes [23,24]. 9(Z),12(Z),15(Z)-octadecatrienoic acid have the property of anti-inflammatory and anti arthritic as reported by earlier workers [25,26].

The volatile oils from the aerial parts of *Gundelia tournefortii* were identified by GC-MS analysis with terpinyl acetate and methyl eugenol as the major oil components [27]. Presence of thiophenes in the *Tagetus patula* in the solvent extracts was confirmed by GC-MS analysis [28]. 9(Z),12(Z),15(Z)-octadecatrienoic acid have been identified in the methanol fraction of *Vernonia amygdalina* [29] and *Dipteracanthus patulus* [30].

CONCLUSION

GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical value of that plant [31]. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study. Further investigations into the pharmacological importance of *Wedelia chinensis* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.

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