Research Article

Determination of bioactive components of chloroform extract of *Ctenolepsis cerasiformis* by gas chromatography–mass spectrometry

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ABSTRACT

Aim: The aim of this study was to determine the bioactive and pharmaceutical components of the chloroform extract of *Ctenolepsis cerasiformis*. **Methods:** The phytoconstituents of chloroform extract of *C. cerasiformis* were analyzed by gas chromatography–mass spectrometry (GC-MS). **Results:** The present study indicates that the presence of 15 phytocompounds of the chloroform extract of the plant. **Conclusion:** Medicinal plants have been exhaustively studied for their potential value as a source of drugs. Obviously, natural products will continue to be extremely important as sources of medicinal agents for treating many diseases including human cancers. Therefore, it is of interest to investigate the phytocompounds of *C. cerasiformis* chloroform extract by GC-MS.

KEY WORDS: *Ctenolepsis cerasiformis,* Drug development, Gas chromatography–mass spectrometry analysis, Medicinal agents, Molecular structure, Phytomolecules

INTRODUCTION

Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic form. Plant secondary metabolites can also serve as drug precursors, drug prototypes, and pharmacological probes. Recent developments in drug discovery from plants, including information on approved drugs and compounds now in clinical trials, are presented. There are also several plant extracts or phytomedicines in clinical trials for the treatment of various diseases. In future, plant-derived compounds will still be an essential aspect of the therapeutic array of medicines available to the physician. The plant chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites and are not directly involved in the growth, development, or reproduction of plants. These secondary metabolites can be classified into several groups according to their chemical classes, such as alkaloids, terpenoids, and phenolics.[1] Plants have formed the basis of sophisticated traditional medicine practices that have been used for thousands of years by people in China, India, and many other countries.[2] Some of the earliest records of the usage of plants such as drugs are found in the Atharva Veda. Nowadays, plants are still important sources of medicines, especially in developing countries that still use plant-based traditional medicine for their health care.[3] The correlation between the ethnomedical usage of medicinal plants and modern medicines discovered from those plants has been studied by Fabricant and Farnsworth. Based on their analysis, 88 single chemical entities isolated from 72 medicinal plants have been introduced into modern therapy, many of which have the same or a similar therapeutic purpose as their original ethnomedical use.[4] Therefore, the present study aims to explore the identification of phytoconstituents from the chloroform extract of *Ctenolepsis cerasiformis.*

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MATERIALS AND METHODS

Collection of Medicinal Plants

The Indian medicinal plants *C. cerasiformis* were collected from the medicinal garden, Chennai, India. The parts of the plants were authenticated by the botanist.

Plant Materials

The chloroform extracts of a *C. cerasiformis* (leaves) were used for this study.

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Preparation of Plant Extracts

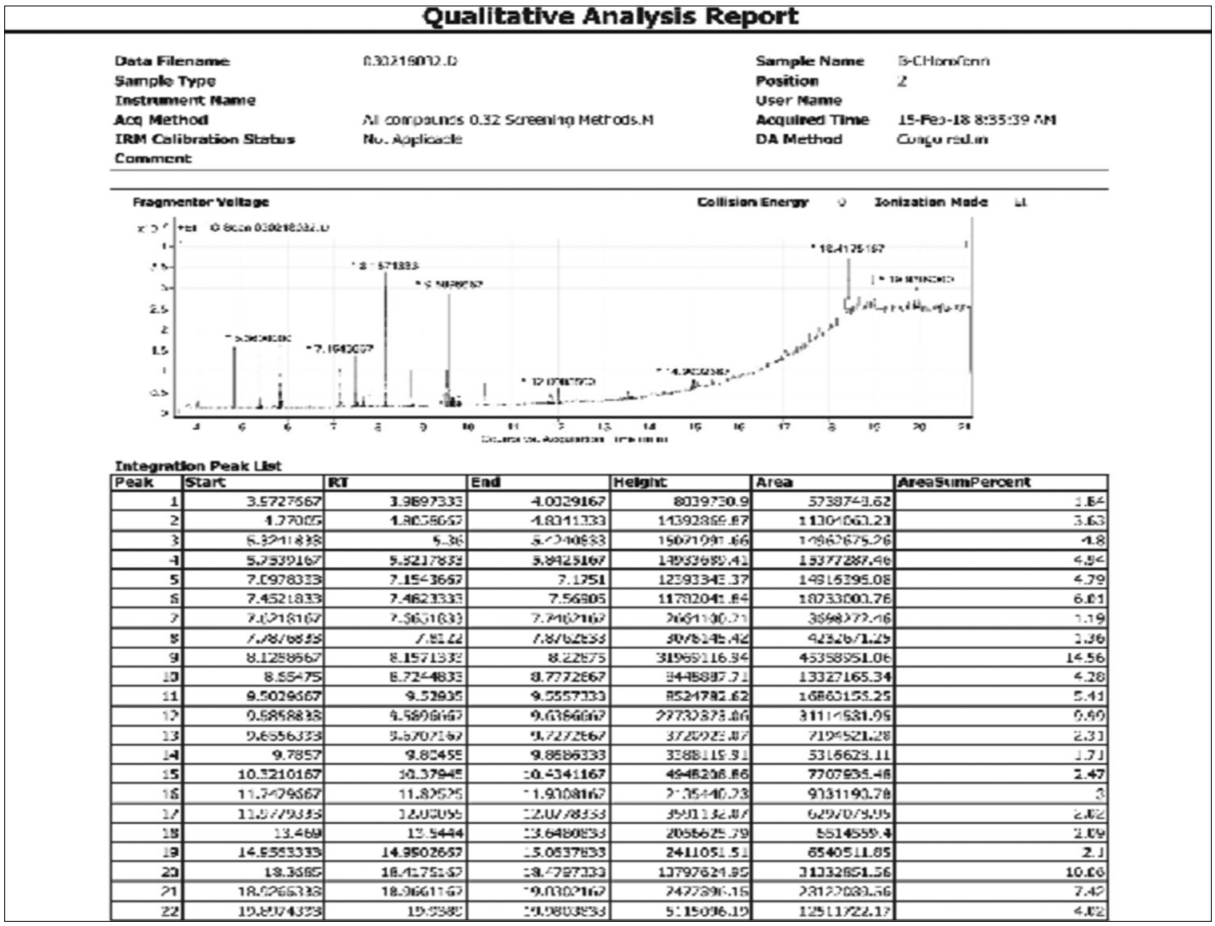
The extraction of the plant material was carried out using the known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with petroleum ether (60–80°C), followed by 900 ml of hydroalcohol using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No. 1). The hydroalcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine the concentration in mg/ml. The extract was preserved at 2–4°C.

Chemicals and Reagents

All chemicals were used for this project were purchased from M/s. Sigma Chemicals, USA. Gas chromatography–mass spectrometry (GC-MS) was used for the analysis of chloroform extract of 66 *C. cerasiformis.*

Preparation of Plant Extract for GC-MS

50 g of the powdered plant material was soaked in 95% chloroform for 12 h. The extract was then filtered through Whatman filter No. 41 along with 2 g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95% ethanol along with sodium sulfate. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents in the plant material. 2 µl of this solution was employed for GC-MS analysis. The herbal powder was extracted with chloroform and analyzed using GC-MS analyzer (GC Clarus 500 Perkin Elmer). The data were obtained on an Elite-1 (100% dimethylpolysiloxane) column. Helium (99.999%) was used as the carrier gas with a flow rate of 1 ml/min in the split mode (10:1). An aliquot of 2 µl of ethanol solution of the sample was injected into the column with the injector temperature at 250°C. GC oven temperature was started at 110°C and holding for 2 min and it was raised to 200°C at the rate of 10°C/min, without holding. Holding was allowed at 280°C for 9 min with program rate of 5°C/min. The injector and detector temperatures were set at 250°C and 280°C, respectively. Ion source



Peak	Start	RT	End	Height	Area	AreaSumPercent
1	3.5727667	3.9897333	4.0029167	8039730.9	5738743.62	1.84
2	4.77005	4.8038667	4.8041333	11393869.87	11304063.23	3.63
3	5.3241833	5.36	5.4040833	15071091.66	14863675.26	4.8
4	5.7539167	5.8217833	5.8425167	14853680.41	15377287.46	4.94
5	7.0978333	7.1543667	7.1751	12393348.37	14816395.08	4.79
6	7.4521833	7.4623333	7.56805	11702041.84	18733003.76	6.01
7	7.6218167	7.9651833	7.9910167	2661130.71	3698372.46	1.19
8	7.8878833	7.8122	7.8762833	3078145.42	4236671.25	1.36
9	8.1258667	8.1571333	8.22875	31969116.34	45358951.06	14.56
10	8.55475	8.7244833	8.7772667	8448887.71	13327165.34	4.28
11	9.5026667	9.52935	9.5557333	8524782.62	16863155.25	5.41
12	9.5858833	9.5896667	9.6366667	27730373.86	31114531.05	9.99
13	9.6556333	9.6707167	9.7272667	3720923.87	7195521.28	2.31
14	9.7857	9.82455	9.8586333	3388115.91	5316623.11	1.71
15	10.3210167	10.37945	10.4341167	4948206.86	7707835.48	2.47
16	11.3479667	11.82525	11.9108167	2705440.73	9131190.78	2.93
17	11.9779333	12.03055	12.0778333	3931134.87	6297079.95	2.02
18	13.469	13.5444	13.6480833	2056625.79	5514559.4	1.77
19	14.8553333	14.9502667	15.0537833	2411051.51	6540511.85	2.1
20	18.3685	18.4175167	18.4787333	13797621.95	31332851.56	10.06
21	18.9266333	18.9661167	19.0302167	2427896.15	23122083.56	7.42
22	19.8974333	19.9588	20.0803833	5115036.19	12511724.17	4.02

Figure 1: Chromatogram of chloroform extract of *Ctenolepsis cerasiformis*

Table 1: The molecular formula and molecular weight of the phytocompounds from chloroform extract of *Ctenolepsis cerasiformis*

Retention time	Name of the components	Molecular formula	Molecular weight
3.9727667	6-Tridecene, (Z)-	$C_{13}H_{26}$	182.34
4.77005	1-Tetradecene	$C_{14}H_{28}$	196.37
5.3241833	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32
5.7539167	5-Eicosene,(E)-	$C_{20}H_{40}$	280.53
7.0978333	5-Eicosene,(E)-	$C_{20}H_{40}$	280.53
7.4521833	Phytol, acetate	$C_{22}H_{42}0_2$	338.56
7.6218167	13-Heptadecyl-1-ol	$C_{17}H_{32}O$	252.43
7.7876833	Ethanol, 2-(9-Octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312.53
8.1288667	Hexadecanoic acid, methylester	$C_{17}H_{34}O_2$	270.45
8.65475	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256.46
9.5029667	17-Octadecynoic acid, methylester	$C_{19}H_{34}O_2$	294.47
9.5858833	9-Octadecenoic acid (Z)-, methylester	$C_{19}H_{36}O_2$	296.48
9.6556333	Ethanol, 2-(9-Octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312.53
9.7857	Tetradecanoic acid, 12 methyl-, methylester	$C_{16}H_{32}O_2$	256.42
10.3210167	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256.46
11.7479667	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl- heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428.73
11.9779333	Octadecanol, 2-bromo-	$C_{18}H_{37}$	349.39
13.469	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	579.24
14.9563333	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	579.24
18.3685	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	579.24
18.9661167	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	579.24
19.9389	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	579.24

Table 2: The presence of phytocompounds and their area percentage in the chloroform extract of *Ctenolepsis cerasiformis*

Name of the compounds	Area percentage
6-Tridecene, (Z)-	1.84
1-Tetradecene	3.63
Phenol, 2,4-bis (1,1-dimethylethyl)-	4.8
5-Eicosene,(E)-	4.94
5-Eicosene,(E)-	4.79
Phytol, acetate	6.01
13-Heptadecyl-1-ol	1.19
Ethanol, 2-(9-Octadecenyloxy)-, (Z)-	1.36
Hexadecanoic acid, methylester	14.56
1-Hexadecanol, 2-methyl-	4.28
17-Octadecynoic acid, methylester	5.41
9-Octadecenoic acid (Z)-, methylester	9.99
Ethanol, 2-(9-Octadecenyloxy)-, (Z)-	2.31
Tetradecanoic acid, 12 methyl-, methylester	1.71
1-Hexadecanol, 2-methyl-	2.47
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	3
Octadecanol, 2-bromo-	2.02
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	2.09
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	2.1
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	10.06
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	7.42
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	4.02

temperature was maintained at 200°C. The mass spectrum of compounds in samples was obtained by electron ionization at 70 eV, and the detector was operated in scan mode from 45 to 450 amu (atomic mass units). A scan interval of 0.5 s and fragments from 45 to 450 Da was maintained. The total running time was 36 min.[5]

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 the National Institute Standards 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.[6]

RESULTS AND DISCUSSION

Plants are natural and healthy resource of life, especially herbal medicinal plants are of great importance with endless therapeutic potentials and useful for curing various diseases with an advantage of being natural.[7] There are number of products in market with adverse side effects on one's health.

Figure 1 shows that the chromatogram of chlororoform extract of *C. cerasiformis*. Tables 1 and 2 shows the molecular formula , molecular weight and retention time of phytocompounds from the chloroform extract of *C. cerasiformis*.

Hence, the use of secondary metabolites or phytochemicals from plant origin could be an advantage and best solution to narrow down the use of unhealthy products. Moreover, plants have always been a source of a wide array of secondary metabolites with potential biological and pharmacological properties. The chloroform extract of *C. cerasiformis* contains 15 phytocompounds such as 6-Tridecene, (Z)-; 1-Tetradecene; Phenol, 2,4-bis(1,1-dimethylethyl)-; 5-Eicosene, (E)-; Phytol, acetate; 13-Heptadecyl-1-ol; Ethanol, 2-(9-Octadecenyloxy)-, (Z)-; Hexadecanoic acid, methylester; 1-Hexadecanol, 2-methyl-; 17-Octadecynoic acid, methylester; 9-Octadecenoic acid (Z)-, methylester; Tetradecanoic acid, 12 methyl-, methylester; 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol; Octadecanol, 2-bromo-; and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexad ecamethyl-. In the past, the plant extracts in crude or partially purified forms were the only sources of medication available for the treatment of human and animal diseases. This gave an idea that the effect of a drug in human body is due to an interaction of drug with biological molecules. This opened new doors in pharmacology, as pure, isolated chemicals, instead of extracts, as the standard for the treatment of diseases. At present, there are innumerable number of such bioactive compounds isolated form crude extracts and their chemical structure were elucidated.

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