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Research article

GC-MS analysis of the ethanol extracts of *Dyschoriste littoralis* Nees. (Acanthaceae)

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Key words: *Dyschoriste littoralis,* bioactive compound, Gas Chromatography (GC), Mass Spectroscopy (MS).

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Abstract

In the present study, ethanol extracts from *Dyschoriste littoralis* Nees. were subjected to GC-MS analysis to study the important phytochemical constituents responsible for the various pharmacological activities. The crude extracts of ethanol were obtained by soxlet method. The GC-MS analysis of ethanol extract from *D. littoralis* revealed the presence of nine phytocompounds in the underground part and twenty six compounds in the aerial parts. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. acetyl chloride, dichloro ($CHCl_2COCl$) (Rt 9.49); Ethane, 1,1-diethoxy- and Methoxymethyl isothiocyanate are prominent compounds in underground part. Three major phytochemical constituent's mass spectra in aerial part are Ethyl Acetate, (3-Methyl-oxiran-2-yl)-methanol, Hydroperoxide and 1-methylbutyl.

Introduction

Dyschoriste littoralis Nees. belongs to the family Acanthaceae. Its antipyretic, analgesic and anti-inflammatory activity were may be due to its bioactive principles lutolin and quercetin present in the extract and reveals that *Dyschoriste littoralis* could be used as potential drug for the treatment of pain, fever and inflammation. *Dyschoriste littoralis* Nees. are considered a very efficacious remedy for all sorts of coughs being administrated along with ginger. The leaves are used for rheumatism. The leaves were dried made into cigarettes and smoked in asthma and their juice is used treatment of diarrhea and dysentery [1]. Identification of the biologically active compounds form the plants is essential to study biological and pharmacological activities [2–4]. In recent years, gas chromatography mass spectrometry (GC-MS) has develop into a vital technological platform for secondary metabolite profiling in both plant and non-plant species [5]. Review of literature record that information on the GC-MS analysis of *Dyschoriste littoralis* is lacking. Hence, the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique. This work will help to identify the compounds of therapeutic value.

Description of selected plant

Slender under shrubs with green branches from a woody root stocks; plant with prostrate stems often rooting at the nodes; leaves elliptic or obovate, 75 - 1.5 in long; flowers in clusters or cymes; flowers in axillary clusters with long – acuminate nearly glabrous; calyx – lobes, small funnel shaped; corolla about 25 in long; anthers. minutely mucoronate at base.

Experimental

Materials and methods

Plant material

The medicinal plant *Dyschoriste littoralis* (Plate: 1) was collected during January - March from Tirunelveli District, Tamil Nadu, India. The identified plant species was confirmed with Voucher specimen available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu (voucher no: 25834). The taxonomic features of the plant confirmed with the Flora of Presidency of Madras [6] and The Flora Tamil Nadu Carnatic [7].



Plate 1. Flowering twig of *D. littoralis*

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Soxhlet extraction

60 gram of shade dried *Dyschoriste littoralis* powder was refluxed with 250 ml of the ethyl alcohol for five hours on a steam bath. The collected extract was concentrated.

Procedure

Shimadzu GC – MS - QP 2010 was used for The GC - MS analysis. Analysis was performed with a DB1fused methylphenylsiloxane, (30 m × 0.25 mm i.d.) capillary column in Gas chromatograph. The column is directly coupled with mass spectrophotometer. Noble gas was used as carrier gas, with a flow of one ml/min. The column temperature was 70°C. The column was programmed for 5 minutes in 180°C, 180-260°C at 3°C/min, 5 minutes in 260°C, 260-280°C at 0.2°C/min, and finally 5 minutes in 280°C. The sample was injected at temperature 280°C without splitting sample. The detector temperature was 290°C. 2 µL of sample was injected. The split magnitude relation was 10:1. The Mass Spectra operative parameters includes ionization potential seventy eV. The ion supply temperature was 200°C and quadrupole 100°C. The solvent delay was 6.0 minutes. Scan speed was 2000 amu/s. Total MS time period for scanning was 36 minutes. The reaction time of scan varies from 30 to 600 amu/s. The electron volt voltage was 3000 volts.

The targeted *Dyschoriste littoralis* extract is injected into the GC/MS instrument (Hewlett Packard 5890 GC/MS with Mass Selective Detector with Turbo mass gold-perkin Elmer). The ethanolic extract is vaporised at the injection port. By increasing temperature, the extract was eluted through a capillary column. Since the extract run all the way through the column, based on the affinity with the stationary phase of the column varied elements separated. It was known by retention time. Retention time means the time sample takes for a compound to move through the column and gas chromatograph system. Each chemical component in the sample has a unique retention time and it was measured in minutes. The result was revealed in a peak on a graph. It measures abundance on the ordinate against retention time on the abscissa. The integrated peak is correlated to the concentration of the chemical. A mass spectroscopic selective detector divides each chromatographic constituent into fragment ions. It was revealed by their abundance, with each particle depicted as a vertical line in the order of increasing relative molecular mass. The peak of each line has a close similarity to the abundance of that particle. The resulting spectrum is selective to particular chemical. In ‘‘Scan’’ mode all chemical constituents are present in the sample was listed first.

Compound Identification

Identification of components of the methnolic extracts was depend on the comparison of their mass spectra data and retention indices with those reported in the literature and by matching the data in the NIST 2005 MS computer library (Wiley).

Results and discussion

The compounds present in the ethanolic extract of *Dyschoriste littoralis* aerial parts were identified by GC-MS analysis presented in Table 1. More than nineteen compounds were identified in the extract. The prevailing compounds were Ethanol, Pentane, 2-methyl, Hexane, 1-Propene, 2-methyl-, Hydrazine, 1,2-dimethyl-, Cyclopentane, methyl-, Ethyl Acetate, (3-Methyl-oxiran-2-yl)-methanol Hydroperoxide, 1-methylbutyl, Methane, oxybis (dichloro-, Acetyl chloride, dichloro, 1-Methoxy-5-trimethylsilyloxyhexan, Methoxymethyl isothiocyanate, Silane, triethylmethoxy- 5H-1-Pyrindine, 2-Furancarboxaldehyde, 5-(hydroxym, 1,6,10-Dodecatrien-1-01, 3,7,11-tr, 3-Eicosene, (E)- 1-Nonadecene, 1-Hexacosanol, Pentadecanoic acid, ethyl ester, Nonadecanoic acid, Undecanoic acid, Benzene, 1-methoxy-4-(1-methylethy Phenol, 2-methyl-5-(1-methylethyl). The Chromatogram (Figure 1) shows 5 prominent peaks in the retention time range 6.36 - 9.52. The peak at 6.36, 6.62, 6.73 retention time is having the peak area 12.61, 45.81 and 29.40 respectively. The fourth less prominent peak at 26.008 retention time with the peak area 9.52 denotes Ethyl Acetate, (3-Methyl-oxiran-2-yl)-methanol, Hydroperoxide, 1-methylbutyl.

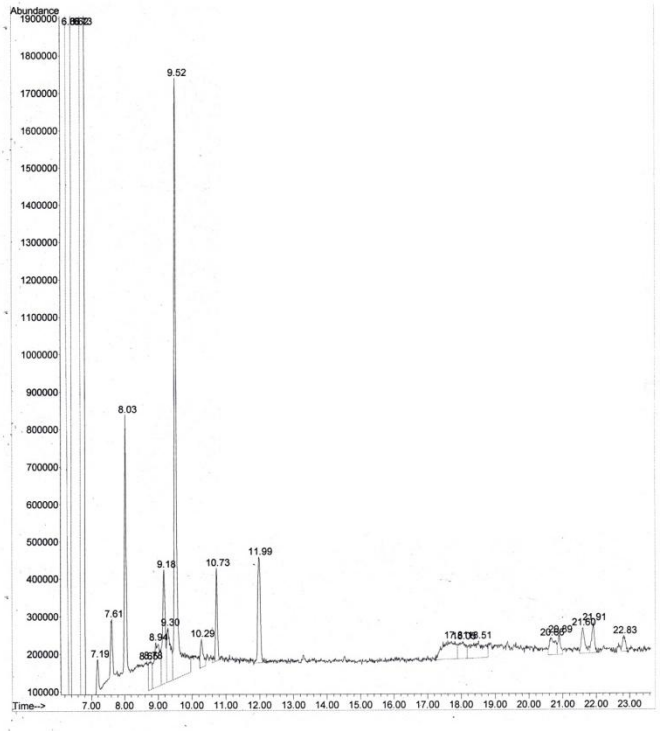


Figure 1. Chromatogram for *D. littoralis* aerial part of ethanol extract

Table1. GC-MS studies on ethanol extract of Aerial part of *D. littoralis*

S. No	RT value	Area	Chemical compound
1	6.36	12.61	Ethanol
2	6.62	45.81	Ethanol
3	6.73	29.40	Ethanol
4	7.19	0.45	Pentane, 2-methyl-
5	7.61	1.03	Pentane, 3-methyl-
6	8.03	1.85	Hexane 1-Propene, 2-methyl- Hexane
7	8.65	0.83	Ethanol
8	8.79	0.20	Ethanol
9	8.94	0.58	Acetic acid Hydrazine, 1,2-dimethyl-
10	9.18	0.69	Cyclopentane, methyl-
11	9.29	0.45	Ethyl Acetate (3-Methyl-oxiran-2-yl)-methanol Hydroperoxide, 1-methylbutyl
12	9.52	2.90	Methane, oxybis(dichloro- Acetyl chloride, dichloro- Chloroform
13	10.28	0.17	Cyclohexane
14	10.73	0.34	Benzene
15	11.99	0.61	Ethane, 1,1-diethoxy- Methoxymethyl isothiocyanate
16	17.81	0.52	1-Methoxy-5-trimethylsilyloxyhexane Benzonitrile, 3-methyl-
17	18.05	0.26	1-Methoxy-5-trimethylsilyloxyhexane Silane, triethylmethoxy- 5H-1-Pyrindine
18	18.51	0.48	1-Methoxy-5-trimethylsilyloxyhexane Benzene, 1-isocyano-2-methyl- Tetrahydro-1, 3-oxazine-2-thione
19	20.66	0.21	2-Furancarboxaldehyde, 5-(hydroxym
20	20.89	0.11	2, 5-Diethylphenol Benzene, 1-methoxy-4-(1-methylethyl) Phenol , 2-methyl-5-(1-methylethyl)
21	21.59	0.21	1,6,10-Dodecatrien-1-01, 3,7,11-tr 2,6,10-Dodecatrien-1-01, 3,7,11-tr
22	21.91	0.20	3-Eicosene, (E)- 1-Nonadecene 1-Hexacosanol
23	22.82	0.11	Pentadecanoic acid, ethyl ester Nonadecanoic acid, ethyl ester Undecanoic acid, ethyl ester

GS-MS chromatogram of the ethanolic extract study showed 9 peaks in underground part powder of *Dyschoriste littoralis*, besides a number of peaks with very narrow retention time. The ethanol extract constituents along with their retention time and percentage area obtained from the GC/MS analyzer are tabulated in Table 2 and Figure 2. The GC/MS profiles were used and identified nineteen constituents. The percentage content of compounds are acetyl chloride, dichloro ($CHCl_2COCl$) (Rt. 9.49); Ethane, 1,1-diethoxy- and Methoxymethyl isothiocyanate (Rt. 11.93); 2-Penten-4-yne, 2-methyl-, 2H-Pyrano(3,2-b) pyridine, Thiocyanic acid, 2-propynyl ester (Rt. 10.69) observed found to be 53.46 10.99 and 10.69% respectively. Other compound 2, 4-Hexadiyne (Rt. 10.72) was found to be 8.81%. Acetyl chloride, dichloro is an autofluorescence compound (green). Methoxymethyl isothiocyanate ($C_3H_5NO_2$) are phytochemicals with a broad array of effects in biological systems. Bioactivity includes the stimulation of cellular antioxidant systems, induction of apoptosis and interference with cytokine production and activity. Epidemiological evidence and experimental studies indicated that naturally occurring isothiocyanates and synthetic derivatives have anti-cancer and anti-

inflammatory properties [8]. 2H-Pyrano (3,2-b) pyridine (C_8H_7NO) exhibits antimicrobial activity [9].

Conclusion

Ethanol extracts of *Dyschoriste littoralis* contains nineteen and nine compounds respectively on aerial and underground part dry powder by (GC) and Mass Spectroscopy (MS) method. Components of the ethanolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST '2005 MS computer library (Wiley). Many fatty acids fractions were identified. Further experiments, are planned to establish the influence of the components of these mixtures on the pharmacological activity.

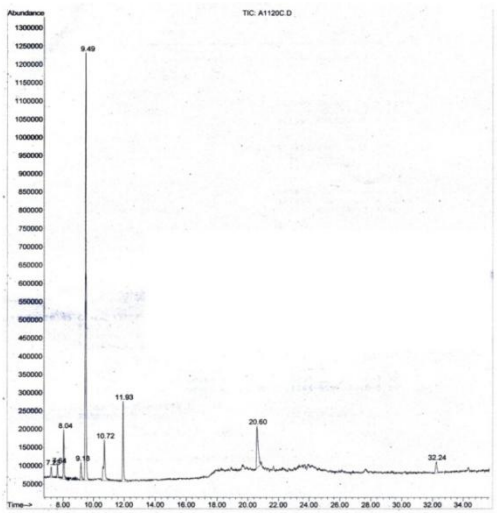


Figure 2. Chromatogram for *D. littoralis* underground part of ethanol extract

Table 2. GC-MS studies on ethanol extract of underground part of *D. littoralis*

S. No	RT value	Area	Chemical compound
1	7.22	2.20	Hydroxylamine, 0-(2-methylpropyl)- Pentane, 2-methyl- Ethanol, 2-(2-propynyloxy)-
2	7.63	1.63	2-Heptene 1-Hexene, 4-methyl-
3	8.05	6.84	1-Propene, 2-methyl- 1-Butene
4	9.18	2.80	1-Butene 1-Proene, 2-methyl-
5	9.49	53.46	Chloroform Acetyl chloride, dichloro- Chloroform
6	10.72	8.81	Benzene 2, 4-Hexadiyne
7	11.93	10.99	Ethane, 1,1-diethoxy- Methoxymethyl isothiocyanate
8	20.60	10.69	2-Penten-4-yne, 2-methyl- 2H-Pyrano(3,2-b)pyridine Thiocyanic acid, 2-propynyl ester
9	32.24	2.57	Ethanol, 2-bromo- Dodecanal Cyclohexanone, 2,5-dimethyl-2-(1-methanol)

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