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GC-MS Chemical Profiling, Antifungal and Antioxidant Activity of *Cleome aspera* Aerial Parts Extracts

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Cleome aspera belongs to the *Capparidaceae* family. Many species of this family have been used in traditional folk medicine for a long period of time. This study aims to evaluate the antifungal and antioxidant properties of *C. aspera* and to identify various chemical constituents through GC-MS chemical profiling. In present study, aerial parts of *Cleome aspera* plant were extracted by petroleum ether, ethyl acetate and ethanol. *In vitro*, antifungal activity was investigated by agar disc diffusion method and minimum inhibitory concentration, whereas *in vitro* assay techniques, including total phenolic content (TPC) and total flavonoid content (TFC), were employed to evaluate antioxidant activity. Against all of the chosen strains, the ethanol (polar) extract showed prominent antifungal activity and had the strongest antioxidant activity, which is attributed due to the presence of significant levels of total flavonoids and phenolic contents. The findings indicate that the plant has considerable antioxidant and antifungal properties, along with a range of pharmacologically significant chemical compounds.

Keywords: GC-MS chemical profiling, Antioxidant activity, Antifungal activity, *Cleome aspera*, DPPH assay, ABTS assay.

INTRODUCTION

Antibiotic resistance is a major public health issue, particularly in under developed nations [1]. Thus, there is significant interest in discovering novel chemicals from therapeutic plants. Recent investigations have shown that organic herbs contain secondary metabolites that can be used as antibacterial and antifungal agents in traditional medicines [2]. Studies show that therapeutic plant based drugs can effectively suppress harmful microorganisms. Therefore, it is essential to evaluate the antifungal activity by analyzing various medicinal plant components and extracts [3].

Free radicals are extremely reactive atoms or groups with unpaired electrons and are highly unstable. However, overproduction of these species may result in damaged proteins, lipids and DNA, which can cause several diseases, including membrane damage, cardiac difficulties, aging as well as cancer. Antioxidants can help to prevent free radical damage [4,5]. The majority of these free radicals are scavenged or inactivated by endogenous systems, including superoxide dismutase, catalase and the peroxidase-glutathione system. However, these mechanisms may not be efficient enough to meet the demands of the body, resulting in a dependence on exogenously accessible antioxidants. There has been a lot of attention in recent years for antioxidants derived from natural sources as a result of restrictions on the use of synthetic antioxidants due to potential adverse effects. As a result, the production of antioxidants from plant species has received a lot of attention.

Capparidaceae is a family of plants with over 200 species around the world. Various species of the *Cleome* genus have been utilized for millennia in traditional medicine because they have anthelmintic, carminative, antidiarrheal, antimicrobial, antioxidant and wound healing activity [6]. Several species of this genus, including *C. rutidosperma* DC, *C. gynandra* L, *C. arabica* L, along with *C. viscosa* Linn and *C. droserifolia* (Forssk.) were used to treat scabies, inflammation, blood issues, uterine issues, malaria, diabetes, paralysis, anthelmintic issues, epilepsy, spasms, pain and skin diseases and possess anti-retroviral and anti-diarrheal properties [6]. The traditional applications of the genus *Cleome* have been according to the application of different plant components, including leaves, roots and seeds, as carminatives, stimulants, antiscorbutic agents, anti-anthelmintics

and rubefacients [7]. Phytochemical screening of the *Cleome* genus revealed a range of compounds, including alkaloids, phenols, terpenoids and flavonoids as well as fatty acids and coumarin-lignan. Furthermore, plants contain several nutrients, including vitamins A, vitamin C, protein and gallotannin, as well as saponins and iridoids [8,9].

Therefore, a viable approach is to find safer and more effective natural antioxidant and antifungal treatments that have similar therapeutic effects [10-12]. Up till now, research has been done on the chemical constituents and pharmacological effects of many *Cleome* species. The plant *Cleome aspera* J. Koenig ex DC. is part of the *Cleome* genus. So far, no studies have been published on the chemical components or potential applications of *C. aspera* extracts. This investigation focuses on the antioxidant and antifungal properties of different extracts of *C. aspera*. In this work, GC-MS technique was employed to analyze the chemical composition of the most potent ethanol extract of *C. aspera*.

EXPERIMENTAL

Collection of plant material: Fresh aerial parts of the *Cleome aspera* were collected from Sri Venkateswara National Park, Tirumala hills of Tirupati (13º45′4″N 79º20′16″E), India. The taxonomy of the collected plant material was determined and validated by a taxonomist of the Department of Botany, Shri Venkateswara University, Tirupati, India and voucher specimen (0506) deposited in the herbarium. The obtained plant components (aerial sections excluding roots) were thoroughly rinsed with running tap water to eliminate any dust particles. Following washing, the plant material was dried in the shade. After drying was complete, the plant material was carefully ground into a fine powder with a mechanical mixer and placed in an appropriately labeled and sealed container for future use.

Preparation of extracts: In a Soxhlet apparatus, 150 g of powdered, dried aerial parts of *C. aspera* were weighed, loaded and extracted using 1 L portions of petroleum ether, ethyl acetate and ethanol successively following the hierarchy of polarity of solvents. Extractions were carried out for 72 h, or until the solvent that came out of the siphoning tube was colourless. The determination of phytochemical screening, antifungal and antioxidant activities was done using the prepared extracts.

Determination of phytoconstituents: In order to identify preliminary phytochemicals, a standardized method was used to screen the various phytoconstituents [13,14].

Antifungal activity

Microorganisms: Four distinct fungal strains *viz. Candida albicans* (C.-P. Robin) Berkhout, *Aspergillus niger* (Tiegh), *Penicillium notatum* (Alexander Fleming) and *Helminthosporium solani* (Durieu & Mont.) were received from Department of Microbiology, Dr. B.V. Raju College, Bhimavaram, India.

Agar disc diffusion method: The chosen pathogenic fungi were employed to evaluate the antifungal properties of the extracts by agar disc diffusion method. At concentrations of 2, 5 and 10 mg/mL, each extract was separately dissolved in DMSO. As a reference control for the antifungal study, cotrimazole (25 µg/mL) in DMSO was also used [15]. The antifungal investigation was conducted using PDA media. The melted media was then put into the sterile petri plates after being inoculated with 200 µL of the inoculums (1×10^8 CFU). The disc was added to the top layer of the seeded agar plate after being saturated with 20 µL of the extract separately and given time to dry. The zones of inhibition were measured after the plates had been incubated for 48 h at 280 ºC [16-18].

The broth dilution method was employed to assess the minimum inhibitory concentration (MIC) of the ethanol extract against all test microorganisms at concentrations between 25 µg/mL and 500 µg/mL in DMSO. Briefly, all four test microorganisms were grown in Sabouraud dextrose broth at 37 ºC for 20-24 h, then diluted to 0.5 McFarland at 1×10^8 CFU/mL of microorganisms. The specimens were cultured with *C. aspera* ethanol extract at various concentrations (ranging from 25 µg/mL to 500 µg/mL) at 37 ºC for about 20-24 h. After incubation, the microbial growth was observed by measuring the turbidity of liquid formed in the test tubes after incubation.

In vitro antioxidant and free radical scavenging activity

Antioxidant activity: In a radical scavenging assay, the antioxidant potential of *C. aspera* aerial parts extract was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-*bis*-ABTS radicals. The plant extract was prepared at 0.3125-1.0 mg/mL concentration. The antioxidant activity of plant extract was investigated in the 2.5-0.1953 µg/mL concentration range using gallic acid as a standard benchmark [19,20]. The spectrophotometric determinations were performed using a double-beam spectrophotometer with split-beam technology, model SPECORD® 50 PLUS, manufactured by Analytik Jena AG, Germany.

DPPH assay: The DPPH free radical-scavenging assay, adapted by Omokhua-Uyi *et al.* [21], was employed in this investigation. Six different concentrations of extract (0.5, 0.25, 0.125, 0.625 and 0.3125 mg/mL) were evaluated. After preparing the DPPH solution to a concentration of 60 µM, 40 µL of ethanol extract was added. Then, the DPPH free radical-scavenging assay was evaluated using the following equation [22]:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample. By using a dose-response curve with a concentration on the X axis and the percentage of inhibition on the Y axis, linear regression analysis was used to determine the effective concentration of sample needed to scavenge DPPH radical by 50% (IC_{50} value).

ABTS assay: The modified version of ABTS assay reported by Arumugam *et al.* [23] was used in this investigation. Breifly, a 7 mM ABTS solution was prepared and then left in the dark for around 18 h at a room temperature of 25 ºC. A160 µL of 7 mM ABTS solution was combined with various quantities of *C. aspera* extracts (0.3125-1 mg/mL) and placed in 96-well microplates for analysis. At 734 nm, the ultimate absor-

bance (Abs) was calculated and the ABTS radical-scavenging activity was computed using the formula shown below [23-26]:

$$\text{ABTS radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of ABTS radical and methanol, A_{sample} is the absorbance of ABTS radical and *C. aspera* extract. All the experiments were performed in triplicate.

Estimation of total phenolic content: Using the Folin-Ciocalteu method, the intensity of the generated blue colour was used to determine the total phenolic content. In brief, after a 30 min dark incubation period with continuous shaking, 2.5 mL of diluted Folin-Ciocalteu reagent (10-fold) and 2 mL of 7.5% Na_2CO_3 were mixed with 200 µg/mL of plant extract dissolved in methanol (0.5 mL). At 760 nm, the absorbance was measured with reference to gallic acid solution. The total phenolic content (TPC) of the different plant extracts was determined by an average of three independent analyses. The result was expressed as mg of gallic acid equivalent/g dry weight extract (mg GAE/g extract) [27,28].

Estimation of total flavonoid content: The method aluminum chloride colorimetric assay was utilized to ascertain the total flavonoid content. In brief, 1.3 mL of distilled water, 250 µL of plant extract in methanol (500 µg/mL) and 75 µL of $NaNO_2$ (5%) were mixed followed by the addition of 150 µL of $AlCl_3$ (10%) after 5 min. The after few minutes later, 0.5 mL of 1 M NaOH and 275 µL of distilled water were added to dilute the reaction mixture. Using a standard rutin solution, the absorbance was measured at 510 nm after 15 min. The total flavonoid content (TFC) was expressed as mg rutin equivalent per gram extract (mg RE/g extract) for each experiment, which was conducted in triplicate [29].

Statistical analysis: Version 2010 of Microsoft Excel and SPSS (version 16) were used for the statistical analyses. All the experiments were run in triplicate and the data were reported as means ± standard deviation (SD) except MIC values which were expressed as values of the average of three determinations.

GC-MS chemical profiling: The GC-MS was used to examine 1 µL of extract diluted in 10 µL of GC-grade *n*-hexane. For quantitative analysis, a Hewlett-Packard HP-8590 GC equipped with an optima-5 column (95% dimethyl polysiloxane, 5% diphenyl) (30 m × 0.25 mm, 0.25 µm film thickness) and a split-split less injector (split ratio 1:50) was utilized in conjunction with a system which was equipped with a DB-5 GC capillary column. The injector temperature was set at 260 ºC for the chromatographic run. After injecting the extracted sample (1 µL) into the device, the oven temperature was set to 60 ºC for 2 min, afterward 300 ºC at a rate of 10 ºC per min and then again at 300 ºC, which was maintained for around 6 min. Conditions for the mass detector were transfer line: 240 ºC; ion source: a 240 ºC; ionization mode electron impact: 70 eV, scan time: 0.2 s and scan interval time: a 0.1 s. It was observed that the scanned components fell between the 40-600 Da range [30]. The bioactive compounds isolated in the ethanol extract of *C. aspera* aerial parts were identified based on GC retention time and compared to the database reported in the GC-MS NIST (2008) library [31-33].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis: To ascertain the primary chemical components and to determine their capability to scavenge free radicals the current study was conducted with a preliminary phytochemical screening of different extracts of *Cleome aspera* aerial parts. The preliminary phytochemical screening detected the presence of steroids, phenols, alkaloids, flavonoids, terpenoids, saponins and tannins. The results showed the presence of several key phytoconstituents like alkaloids, flavonoids, glycosides, terpenoids, phenols, saponins, steroids and tannins (Table-1).

TABLE-1
QUALITATIVE PHYTOCHEMICAL SCREENING FOR AERIAL PARTS OF *Cleome aspera*

Phytochemicals	Inference			
	Test	Petroleum ether	Ethyl acetate	Ethanol
Alkaloid	Dragondraffs	–	–	+
Steroid	Salkowski	+	+	+
Flavonoid	Alkaline reagent	+	+	+
Phenols	Ferric Chloride	+	+	+
Tannin	Ferric Chloride	+	+	+
Glycoside	Borntrager's	–	+	+
Terpenoid	Salkowski	–	+	+
Saponin	Froth	–	+	+

NOTE: '+' implies presence of phytochemical, '-' implies absence of phytochemical

Antifungal activity: Table-2 shows the results of the zone of inhibition values, whereas Table-3 shows the MIC values. The results indicated that only the ethanol extract of *C. aspera* exhibited significant antifungal activity against all four tested microorganisms with efficacy dependent on concentration, as demonstrated by the zone of inhibition, and this activity is comparable to that of the standard drug cotrimazole. Other extracts did not display antifungal activity against all the tested microorganisms, except ethyl acetate extract, which could inhibit only *A. niger*. Also, the MIC investigations showed that the ethanol extracts had antifungal activity against the tested strains of microorganisms at concentrations between 150 and 350 µg/mL.

The antifungal activity observed only for ethanol extract, is attributed to the presence of several primary and secondary metabolites. In case of the non-satisfactory results of antifungal activity exhibited by petroleum ether and ethyl acetate extracts the reason is might be due to the absence of sufficient amounts of tannins, flavonoids and phenolic compounds.

Antioxidant activity: The antioxidant activity of *C. aspera* aerial parts extracts was assayed using DPPH and ABTS tests and the results are shown in Figs. 1 and 2, respectively. The ethanol extract exhibited the highest level of antioxidant activity among the extracts (IC_{50} values of 27.53 ± 5.03 and 219.13 ± 6.63 mg/mL for ABTS and DPPH, respectively) (Table-4). Based on the GC analysis, the ethanolic extract has higher phenolic as well as flavonoid contents, thus, the ethanol extract outperformed the petroleum ether and ethyl acetate extracts in terms of antioxidant activity.

TABLE-2
ZONE OF INHIBITION (mm) OF VARIOUS EXTRACTS OF AERIAL PARTS OF *Cleome aspera*

Test substance	Conc. (mg/mL)	*Aspergillus niger*	*Candida albicans*	*Helminthosporium solani*	*Penicillium notatum*
Petroleum ether extract	2	–	–	–	–
	5	–	–	–	–
	10	–	–	–	–
Ethyl acetate extract	2	5.2 ± 019	–	–	–
	5	7.1 ± 019	–	–	–
	10	9.4 ± 0.22	–	–	–
Ethanol extract	2	7.3 ± 0.21	7.2 ± 0.19	7.1 ± 0.12	7.5 ± 0.26
	5	11.3 ± 0.35	10.3 ± 0.21	10.5 ± 0.35	11.1 ± 0.19
	10	12.5 ± 0.52	13.4 ± 0.41	11.6 ± 0.38	13.2 ± 0.16
Cotrimazole	25 µg/mL	27 ± 0.25	24 ± 0.14	26 ± 0.25	26 ± 0.25

Values are mean ± standard deviation (SD) of three readings, Standard used for antifungal studies; Cotrimazole -25 µg/mL; (-) indicates no activity

TABLE-3
MIC VALUES OF ETHANOL EXTRACT OF *Cleome aspera*

Microorganisms	MIC values (µg/mL)
Aspergillus niger	225
Candida albicans	175
Helminthosporium solani	150
Penicillium notatum	175

All values are average of three determinations.

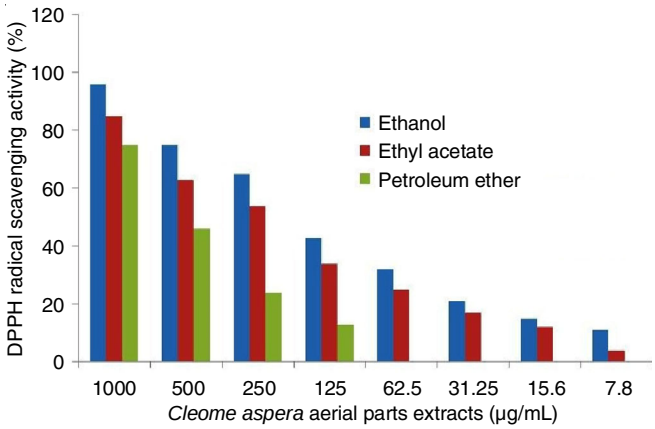

Fig. 1. Antioxidant activity of *Cleome aspera* aerial parts extracts produced using different solvents was tested by DPPH assay

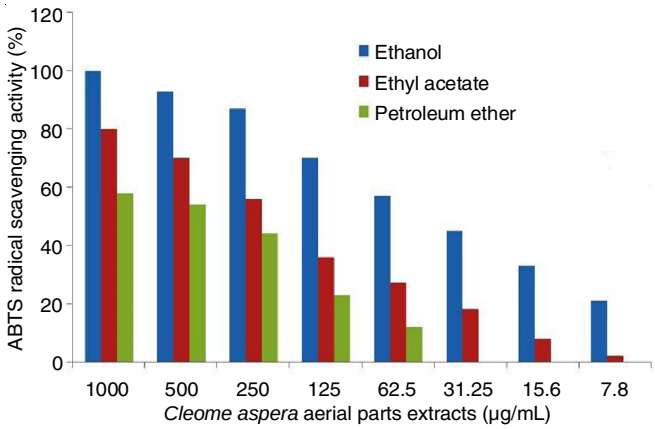

Fig. 2. Antioxidant activity of *Cleome aspera* aerial parts extracts produced using different solvents was tested by ABTS assay

TABLE-5
TOTAL PHENOLIC AND FLAVONOID CONTENTS OF VARIOUS AERIAL PARTS EXTRACTS OF *Cleome aspera*

Extract	Yield (%)	Total phenols (mg gallic acid equivalent (GAE/g ext)	Total flavonoids mg rutin equivalent (RE/g ext)
Petroleum ether	1.76 ± 0.23	32.19 ± 0.49	38.04 ± 0.35
Ethyl acetate	3.92 ± 0.12	82.65 ± 0.43	21.63 ± 0.33
Ethanol	4.85 ± 0.18	124.43 ± 0.53	51.63 ± 0.54

Results from three independent experiments were given as mean ± standard deviation (SD).

Total phenolic and flavonoid contents: The total flavonoid contents of various extracts of *C. aspera* aerial parts were determined by the aluminum chloride method. The results showed that ethanol extract has the highest total flavonoid content (51.63 ± 0.54 mg RE/g ext.), followed by petroleum ether extract (38.04 ± 0.35 mg RE/g ext) whereas the ethyl acetate extract had the lowest flavonoid content (21.63 ± 0.33 mg RE/g ext.) (Table-5). The total phenolic contents were determined by the Folin-Ciocalteu method for the different extracts. The results showed that ethanol extract has the highest total phenolic content (124.43 ± 0.53 mg GAE/g ext.) followed by ethyl acetate extract (82.65 ± 0.43 mg GAE/g ext.) whereas, the petroleum extract had the lowest phenolic content (32.19 ± 0.49 mg GAE/g ext.).

The relationship between the total phenolic content and the antioxidant activity of *C. aspera* extracts showed a positive correlation. A significant as well as linear relationship was found between the antioxidant capacity and the total phenolic content from this study.

GC-MS chemical profiling: The GC-MS chromatogram (Fig. 3) displayed the components found in the ethanol extract

TABLE-4
IC_{50} VALUES (µg/mL) OF *Cleome aspera* AERIAL PARTS EXTRACT DETERMINED BY DPPH AND ABTS ASSAYS

	Ethanol	Ethyl acetate	Hexane	Gallic acid (positive control)
DPPH	219.02 ± 6.63	303.26 ± 8.69	1273.87 ± 159.03	9.53 ± 0.43
ABTS	27.43 ± 5.14	86.84 ± 7.43	517.76 ± 67.32	6.05 ± 0.06

Results from three independent experiments were given as mean ± standard deviation (SD).

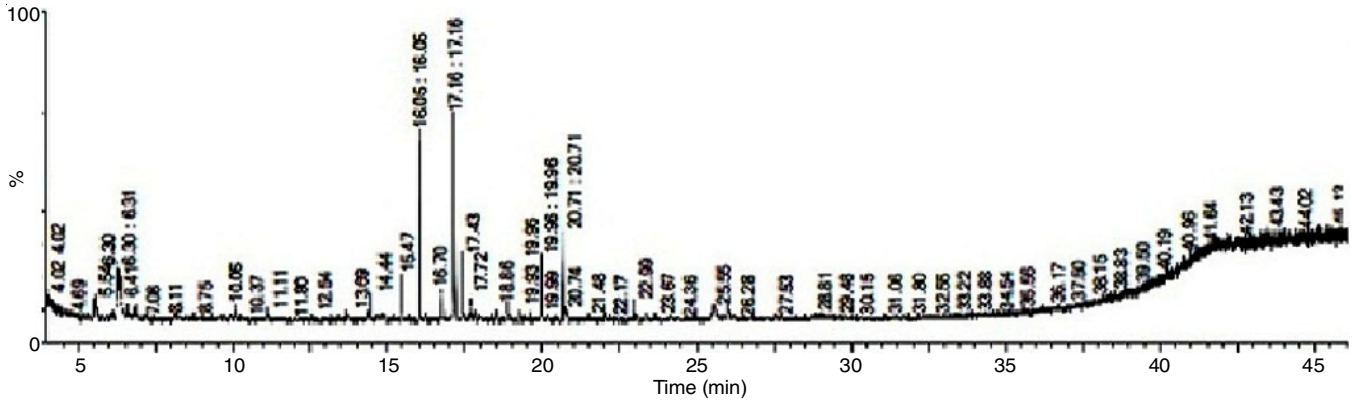

Fig. 3. GC-MS chromatograms of the bioactive compounds present in ethanol extract of *Cleome aspera* aerial parts

of *C. aspera* aerial parts. Table-6 lists the active ingredients along with their molecular formula, retention time (RT) and peak area as percentage. The major constituents identified were 4-undecene-(Z) (3.54%), 1-chloro-7-heptadecene (3.22%), 17-chloro-7-heptadecene (3.05%) and 1,5-pentanediol (1.45%). Other chemicals are found in trace quantities.

TABLE-6
NAME, MOLECULAR FORMULA, RETENTION TIME (min) AND AREA (%) OF COMPOUNDS OBTAINED FROM GC-MS SPECTRA OF ETHANOLIC EXTRACT OF *Cleome aspera*

Name of the compound	Molecular formula	Retention time (min)	Area (%)
1,5-Pentanediol	$C_6H_{14}O_2$	4.01	1.45
3-Methyl-4-tridecene	$C_{13}H_{26}$	4.08	0.73
(Z)-4-Tetradecene	$C_{14}H_{28}$	4.13	0.57
(E)-2-Decene-1-ol	$C_{10}H_{20}O$	4.19	0.35
(E)-Erucic acid	$C_{22}H_{42}O_2$	4.22	0.73
4-Tetradecene	$C_{14}H_{28}$	4.28	0.35
(Z)1-Chloro-8-heptadecene	$C_{17}H_{33}Cl$	4.33	0.34
8-Oxabicyclo[5.1.0]octane	$C_7H_{12}O$	4.39	0.36
cis-2-Methyl-7-octadecene	$C_{19}H_{38}$	4.82	0.36
1,2:4,5:9,10-Triepoxydecane	$C_{10}H_{16}O_3$	5.54	0.73
3-Methyl-2-(2-Oxopropyl)furan	$C_8H_{10}O_2$	5.60	0.50
17-Chloro-7-heptadecene	$C_{17}H_{33}Cl$	6.30	3.05
9-Octadecenoic acid-2,2,2-trifluoroethylester	$C_{20}H_{35}O_2F_3$	6.40	0.89
Undecanal	$C_{11}H_{22}O$	10.05	0.40
7-Tetradecene	$C_{14}H_{28}$	14.44	0.44
2,6,6-Trimethyl-bicyclo[3.1.1]hept-3-ylamine	$C_{10}H_{19}N$	15.47	0.84
1-Chloro-7-heptadecene	$C_{17}H_{33}Cl$	16.05	3.22
Dodecanal	$C_{12}H_{24}O$	16.70	0.63
4-Undecene-(Z)	$C_{11}H_{22}$	17.16	3.54
Cyclopentaneethanol, β,2,3-trimethyl-	$C_{10}H_{20}O$	17.43	1.19
Cyclopentane, 2-(1-hydroxy-2-propyl)-1,3-dimethyl-	$C_{10}H_{20}O$	17.72	0.45
Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	$C_{19}H_{34}$	18.86	0.49
Cyclohexaneethanol, β,4-dimethyl-, *cis*-	$C_{10}H_{20}O$	19.30	0.29
Cholestan-3,26-diol-22-one	$C_{27}H_{46}O_3$	19.96	1.36
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-,[1s(1α,2β,5α)]-	$C_{10}H_{18}$	20.71	1.72
Cyclohexaneethanol, β,4-dimethyl-, *trans*-	$C_{10}H_{20}O$	22.99	0.51
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1α,2β,5α)-	$C_{10}H_{16}O$	25.59	0.32
Glutaric acid, (cyclohex-3-enyl)methyl cyclohexyl ester	$C_{19}H_{30}O$	25.61	0.27
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-(1α,2α,5α)-	$C_{10}H_{16}O$	26.02	0.60
Cyclohexanemethanol, 4-(1-methyl ethyl)-, *cis*-	$C_{10}H_{20}O$	42.21	0.28
Cyclohexane,1-(1,5dimethylhexyl)-4-(4-methylpentyl)-	$C_{20}H_{40}$	–	–
Cholestan-3,26-diol-22-one diacetate	$C_{31}H_{50}O_5$	–	–
Androst-1-en-3-one, 4,4-dimethyl-, (5α)-	$C_2H_{32}O$	–	–
Divinyl*bis*(cyclopropyl)silane	$C_{10}H_{16}Si$	–	–
16-Deoxo-5,6-dihydrokryptogenin diformate	$C_{29}H_{46}O_5$	–	–
trans-1,4-Cyclohehanedimethanol, mono-*o*-trifluoroacetyl	$C_{10}H_{15}O_3F_3$	–	–
5-Methylene-9-decen-2-one	$C_{11}H_{18}O$	–	–
5,9-Tridecadien-1-ol, 10-propyl-, acetate	$C_{18}H_{32}O_2$	–	–

Conclusion

The antifungal, antioxidant and GC-MS analysis of *Cleome aspera* aerial parts extracts were investigated. The obtained results showed that ethanolic extract contains flavonoids and phenolic compounds along with steroids in addition to other phytoconstituents which is further confirmed with the GC-MS analysis. Both significant antifungal activity and a robust antioxidant capacity were observed in the ethanol extract of *C. aspera* aerial parts. The presence of a major ingredients in the extracts may have a synergistic impact with smaller amounts of other compounds, indicating that the extracts contain various natural product components.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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