

## Characterization and Bioactivity of Essential Oils Extracted from *Cleome Brachycarpa* Plant

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**Abstract.** Exploration of new bioactive compounds is a need of the day to meet the medicinal requirements of the world. The present study deals with the identification and assessment of phytochemical, antibacterial, and antifungal activities of the constituents present in the essential oil, water, n-hexane, and ethyl acetate extract of *Cleome brachycarpa*. The plant was collected from four different locations within Pakistan. The major constituents that have been identified were D-limonene,  $\beta$ -linalool, *p*-menth-1-en-8-ol, bergamiol,  $\gamma$ -eudesmol, viridiflorol,  $\alpha$ -caryophyllene, elemol, and globuol. Among these  $\gamma$ -eudesmol known as an anticancer agent, was in high contents that in fact highlights the medicinal importance of this plant. The biological study against gram-negative (*E. coli*, *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa*, *E. carotovora*, *A. tumefaciens*), gram-positive (*B. subtilis*, *B. atrophaeus*, *S. aureus*) bacterial species and one fungal (*C. albicans*) strain revealed a significant bioactivity of the extract in inhibiting the bacterial and fungal growth.

**Keywords:** *Cleome brachycarpa*; antibacterial; antifungal; essential oil; anti-microbial activities.

**Resumen.** La exploración de nuevos compuestos bioactivos es una necesidad actual para satisfacer las necesidades medicinales mundiales. El presente estudio aborda la identificación y evaluación de las actividades fitoquímicas, antibacterianas y antifúngicas de los constituyentes presentes en el aceite esencial y el extracto de agua, n-hexano y acetato de etilo de *Cleome brachycarpa*. La planta se recolectó en cuatro lugares diferentes de Pakistán. Los principales constituyentes identificados fueron D-limoneno,  $\beta$ -linalool, *p*-menth-1-en-8-ol, bergamiol,  $\gamma$ -eudesmol, viridiflorol,  $\alpha$ -cariofileno, elemol y globuol. Entre estos, el  $\gamma$ -eudesmol, conocido como agente anticancerígeno, se encontraba en altos contenidos que, de hecho, resaltan la importancia medicinal de esta planta. El estudio biológico frente a especies bacterianas gram-negativas (*E. coli*, *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa*, *E. carotovora*, *A. tumefaciens*), gram-positivas (*B. subtilis*, *B. atrophaeus*, *S. aureus*) y una cepa fúngica (*C. albicans*) reveló una bioactividad significativa del extracto en la inhibición del crecimiento bacteriano y fúngico.

**Palabras clave:** *Cleome brachycarpa*; antibacterial; antifungico; aceite esencial; actividades antimicrobianas.

## Introduction

The *Cleome brachycarpa* of the family Cleomaceae is a wild-growing flowering and perennial herb found worldwide, including Pakistan (Fig. 1). The *Cleome brachycarpa* exhibits a special pleasant aroma hence in Hindi it is called Panwar, while in Pakistan mostly known as Gandhi booty [1-3]. *Cleome brachycarpa* is a well-recognized bioactive plant and hence very commonly used for ailments of humans and animals particularly for cancer [1,2]. Quite a high percentage of the reported work focuses on the identifications of chemical constituents from the extracted essential oil of the plant [1-14]. Unfortunately, very few investigations deal with the bioactive properties of the extracted oil [8,9,13,14]. However, almost no research work about water-extracted components has been reported [12]. It is documented that plants harvested /collected from various places may not have the same composition/ components [15-17]. Therefore, the purpose of the current research work was to explore essential oil / volatile components, and ethanolic extract of *Cleome brachycarpa* for the composition as well as phytochemical, antibacterial, and antifungal activities.



Fig. 1. A view of the *Cleome brachycarpa* plant [3].

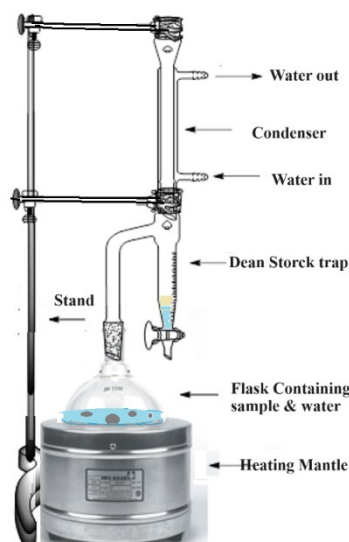
## Material and methods

The aerial parts with composition as stem 40 %, leaves 35 %, and flower 25 % of the plant *Cleome brachycarpa* were collected in October 2020, from Kot Musa (31° 35' 9" North, 70° 25' 24" East) and Ramak (31° 26' 0" North, 70° 41' 0" East) towns of district Dera Ismail Khan, KPK, and Bhakkar (31° 37' 59.9988" N and 71° 3' 59.9976" E) and Karor Lal Esan (31° 13' 38.0712" N and 70° 57' 6.1416" E) a city of Layyah District, Punjab, Pakistan (Fig. 2).



Fig. 2. Locations of the plant collection.

The freshly collected plant was cut into small pieces and dried in the Laboratory under shade. 500 g of dried plant material was placed in a flask of 5-litre capacity containing 3000 mL of distilled water. The essential oil was hydro-distilled for approximately six hours at the boiling temperature of suspension, using a Clevenger-type apparatus (Fig. 3). The collected essential oil was dehydrated using anhydrous sodium sulfate [18-21]. The process was repeated several times using fresh samples of plant material till the collection of sufficient amounts of essential oil. All the procedure was carried out in the Organic Chemistry Laboratory of ICS, Gomal University, Dera Ismail Khan, Pakistan.



**Fig. 3.** Schematic representation of hydro distillation apparatus.

### GC-MS analysis of the essential oil

The chemical analysis of essential oil of *Cleome brachycarpa* was performed using GC-MS-QP2010 Plus Shimadzu, Kyoto, Japan instrument, with DB5 capillary column having dimensions 30 m  $\times$  0.25 mm, film thickness 0.25  $\mu$ m, using helium as a carrier gas at a flow rate of 1.5 mL/min. The oven temperature of the GC was set at 40  $^{\circ}$ C for an initial 2 min, then was raised at a rate of 3  $^{\circ}$ C up to 90  $^{\circ}$ C and maintained at this temperature for 10 min. Finally, the temperature was raised to 240  $^{\circ}$ C at a rate of 5  $^{\circ}$ C per min. A solution of essential oil (500 ppm v/v) was prepared in dichloromethane (DCM, Merck, Darmstadt, Germany) and slowly shaken for 2 min and allowed to stabilize for 6 min for injection in to GCMS. 1  $\mu$ L of the essential oil solution was injected into the GC column at a temperature of 240  $^{\circ}$ C, using a split injection mode. The mass spectrum was acquired at 70 eV between the start time of 3.00 min and end time 46.00 min. The mass data was recorded between start m/z 40 and end m/z 500 amu at a sampling rate of 0.5 scan/s. The components of the essential oil were identified by comparing them with a database of the National Institute of Standards and Technology (NIST) Library installed in the computer of the instrument. These were further confirmed by comparing the retention index (RI) of the straight-chain alkanes C7-C40 with the retention index (RI) of the components of the essential oil [18].

### Extraction and fractionation

The air-dried powdered plant material (500 g) was exhaustively extracted with 95 % ethanol (3  $\times$  1000 mL) using the Soxhlet apparatus. The combined ethanol extract was concentrated under the vacuum at 40  $^{\circ}$ C to give a brown residue using a rotary apparatus. The obtained residue was suspended in 250 mL of distilled water and then partitioned successively with n-hexane (3  $\times$  100 mL) and Ethyl acetate (3  $\times$  100 mL). Each fraction was concentrated under *vacuum* at a temperature not exceeding 40  $^{\circ}$ C to afford 12, 20, and 25 g, respectively.

### Biological activities of essential oil and extracts

The biological activities were carried out at PCSIR labs, Peshawar, Pakistan, The Antibacterial activities in terms of the diameter of the inhibition zone (mm) were performed by the reported protocol applying

the agar disc diffusion method [22-25]. Commercially available agar preincubated (for half an hour at room temperature) Petri dishes were inoculated by swabbing with microbes comparable to McFarland (0.5 mg/mL). The activity of essential oil and extracts were procured using 6 mm diameter discs of filter paper dipped in essential oil and extract dilutions. The dilutions of essential oils (w/v) and extracts (w/v) in 2 mL of distilled water having 0.5 % DMSO were 25 %, 50 %, 75 %, and 100 % (pure essential oil or extract). Afterward, the Petri dishes were incubated at 37 °C for 24 hours. Antibacterial activities were established by measurement of the diameter of the zone of inhibition (mm); a transparent circular area around the microbes at the disc. Positive control for bacteria was Gentamicin and one fungal strain i.e. *C. albicans* was used to measure the anti-fungal activities using miconazole as a standard. Pure water was used as a control [22].

### Antimicrobial activity (MICs) of extracts and essential oil

The agar well dilution method was applied to determine the minimum inhibitory concentrations (MICs) values of plant extracts and essential oils [22].

## Results and discussion

### GC-MS analysis of the essential oil

Essential oil collected from the plant was analyzed by employing GC-MS. The results for the plant collected from Ramak are displayed in Fig. 4. The same results are displayed in the form of histogram in Fig. 5. The same procedure was adopted, and the components identified from the plants collected from various sites are reported in Table 1. The analysis showed that oil from the plant contained about 10 different components and the concentration of these components was found to be 2 % to 36 %. The main component that is present in the plants is D-limonene having the percent composition as 2.57 %. The essential oil extracted from the plant also contained 6.19 %  $\beta$ -linalool, 2.68 % *p*-menth-1-en-8-ol, and 2.13 % bergamiol. The contents of caryophyllene was 5.25 % and that of  $\alpha$ -caryophyllene was 12.43 %. GC-MS analysis also revealed that the plant also contained 24.55 % elemol and 4.12 % globuol. The effective concentration of viridiflorol in the essential oil of the plant was 4.21 %. The highest percentage was of  $\gamma$ -eudesmol (36.21 %) whereas the concentration of the bergamiol was lowest in the essential oil. The D-limonene presented a non-significant effect for all the sites. The mean concentration of D-limonene (%) among the four sites ranged from 3.03 to 4.3 (%). The values of D-limonene investigated were greater at site 2 as compared to others. The  $\beta$ -linalool showed non-significant results in *Cleome brachycarpa* for all the sites assessment. Mean values of  $\beta$ -linalool among the four sites varied from 5.49 (Site 3) to 6.18 % (Site 1). It can be noted that though the values of various components were different for the plants collected from different locations, the difference is not significant contrary to other conclusions [15-17,22]. The reason behind it can be that the environment of the four locations is almost the same.

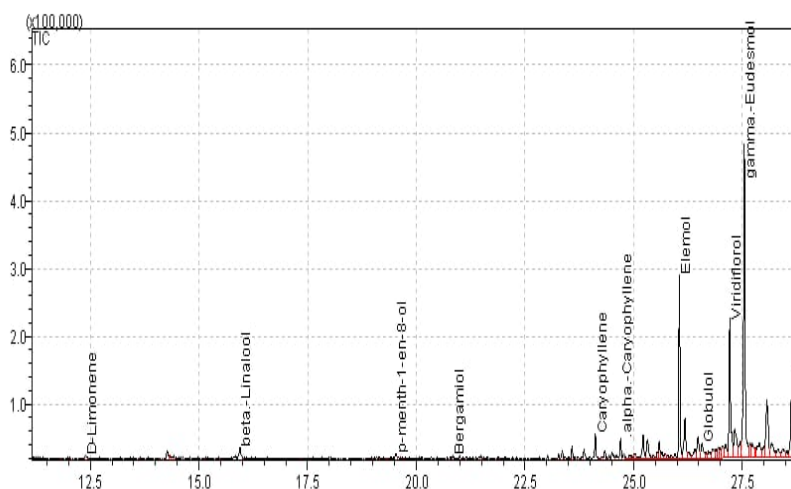
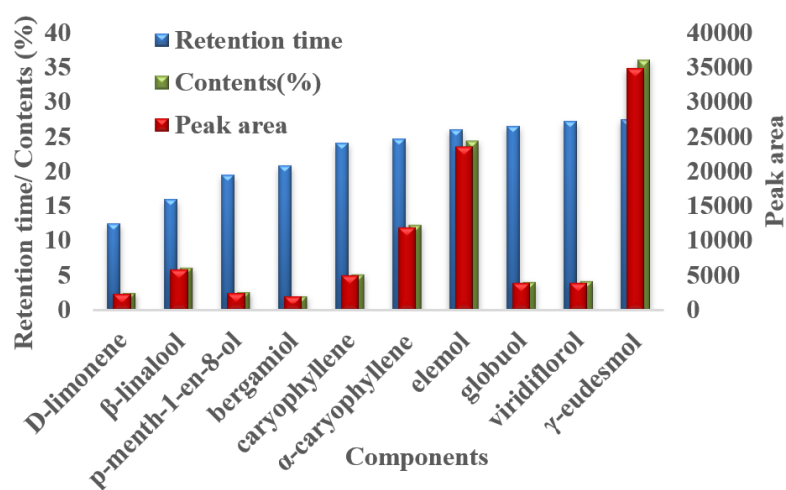


Fig. 4. GC-MS spectra of essential oil extracted from *Cleome brachycarpa*.



**Fig. 5.** Histogram showing the peak area and composition.

**Table 1.** Mean percent composition of compounds identified from essential oil of *Cleome brachycarpa* collected from various sites.

S. No	Compound	Sites name	Minimum	Maximum	Mean
1	D-limonene	Kot Musa	2.31	3.77	3.04
		Ramak	2.87	4.01	3.44
		Bhakkar	3.21	3.77	3.49
		Karor Lal Esan	3.25	4.03	3.64
2	β-linalool	Kot Musa	5.37	6.99	6.18
		Ramak	5.23	6.29	5.76
		Bhakkar	5.34	5.64	5.49
		Karor Lal Esan	5.54	6.21	5.88
3	p-menth-1-en-8-ol	Kot Musa	2.37	2.91	2.64
		Ramak	1.65	2.11	1.88
		Bhakkar	2.67	3.22	2.95
		Karor Lal Esan	2.04	2.64	2.34
4	bergamiol	Kot Musa	2.13	2.97	2.55
		Ramak	2.85	3.13	2.99
		Bhakkar	2.92	3.52	3.22
		Karor Lal Esan	2.56	3.43	3.05

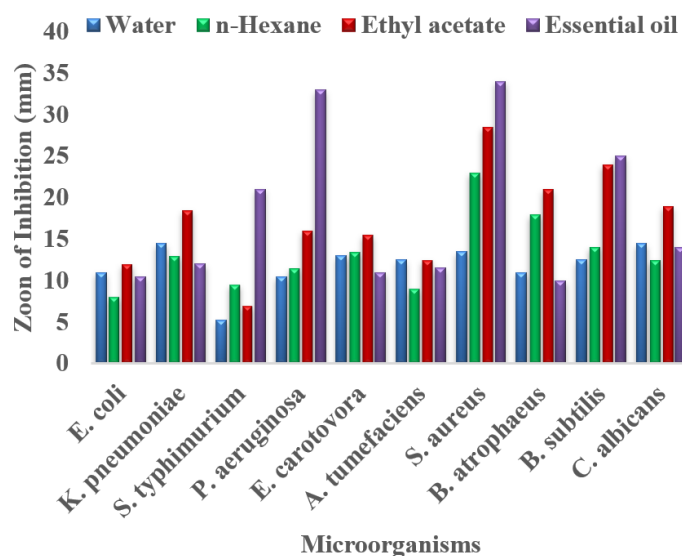
S. No	Compound	Sites name	Minimum	Maximum	Mean
5	carophyllene	Kot Musa	4.41	5.87	5.14
		Ramak	4.79	5.97	5.38
		Bhakkar	4.50	5.20	4.85
		Karor Lal Esan	5.01	5.42	5.22
5	$\alpha$ -caryophyllene	Kot Musa	12.43	14.99	13.71
		Ramak	12.41	15.89	14.15
		Bhakkar	12.01	13.01	12.51
		Karor Lal Esan	12.32	13.84	13.08
7	elemol	Kot Musa	24.23	25.51	24.87
		Ramak	23.71	27.01	25.36
		Bhakkar	24.23	26.32	25.28
		Karor Lal Esan	24.12	25.85	24.99
8	globulol	Kot Musa	3.21	6.13	4.67
		Ramak	3.11	6.13	4.62
		Bhakkar	2.93	3.73	3.33
		Karor Lal Esan	3.05	3.82	3.44
9	viridiflorol	Kot Musa	3.98	4.29	4.14
		Ramak	4.11	5.67	4.89
		Bhakkar	4.52	5.63	5.08
		Karor Lal Esan	4.01	5.21	4.61
10	$\gamma$ -eudesmol	Kot Musa	35.53	39.57	37.55
		Ramak	36.97	38.71	37.84
		Bhakkar	36.43	37.52	36.98
		Karor Lal Esan	36.52	37.54	37.03

The biological activities of extracts and essential oils were measured against various bacteria and fungi and reported in terms of zone of inhibition (Table 2). The results indicated that the essential oil and the extract values were lower than the reported in the literature for the same plant [26-28]. The results further highlighted that essential oil showed high activity as compared to extracts.

**Table 2.** Biological activity of the Essential oil and different extracts in terms of zone of inhibition (mm) from the *Cleome brachycarpa*.

Serial No.	Bacteria/ Fungus	Water extract	n-Hexane extract	Ethyl acetate extract	Essential oil
1	<i>Escherichia coli</i>	11 ± 0.09	8 ± 0.01	12 ± 0.06	10.5 ± 0.07
2	<i>Klebsiella pneumoniae</i>	14.5 ± 0.1	13 ± 0.11	18.5 ± 0.02	12 ± 0.14
3	<i>Salmonella typhimurium</i>	5.2 ± 0.02	9.5 ± 0.01	7 ± 0.1	21 ± 0.05
4	<i>Pseudomonas aeruginosa</i>	10.5 ± 0.09	11.5 ± 0.07	16 ± 0.07	33 ± 0.03
5	<i>Erwinia carotovora</i>	13.0 ± 0.02	13.5 ± 0.08	15.5 ± 0.05	11 ± 0.20
6	<i>Agrobacterium tumefaciens</i>	12.5 ± 0.04	9 ± 0.01	12.5 ± 0.03	11.5 ± 0.06
7	<i>Staphylococcus aureus</i>	13.5 ± 0.01	23 ± 0.08	28.5 ± 0.03	34 ± 0.13
8	<i>Bacillus atrophaeus</i>	11.0 ± 0.06	18 ± 0.14	21 ± 0.07	10 ± 0.01
9	<i>Bacillus subtilis</i>	12.5 ± 0.1	14 ± 0.02	24 ± 0.07	25 ± 0.20
10	<i>Candida albicans</i>	14.5 ± 0.06	12.5 ± 0.03	19 ± 0.12	14 ± 0.09

The inhibition activity of the essential oil was in the range of  $10 \pm 0.09$  mm to  $34 \pm 0.03$  mm with a mean index of  $23.2 \pm 0.08$  mm. The growth inhibition of the water extract was in the range of  $10 \pm 0.09$  mm to  $14 \pm 0.06$  mm and showed a little bit of low inhibition activity. The observed growth inhibition activity of n-hexane extract was in the range of  $9 \pm 0.01$  to  $23 \pm 0.09$  mm. This showed a greater inhibition activity than water extract on similar mediums and cultures. The inhibition activity of the ethyl acetate extract examined was between the range of  $12 \pm 0.06$  mm to  $28 \pm 0.03$  mm which was greater among all extracts except the essential oil. The results also describe that the essential oil showed a greater inhibition in the growth of the chosen bacterium.

**Fig. 6.** Zoon of inhibition (mm) of essential oil, water, ethyl acetate, and hexane extract of *Cleome brachycarpa* extracts and against some pathogenic microorganisms.



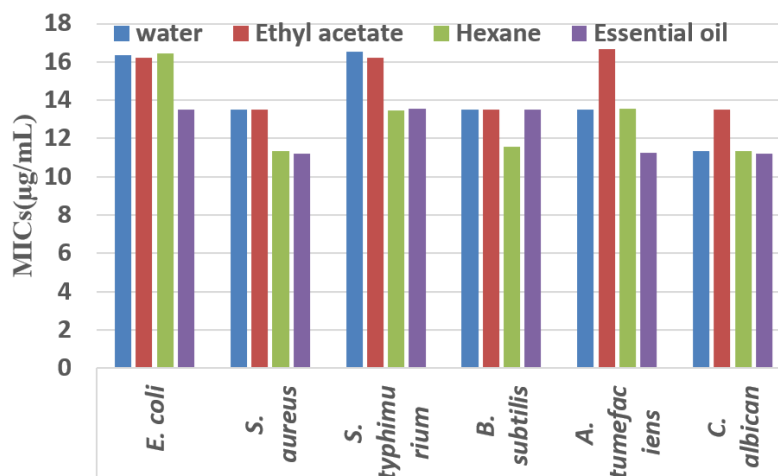
The essential oil extracted from this plant showed adequate inhibition activity. *E. coli* at the rate of  $10.5 \pm 0.09$  mm inhibition diameter with the *Klebsiella pneumoniae* the growth inhibition rate is  $12 \pm 0.06$  mm. The inhibition against *Salmonella typhimurium* was  $21 \pm 0.05$  mm which indicated moderate activity against a given bacterium. The growth inhibition activity of essential oil against *Pseudomonas aeruginosa* is  $33 \pm 0.03$  mm with the highest growth inhibition rate in the selected bacterium. The inhibition against the *Erwinia carotovora*, is measured in the range of  $11 \pm 0.20$  mm and against the *Agrobacterium tumefaciens* it is  $11.5 \pm 0.06$  mm. The activity against the *Staphylococcus aureus* is 34 mm, the antibacterial activity of essential oil for the *Bacillus atrophaeus* was  $10 \pm 0.01$  mm. The resistivity of the extracted oil against the *Bacillus subtilis* was  $25 \pm 0.02$  mm and the inhibition activity of essential oil against the fungus *Candida albicans* was  $14 \pm 0.09$  mm in the inhibition diameter. The extracts and essential oil exhibited good activity against *Staphylococcus aureus* ( $34 \pm 0.13$  mm), *Pseudomonas aeruginosa* ( $33 \pm 0.03$ mm), ( $12 \pm 0.14$  mm) and *Candida albicans* ( $14 \pm 0.09$  mm). The activity against *Salmonella typhimurium* was  $21 \pm 0.07$  mm. The ethyl acetate indicated marginal higher antibacterial and antifungal activity than the water and n-hxane extracts. The water and ethyl acetate extracts showed the lowest activity against *Salmonella typhimurium*. The ethyl acetate extract exhibited an extended zone against *Staphylococcus aureus* ( $28.5 \pm 0.03$  mm) followed by *B. subtilis* ( $21 \pm 0.07$  mm). n-Hexane extract was found effective against *B. subtilis* ( $24 \pm 0.07$  mm) and *B. atrophoeus*. Water extract was lower in activity as compared to both n-hexane and ethyl acetate. According to the researchers the D-limonens and other components present in the essential oil of the common plants are responsible for the high antibacterial and antifungal activities. The current research result showed that the essential oil of the *cleome barchrappya* contained D- limonens which showed significant antifungal activity. D-limonene exhibits powerful inhibitions against bacteria and fungus [27]. The inhibition ability of oil extracted from the plants of the different areas is different from each other mainly due to differences in the composition of the constituent components and differences in the functional groups of the components present in the essential oil of the plants. The components linalool present in the essential oil exhibited moderate activity and D-limonene the lowest activity. The compound containing ester group shows little bit low activity. Many studies on the activity of different plants illustrate that compounds which have phenolic groups are active against bacteria and the bio activity of plant depend upon the functional group and chemical nature. The different plants show different inhibition activities on the gram-positive or negative species due to different groups and structures [29-31].

The minimal inhibitory concentration (MIC) of essential oils and the extracts of *C. brachycarpa* was determined [32-33]. The essential oil showed MIC values from  $11.23$  to  $13.55 \mu\text{gml}^{-1}$ , for all strains tested. The MICs values varied with the type of bacterium tested. The MIC values of extracts ranged from  $11.34$ – $16.67 \mu\text{gml}^{-1}$ . The results are summarized in Table 3 and displayed in Fig. 7 which indicates that the essential oil is the most biologically active component of the plant, and the second one is hexane extract and least one is water extract.

**Table 3.** MICs ( $\mu\text{gml}^{-1}$ ) of essential oil *Cleome brachycarpa* extracts and against some pathogenic microorganisms.

Extract/ Bacteria	Microorganism					
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>	<i>Bacillus subtilis</i>	<i>Agrobacterium tumefaciens</i>	<i>Candida albicans</i>
Water	$16.34 \pm 0.09$	$13.50 \pm 0.05$	$16.54 \pm 0.10$	$13.52 \pm 0.07$	$13.53 \pm 0.07$	$11.34 \pm 0.07$
Ethyl acetate	$16.24 \pm 0.10$	$13.52 \pm 0.06$	$16.23 \pm 0.12$	$13.53 \pm 0.06$	$16.67 \pm 0.10$	$13.52 \pm 0.3$
Hexane	$16.45 \pm 0.12$	$11.34 \pm 0.04$	$13.45 \pm 0.09$	$11.56 \pm 0.08$	$13.55 \pm 0.08$	$11.34 \pm 0.04$
Essential oil	$13.51 \pm 0.13$	$11.23 \pm 0.05$	$13.55 \pm 0.08$	$13.51 \pm 0.09$	$11.24 \pm 0.06$	$11.23 \pm 0.06$





**Fig. 7.** MIC ( $\mu\text{g mL}^{-1}$ ) of essential oil, water, ethyl acetate, and hexane extract of *Cleome brachycarpa* extracts and against some pathogenic microorganisms.

## Conclusions

Phytochemical, antibacterial and antifungal activities of the essential oil, and water, n-hexane and ethyl acetate extract of *Cleome brachycarpa* collected from Pakistan were performed. The GC-MS analysis of the essential oil revealed the presence of following 10 useful compounds with significant yields: D-limonene,  $\beta$ -linalool, *p*-menth-1-en-8-ol, bergamiol,  $\gamma$ -eudesmol, viridiflorol,  $\alpha$ -caryophyllene, caryophyllene, elemol and globuol.  $\gamma$ -eudesmol known as having anticancer activity was in highest percentage. Further, the essential oil and water, hexane and ethyl acetate extract exhibited antifungal and antibacterial potentials. We expect that the findings of the current study could be useful for the medicinal point of view.

## References

1. Naeem, H.; Perveen, R.; Zaidi, S. S. M.; Zia, Z.; Fatima, K.; Akram, Z.; Hussain, M.; Ishaque, F. *RADS J. Pharm. Pharm. Sci.* **2019**, 7, 107-111. DOI: <https://doi.org/10.1155/2020/768345>.
2. Tesfaye, S.; Belete, A.; Engidawork, E.; Gedif, T.; Asres, K. *Evid. -Based Complementary Altern. Med.* **2020**, 2020, 7683450. DOI: <https://doi.org/10.1155/2020/768345>.
3. Ali, H. K.; Cheruth, A. J.; Mohammed A.; Salem, M. A.; Maqsood, S. *Pharmacologyonline.* **2012**, 3, 167-173.
4. Ahmad, V. U.; Alvi, K. A. *Phytochem.* **1986**, 26, 315-316. DOI: [https://doi.org/10.1016/s0031-9422\(00\)81537-x](https://doi.org/10.1016/s0031-9422(00)81537-x).
5. Ahmad, V. U.; Alvi, K. A.; & Khan, M. A. *J. Nat. Prod.* **1986**, 49, 249-252. DOI: <https://doi.org/10.1021/np50044a009>.
6. Ahmad, V. U.; Qazi, S.; Zia, N. B.; Xu, C.; Clardy, J. *Phytochem.* **1990**, 29, 670-672. DOI: [https://doi.org/10.1016/0031-9422\(90\)85144-5](https://doi.org/10.1016/0031-9422(90)85144-5).
7. Jorshabani, S.; Bagheri, F.; Asgarpanah, J.; Bidgoli, S. *ARC J. Pharm. Sci.* **2017**, 3 22-24. DOI: <https://doi.org/10.20431/2455-1538.0302004>.
8. Sarfaraz, S.; Najam R.; Azhar, I.; Ahmed, S.; Sarwar, G. *J. Anal. Pharm. Res.* **2017**, 5, 00162. DOI: <http://doi.org/10.15406/japlr.2017.05.00162>.

9. Saleem, T.; Sumra, A.; Khan, S.; Zain, M.; Hassan, W.; Mehdi, S.; Wahid, N.; Kanwal, S.; Gull, T. *Sains Malays.* **2020**, *49*, 1915–1924. DOI: <http://dx.doi.org/10.17576/jsm-2020-4908-13>.
10. Zaidi, S.; Khatoon, S.; Shaukat, S. S. *Pak. J. Bot.* **2012**, *44*, 1733–1739. DOI: [https://www.pakbs.org/pjbot/paper\\_details.php?id=3336](https://www.pakbs.org/pjbot/paper_details.php?id=3336).
11. Rassouli, E.; Dadras, O. G.; Bina, E.; Asgarpanah, J. *J. Essential Oil-bear. Plants.* **2014**, *17*, 158–163. DOI: <https://doi.org/10.1080/0972060x.2014.884784>.
12. El-Sharkawy, S. H.; Ghazy, N. M.; El-Fiky, F. K.; El-Lakany, A. M.; Omar, A. A. *Alex. J. Pharm. Sci.* **1993**, *7*, 121–124.
13. Camara, A.; Haddad, M.; Traore, M. S.; Chapeland-Leclerc, F.; Ruprich-Robert, G.; Fourasté, I.; Balde, M. A.; Royo, J.; Parny, M.; Batigne, P.; Salon, M.; Coste, A.; Balde, A. M.; Aubouy, A. *BMC Complement. Med. and Ther.* **2021**, *21*, 3231–3233. DOI: <https://doi.org/10.1186/s12906-021-03231-3>.
14. Elsharkawy, E. R.; Alghanem, S. M.; Elmorsy, E. *Sch. Bip. Biotechnol. Rep.* **2021**, *29*, e00581. DOI: <https://doi.org/10.1016/j.btre.2020.e00581>.
15. Khan, M. H.; Dar, N. A.; Alie, B. A.; Dar, S. A.; Lone, A. A.; Mir, G. H.; Fayaz, U.; Ali, S.; Tyagi, A.; El-Sheikh, M. A.; Alansi, S. *Molecules.* **2023**, *28*, 2404. DOI: <https://doi.org/10.3390/molecules28052404>.
16. Elyemni, M.; Louaste, B.; Nechad, I.; Elkamli, T.; Bouia, A.; Taleb, M.; Chaouch, M.; Eloutassi, N. *Sci. World J.* **2019**, 1–6. DOI: <https://doi.org/10.1155/2019/3659432>.
17. Pesavento, G.; Calónico, C.; Bilia, A.; Barnabei, M.; Calesini, F.; Addona, R.; Mencarelli, L.; Carmagnini, L.; Di Martino, M.; Lo Nostro, A. *Food Control.* **2015**, *54*, 188–199. DOI: <https://doi.org/10.1016/j.foodcont.2015.01.045>.
18. Tran, L. T. T.; Nguyen, T. K.; Pham, T. V.; Ha, T. P.; Tran, P. T. D.; Tam, V. T. T.; Dat, T. T. H.; Thai, P. H.; Cuong, L. C. V. *Appl. Sci.* **2023**, *13*, 11224. DOI: <https://doi.org/10.3390/app132011224>.
19. Panchal, B.; Deshmuk, S.; Sharma, M. *Int. J. Gas and Coal Eng.* **2014**, *2*, 1–6. DOI: <https://doi.org/10.11648/j.ogce.20140201.11>.
20. Ozturka, M.; Tela, G.; Durua, M. E.; Harmandara, M.; Topcu, G. *Nat. Prod. Commun.* **2009**, *4*, 1017–1020.
21. Onyebuchi, C.; Kavaz, G. *Sci. Rep.* **2020**, *10*, 21760. DOI: <https://doi.org/10.1038/s41598-020-78847-5>.
22. Liu, X.; Zhou, S.; Huang, Y.; Chen, M.; Wang, W.; Wang, J.; Hao, E.; Wu, H.; Li, Y. *J. Essent. Oil-Bear. Plants.* **2023**, *26*, 787–801. DOI: <https://doi.org/10.1080/0972060x.2023.2239847>.
23. Afifi, M. S. *Int. J. Pharm. Sci. & Res.* **2014**, *5*, 4008–4014. DOI: [http://doi.org/10.13040/IJPSR.0975-8232.5\(9\).4008-14](http://doi.org/10.13040/IJPSR.0975-8232.5(9).4008-14).
24. Piras, A.; Rosa, A.; Marongiu, B.; Porcedda, S.; Falconieri, D.; Dessì, M.; Ozcelik, B.; Koca, U. *Ind. Crop. Prod.* **2013**, *46*, 317–323. DOI: <https://doi.org/10.1016/j.indcrop.2013.02.013>.
25. Sabo, V. A.; Knezevic, P. *Ind. Crop. Prod.* **2019**, *132*, 413–429. DOI: <https://doi.org/10.1016/j.indcrop.2019.02.051>.
26. Bussmann, R.; Malca-García, G.; Glenn, A.; Sharon, D.; Chait, G.; Díaz, D.; Pourmand, K.; Jonat, B.; Somogy, S.; Guardado, G.; Aguirre, C.; Chan, R.; Meyer, K.; Kuhlman, A.; Townesmith, A.; Effio-Carbajal, J.; Frías-Fernandez, F.; Benito, M. *J. Ethnopharmacol.* **2010**, *132*, 101–108. DOI: <https://doi.org/10.1016/j.jep.2010.07.048>.
27. Andrews, J. M. *J. Antimicrob. Chemother.* **2001**, *48*, 5–16. DOI: [http://doi.org/10.1093/jac/48.suppl\\_1.5](http://doi.org/10.1093/jac/48.suppl_1.5).
28. Ghosh, S.; Ozek, T.; Tabanca, N.; Ali, A.; Rehman, J. U.; Khan, I. A.; Rangan, L. *Ind. Crop. Prod.* **2014**, *53*, 111–119. DOI: <https://doi.org/10.1016/j.indcrop.2013.12.026>.
29. Filipowicz, N.; Kamiński, M.; Kurlenda, J.; Asztemborska, M.; Ochocka, J. R. *Phytother. Res.* **2013**, *17*, 227–231. DOI: <https://doi.org/10.1002/ptr.1110>.

30. Gurbuz, I.; Ozcelik, B.; Gunbatan, T.; Akkol, E. K.; Sahinoz, M.; Akaydin, G. *Pak. J. Pharm. Sci.* **2021**, 34, 1011-1017. DOI: <http://doi.org/10.36721/PJPS.2021.34.3.REG.1011-1017.1>.
31. Joukar, M.; Larijani, K.; Farjam, M. H.; Givianrad, M. H.; Nematollahi, F. *J. Med. Plants By-prod.* **2023**, 12, 251-258. DOI: <https://doi.org/10.22092/jmpb.2022.353596.1336>.
32. Man, A.; Santacroce, L.; Jacob, R.; Mare, A.; Man, L. *Pathogens*. **2019**, 8, 15. DOI: <https://doi.org/10.3390/pathogens8010015>.
33. Naeem, H.; Gul, S.; Khan, M.; Hamid, S.; Leghari, Q. A.; Yasin, H.; Perveen, R. *Jundishapur J. Nat. Pharm. Prod.* **2023**, 18, e132712. DOI: <https://doi.org/10.5812/jjnpp-132712>.