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Chemical profiling of fern *Cheilosoria mysurensis* (Wall. ex Hook.) Ching & Shing and its biological activity

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Abstract

Cheilosoria mysurensis (Wall. ex Hook.) Ching & Shing, a medicinal fern traditionally used to treat burns, throat pain and bone fracture. There is no any scientific report regarding anticancer studies of this species. The aim of the study was to find out the chemical components through GC/MS analysis with their antioxidant and cytotoxic activity. GC-MS analysis shows primary ingredients viz, Cis-9, 10-epoxyoctadecan-1-ol and Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans were present. High free-radical scavenging activity has been discovered in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and showed the IC_{50} value of 52.13±0.33. Furthermore, *C. mysurensis* also showed good cytotoxic effects against DLA and EAC cell lines with values of 72.9% and 79.5% at 200 µg/ml dose respectively. Overall findings suggested that the identified chemical compounds proved to be rich in antioxidant properties. Further, this fern can be a good resource for the pharma industry to produce novel anti-cancer drugs.

Keywords

Antioxidant, *Cheilosoria mysurensis*, cytotoxicity, GC/MS, medicinal fern

Introduction

Cheilosoria mysurensis (Wall. ex Hook.) Ching & Shing is commonly called as lip fern and belongs to family Pteridaceae. The genus *Cheilosoria* consists of about 874 species worldwide and 11 species are reported in Indian subcontinent (1). This fern is commonly found in dry, rocky places, plains and in foot hills (2). Traditionally the ash of the study fern mixed with coconut oil and applied topically on skin to fasten the healing of burns (3) to treat throat pain (4) and bone fracture (5).

Several diseases have become more prevalent in recent years. There is an immediate need for search of novel compounds to treat cancer and infectious diseases caused by microorganisms. Plant sources with diverse natural bioactive ingredients play an important role in curing deadful diseases (6). Many chemical compounds show impressive *in vitro* activity against pathogenic microorganisms resistant to recent allergenic drugs. Plants have a rich source of secondary metabolites (terpenoids, phenolics and alkaloids), that exhibit a broad spectrum of bioactivities including anti-tumor activity (7). The identification and characterization of above said molecules in terms of their biological activity on the spectrum, potency, toxicity and safety is most needed.

Previously the fern *C. tenuifolia* have reported with variety of biologically active compounds including polyphenols, flavonoids and other important phytochemicals responsible for its antioxidant and cytotoxic potential (8). *C. farinosa* has significant cytotoxic activity in the hepatoma cell line Hep 3B but not in the non-cancerous cell line RAW 264.7 (9). The most prevailing compounds reported in *C. farinosa* ethanolic extract are n-hexadecanoic acid, diplopene, 9 octadecanoic acid, n-tetracontane and 3, 7, 11 and 15 tetramethyl-2-hexdecane-1-ol. Hexadecanoic acid has biological activity such as antioxidants, hypocholesterolemic nematicides and pesticides (10). Recently, a few research reports are available on the genus *Cheiloso-ria*. Many Pteridaceae species are being studied for its possible uses and for the extraction of new active components. Till now there is no research articles published on the phytochemistry and pharmacology of *C. mysurensis*. The objective of the work is to identify the chemical compounds that are carried out through GC/MS analysis and to study on antioxidant and cytotoxicity activities of the fern *C. mysurensis* and this is the first report on this species.

Materials and Methods

Study area and Collection of plant materials

The fresh and healthy leaflet of the fern *C. mysurensis* was collected from Gopalaswamy Hills, located near to Anaimalai hills of 10° 13' and 10° 31' north Latitude and between 76° 52' and 77° 22' east longitude of Western Ghats, Tamil Nadu, India. The fern species was identified and authenticated by BSI, Southern circle, Coimbatore, Tamil Nadu. A voucher specimen was deposited as herbarium at the Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi.

Extract preparation

The fern leaflets were cut into small pieces and allowed to shade dried for 2-3 weeks. The shade-dried samples were ground to a fine powder using an electric grinder. The powder was then fed into the soxhlet apparatus using methanol as the solvent (11).

GC/MS Analysis

In this study, Clarius 680 GC was used. Components were separated using a fused silica column packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m 0.25 mm ID, 250 m df) and helium as the carrier gas at a speed of 1 ml/min. The injector temperature was set at 260 °C during the chromatographic cycle. 1 µl of the extract was injected into the apparatus in the oven as follows: 60 °C (2 min); followed by 300 °C, where it was held for 6 min. The conditions of the mass detector were as follows: line temperature of 230 °C; ion source temperature of 230 °C; and electron impact in ionization mode at 70 eV, the scan time was 0.2 S and the scan interval was 0.1 S. The shards range in mass from 40 to 600 Da. Component spectra were compared with a database of known component spectra stored in the NIST GC/MS library (2008) (12).

In vitro Free Radical Scavenging activity

DPPH radical scavenging activity

About 1 mg of *C. mysurensis* extract was dissolved in 1 ml of dimethyl sulfoxide. Samples 125, 250, 500 and 1000 µg/ml were prepared and used for the assay. The free radical scavenging capacity of the evaporated sample of *C. mysurensis* extract was determined using Blois method (13). The DPPH solution was prepared in 95% methanol. Different concentrations (125-1000 µg/ml) methanol extracts of *C. mysurensis* were reacted with 3.0 ml of freshly prepared DPPH solution (0.1 mM) and incubated at room temperature. After 30 min, absorbance was measured at 517 nm using a spectrophotometer (Labman UV-Visible spectrophotometer). Ascorbic acid was used as standard. Standard ascorbic acid and extracts were treated similarly**.** The following formula was used to calculate the % of inhibition.

Percentage of DPPH radical scavenging activity =

$$\frac{\text{A of control- A of sample}}{\text{A of control}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\text{eqn.1}$$

Where as, A- Absorbance

Determination of Cytotoxic activity

The sample was dissolved in dimethyl sulfoxide. The test compound was investigated for short-term *in vitro* cytotoxicity using Dalton's ascites (DLA) and Ehrlich ascites (EAC) carcinomas cell lines. Tumor cells were collected from the peritoneal cavity of tumor-bearing mice and washed three times with PBS or a normal cell line. Trypan blue exclusion was used to check cell viability (14). Viable cell suspensions were added to tubes containing various concentrations of test compounds and volumes were made up to 1 ml using a phosphate-buffered cell line (PBS). The control tube contains only the cell suspension. These test mixtures were incubated. The following formula was used to calculate the percentage of cytotoxicity.

Percentage of cytotoxicity (%) **=**

$$\frac{\text{No. of dead cells}}{\text{No. of dead cells + No. of live cells}} \times 100 \ldots\ldots\ldots\text{(eqn. 2)}$$

Results and Discussion

GC/MS Analysis

The GC/MS chromatogram of *C. mysurensis* methanolic extract displayed 12 peaks (Fig. 1), due to the presence of 12 compounds. (Table 1). Important major compounds were Cis-9,10-Epoxyoctadecan-1-Ol (39.66%) and Oxirane-undecanoic acid, 3-pentyl-, methyl ester, trans (13.69%) and other minor compounds were 8,11,14-Eicosatrienoic acid, methyl ester and Tetradecanoic acid, 12-methyl-, methyl ester, (s)-and other trace compounds were shown in Table 1. Some of the GC/MS constituents are physiologically active metabolites. They have pharmacologic properties that may contribute to the plants ability to heal. Cis-9, 10-Epoxyoctadecan-1-Ol is an alcoholic compound that has been shown to have antimicrobial properties (15). Compound 3-pentyl-, methyl ester, trans (13.69%) was confirmed to be present in other plants and exhibiting antioxidant effects (16).

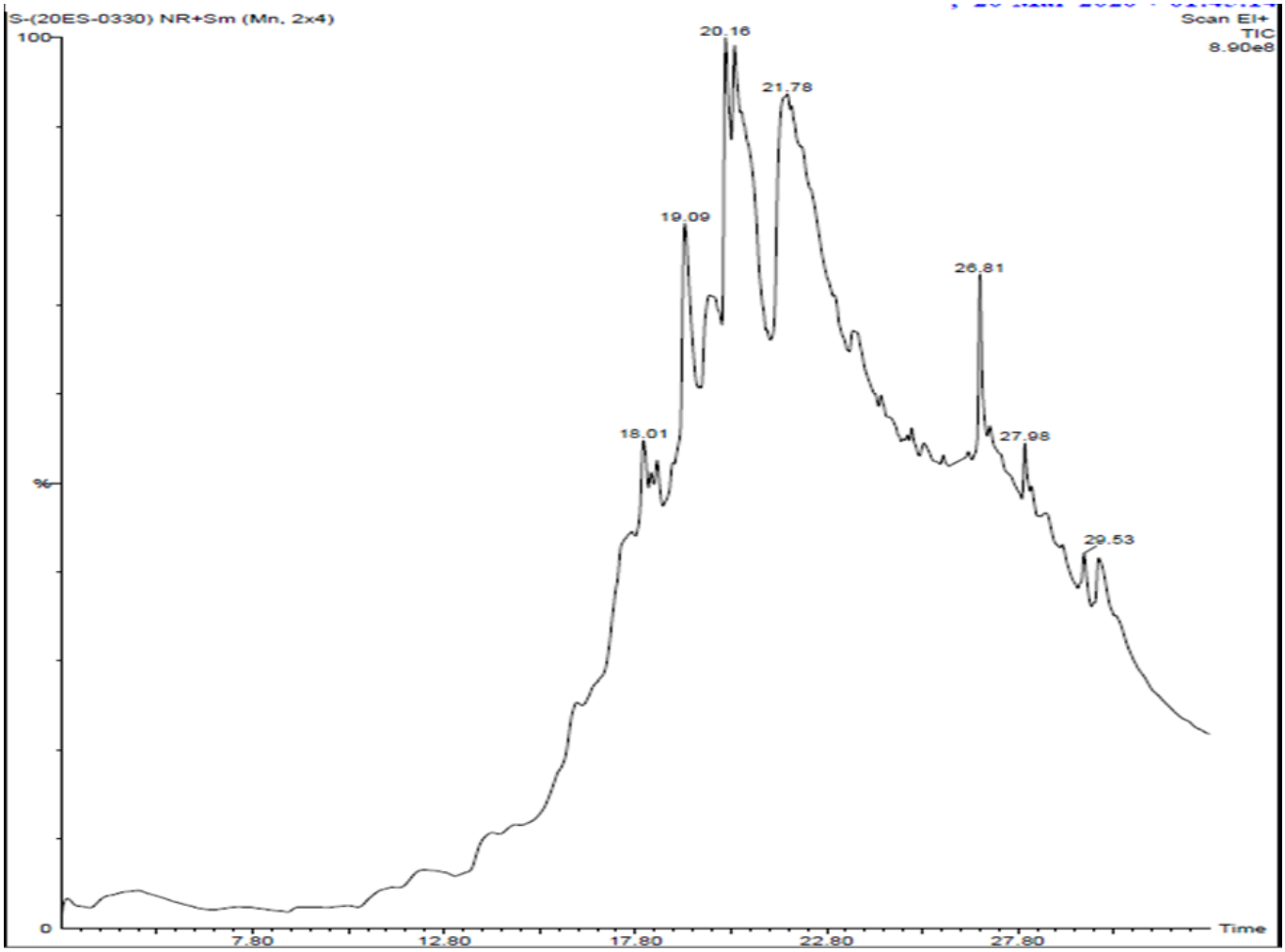

Fig. 1. GC/MS chromatogram of methanolic extract of *Cheilosoria mysurensis.*

Table 1. Compounds identified in the methanolic extract of *Cheilosoria mysurensis*

RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Compound nature
18.019	3,7-Dimethyl-6-nonen-1-ol acetate	$C_{13}H_{24}O_2$	212	8.923	Acetate (Acetate derivative)
18.365	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$	222	2.993	Alcohol (Alcohol derivative)
19.100	Tetradecanoic acid, 12-methyl-, methyl ester, (s)-	$C_{16}H_{32}O_2$	256	9.740	Ester(Ester derivative)
20.160	8,11,14-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320	10.379	Ester(Esterderivative)
20.405	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans	$C_{19}H_{36}O_3$	312	13.691	Ester(Esterderivative)
21.761	Cis-9,10-epoxyoctadecan-1-ol	$C_{18}H_{36}O_2$	284	39.665	Alcohol (Alcohol derivative contain epoxy)
25.047	3-Nonanol, 1,2;6,7-diepoxy-3,7-dimethyl-, acetate	$C_{13}H_{22}O_7$	242	3.959	Acetate (Acetate derivative)
25.843	1-Octadecanesulphonyl chloride	$C_{18}H_{37}O_2ClS$	352	1.968	Sulphur (Sulphur derivative)
26.798	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	6.935	Alkane
27.963	1-Octadecanesulphonyl chloride	$C_{18}H_{37}O_2ClS$	352	1.748	Sulphur (Sulphur derivative)
29.519	Pseduosarsasapogenin-5,20-dien	$C_{27}H_{42}O_3$	414		Ether (Ether derivative)
29.919	Stigmastan-6,22-dien, 3,5-dedihydro-	$C_{29}H_{46}$	394		Steroid

DPPH radical scavenging activity

The radical scavenging activity of the methanolic extracts of *C. mysurensis* and standard ascorbic acid was shown in Table 2. At a dose of 100 µg/ml, the scavenging activity was 79.65 ± 0.78%, while at the same concentration, the standard ascorbic acid was 96.45 ± 0.41%. The Half-maximal inhibitory concentration (IC_{50}) value of the methanol extracts of *C. mysurensis* was 52.13 ± 0.33 µg/ml. The IC_{50} value of ascorbic acid (standard) was 32.85 ± 0.90 µg/ml.

Antioxidants are hypothesised to act on DPPH because of their ability to transfer hydrogen (17). Scavenging activities are critical for preventing the detrimental effects of free radicals in a variety of illnesses, including cancer

Table 2. DPPH radical scavenging activity of *Cheilosoria mysurensis*

Concentration (µg/ml)	**12.5**	**25**	**50**	**100**	**IC $_{50}$**
Methanol extract (% of inhibition)	20.95±0.77	35.033±0.42	47.15±1.38	79.65±0.78	52.13±0.33
Concentration (µg/ml)	**12.5**	**25**	**50**	**100**	**IC_{50}**
Ascorbic acid (% of inhibition)	35.20±2.67	47.01±1.52	60.42±1.51	94.59±1.08	32.85±0.90

(18). The DPPH radical scavenging method is widely used to assess the antioxidant potential of plant extracts. By adding the purple DPPH solution to the extract in a concentration-dependent manner, the extract is reduced to the yellow product, diphenyl picryl hydrazine. Because of its quick processing time, this approach is commonly employed to predict antioxidant activity. The methanolic extracts of *C. mysurensis* possess free radical scavenging activity equivalent to that of normal ascorbic acid, according to our findings. Jarial and his team (19) investigated the antioxidant activity of flavonoids isolated from the fern *Cheilanthes tenuifolia*. The flavonoids extracted with methanol displayed a high DPPH activity; with an IC_{50} value of 39.10 µg/ml. *C. mysurensis* methanol extract was more active than *C. tenuifolia*.

Cytotoxicity assay

Extract toxicity studies were performed on Dalton's ascites carcinoma (DLA) and Ehrlich's ascites (EAC) cell lines. Trypan blue is an acidic colour with two azo pigment cell groups. Trypan blue cannot pass through the cell wall of the plant cells. Trypan blue is an important pigment that used to estimate the number of cells that can exist in a population (20). In this study, *C. mysurensis* inhibited the growth of DLA and EAC in a dose-dependent manner (10, 20, 50, 100 and 200 µg/ml). The trypan blue dye exclusion test results showed that *C. mysurensis* extract strongly inhibited the development of DLA and EAC cells in culture. A concentration of 200µg/ml showed a high degree of cytotoxicity to the DLA and EAC assays with cell death rates of 72.9% and 79.6%, respectively (Table 3, Table 4). This may be because of the higher alcohol derivatives that contain epoxy and ester derivatives in the methanol extract and thus the components may show positive synergistic effects against cancer cell lines.

Table 3. Cytotoxicity of the *C. mysurensis* by DLA assay

Drug concentration (µg/ml)	**Total no.of Live cells**	**Total no.of Dead cells**	**Total no.of cells**	**Percentage of cell death**
10	104	9	113	7.92 ± 1.5
20	90	16	106	14.8 ± 1.5
50	79	29	108	26.9 ± 1.6
100	55	52	107	48.8 ± 0.9
200	29	79	108	72.9 ± 1.6

± = value represents the mean standard deviation (n=4)

Reports are on the *in vitro* cytotoxicity activity of a methanolic extract from the leaves and fruit of *Zanthoxylum ovalifolium* against the cancer cell lines of DLA and EAC (21). The results indicated that cytotoxicity studies on methanolic extracts from leaves and fruits of *Z. ovalifolium* had promising activity against both the cell lines. Both extracts inhibited cancer cell development in a concentration-dependent manner, however the leaf extract was more efficient against cancer cells than the fruit extract. The fruit extract at 200 µg/ml showed a significant increase of 80 and 82% in cell death for the DLA and EAC cell lines and 72 and 75% inhibition of tumor cells were observed in the fruit extract at 200 µg/ml for the DLA and EAC cell lines. The results of the methanol extraction of *C. mysurensis* also showed good cytotoxic effects against DLA and EAC cell lines with values of 72.9% and 79.5% at 200 µg/ml dose respectively.

Table 4. Cytotoxicity of the *C. mysurensis* by EAC assay

Drug concentration (µg/ml)	**Total no.of live cells**	**Total no. of dead cells**	**Total no. of cells**	**Percentage of cell death**
10	101	7	108	6.48 ± 2
20	88	17	105	16.6 ± 2.1
50	70	31	101	30.6 ± 2.4
100	47	53	100	53.2 ± 2
200	20	80	100	79.6 ± 1.8

± = value represents the mean standard deviation (n= 4)

Conclusion

The chemical composition, antioxidant and cytotoxicity activities of the methanol extract of *C. mysurensis* from the Western Ghats of India are reported for the first time in this study. The GC/MS analysis of methanolic extract showed that presence of alcohol derivatives that contain epoxy and ester derivatives. Cell death was evidenced by the cytotoxic effects of *C. mysurensis* methanol extract on DLA and EAC cell lines. Hence, these phytochemicals are responsible for several pharmacological activities, including antioxidant and anticancer effects. The overall finding suggests that the methanol extract of *C. mysurensis* leaflets provided positive initial data for future use as an anticancer agent.

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Authors contributions

For the Authors' contributions RR, AP and SD conducted the experiments, analysed the data statistically and prepared the manuscript. SP and AAM carryout the correction and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare no conflict of interests.

Ethical issues: None.

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