Cleome droserifolia (Forssk.) Del.

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The antioxidant, antimicrobial, and immunomodulatory activities of the Cleome droserifolia (Forssk.) Del. (Cd) shrub were investigated considering the biological activity of its phytogenic compounds.

Keywords: phytogenic ; phenols ; antioxidant ; antimicrobial activity ; immunity

1. Introduction

Medicinal plants can serve as a natural source of therapeutic drugs, nutraceuticals/food supplements, and feed additives that can be safely used to improve human and animal health. The interest in exploring plants as a new source of different drugs, specifically antimicrobials, has increased in recent decades as an attempt to fight multidrug-resistant bacteria [1][2]. Among the medicinal plants, the *Cleome* genus is one of the largest genera belonging to the family Cleomaceae. This genus encompasses about 180–200 species that are geographically distributed in Egypt, Libya, Palestine, Syria, and other arid and semi-arid regions [3]. Moreover, they are perennial, low, and aromatic cushion-like shrubs with a length of 25–60 cm that exhibit intricately branched stems and broad oval-shaped, three-nerved leaves with swollen glandular hairs [3][4]. The shrubs that belong to this genus have medicinal and ecological importance. *Cleome* genus shrubs are well-known in folk medicine for treating stomachache, skin allergies, and open wounds, as well as for exhibiting anticancer and hepatoprotective properties [5][6][7]. In addition, *Cleome* genus shrubs have shown strong antidiabetic properties; the aqueous extract of *Cleome* has been found to contain a very high percentage of flavonols that showed 63.3% activity, similar to that of the metformin synthetic drug [8]. *Cleome* genus shrubs have antioxidant, antiparasitic, and antimicrobial activities [6]. These biological effects are related to the vast array of secondary metabolites that occur naturally in *Cleome* genus shrubs. Several terpenes, flavonoids, glucosinolates, anthocyanin alkaloids, and polyphenols have been isolated from *Cleome* genus shrubs [3]. Given these biological activities of *Cleome* genus shrubs, additional studies are required to explore the active secondary metabolites of these shrubs and their eligibility to innovate natural feed and food supplements that could be applied for improving animal and human health.

2. RP-HPLC Assessment of Total Phenol and Flavonoid Contents and Phenolic Compound Profile

The values of TPC and TFC of the Cd methanolic extract were 32.55- ± 2.26-mg GA/g DM and 12.78- ±1.86-mg CAT/g DM, respectively (**Table 1**). The phenolic profile of the Cd methanolic extract detected by RP-HPLC is shown in **Table 1**. These results revealed that, among the 16 phenolic compounds identified here, the most abundant phenolic compounds, ranging between 1460.62 and 7657.15 µg/g DM, were benzoic acid, rutin, ellagic acid, naringenin, and o-coumaric acid. The second-most abundant phenolic compounds, ranging between 432.14 and 264.06 µg/g DM, were rosmarinic acid, p-hydroxybenzoic acid, resveratrol, kaempferol, quercetin, and ferulic acid. The third-most abundant phenolic compounds were caffeic acid, p-coumaric acid, chlorogenic acid, catechin, syringic acid, and catechin, which were detected in low quantities, ranging between 10.43 and 59.59 µg/g DM.

Table 1. Contents of the total phenolic, total flavonoid, and individual phenolic compounds (as detected by reverse-phase high-performance liquid chromatography; RP-HPLC) in the *Cleome droserifolia* (Forssk.) Del. methanolic extract (Cd extract).

Analysis	Content
Total phenols (mean ± SE, mg GA equivalent/g DM)	**32.55 ± 0.23**
Total flavonoids (mean ± SE, mg CAT equivalent/g DM)	**12.78 ± 0.19**
Individual detected phenolic compounds (µg/g DM)	

Analysis	Content
Benzoic acid	7657.15
Rutin	2987.63
Ellagic acid	1641.98
Naringenin	1516.25
o-Coumaric acid	1460.62
Rosmarinicacid	955.27
p-Hydroxybenzoic acid	924.57
Resveratrol	895.77
Kaempferol	778.80
Quercetin	432.14
Ferulic acid	264.06
Caffeic acid	59.59
p-Coumaric acid	39.55
Chlorogenic acid	29.33
Syringic acid	19.29
Catechin	10.43

GA = gallic acid, CAT = catechol, and DM = dry matter.

3. Antioxidant Activity of the Cd Extract

The antiradical capacity (scavenging activity) of the Cd methanolic extract, as determined by the DPPH and ABTS colorimetric tests, is shown in **Table 2**. The percent inhibition values of the Cd extract were not much greater than those of the standard antioxidant (ascorbic acid). The Cd extract showed a linear increase in the DPPH and ABTS radical scavenging activities with increasing concentrations, reaching 66.09% ± 1.92% and 81.14% ± 1.26% scavenging activity for DPPH and ABTS, respectively, at concentrations of 1000 µg/mL vs. 87.52% ± 0.62% and 92.44% ± 0.14% for ascorbic acid. The half-maximal inhibitory concentration (IC_{50}) of the Cd extract was 470.27 ± 2.24 µg/mL for DPPH and 387.53 ± 3.11 µg/mL for ABTS vs. 16.62 ± 0.91 µg/mL and 14.03 ± 0.67 µg/mL for ascorbic acid, respectively.

Table 2. Antioxidant activity of the *Cleome droserifolia* (Forssk.) Del. methanolic extract (Cd extract), as assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) tests.

Antioxidant Concentration (µg/mL)	DPPH Scavenging Activity, %		ABTS Scavenging Activity, %	
	Cd Extract	Ascorbic Acid	Cd Extract	Ascorbic Acid
7.81	12.87± 0.91 ^{b}	62.66 ± 0.12 ^{a}	23.16 ± 0.76 ^{b}	64.58 ± 0.24 ^{a}
15.6	16.03 ± 0.84 ^{b}	75.68 ± 0.45 ^{a}	25.09 ± 0.84 ^{b}	76.10 ± 0.92 ^{a}
31.25	26.88 ± 0.86 ^{b}	77.6 ± 0.86 ^{a}	34.16 ± 0.92 ^{b}	80.21 ± 1.04 ^{a}
62.5	31.45 ± 1.12 ^{b}	79.11 ± 1.14 ^{a}	43.79 ± 1.16 ^{b}	82.30 ± 0.86 ^{a}
125	34.56 ± 1.24 ^{b}	80.20 ± 0.88 ^{a}	49.28 ± 1.13 ^{b}	85.12 ± 0.45 ^{a}
250	46.87 ± 1.16 ^{b}	83.2 ± 0.62 ^{a}	54.03 ± 0.76 ^{b}	88.07 ± 0.93 ^{a}
500	53.16 ± 0.85 ^{b}	85.4 ± 0.56 ^{a}	64.51 ± 0.85 ^{b}	89.02 ± 0.88 ^{a}
1000	66.09 ± 1.92 ^{b}	87.52 ± 0.62 ^{a}	81.14 ± 1.26 ^{b}	92.44 ± 0.14 ^{a}
Half-maximal inhibitory concentration				
(IC_{50}) (µg/mL)	470.27 ± 2.24 ^{a}	16.62 ± 0.91 ^{b}	387.53 ± 3.11 ^{a}	14.03 ± 0.67 ^{b}

The mean values indicated in the same rows within variable with different superscripts (a and b) were significantly different ($p < 0.05$).

4. In Vitro Antimicrobial Activity

The methanolic extract of Cd exhibited striking inhibitory actions against *Staphylococcus aureus* NCTC 10788, *Salmonella senftenberg* ATCC 8400, *Escherichia coli* BA 12296, and *Candida albicans* ATCC MAY-2876 (**Table 3** and **Figure 2**). Conversely, the Cd extract was inactive against *Lissteria monocytogenes* ATCC 19116 (**Table 3** and **Figure 2**).

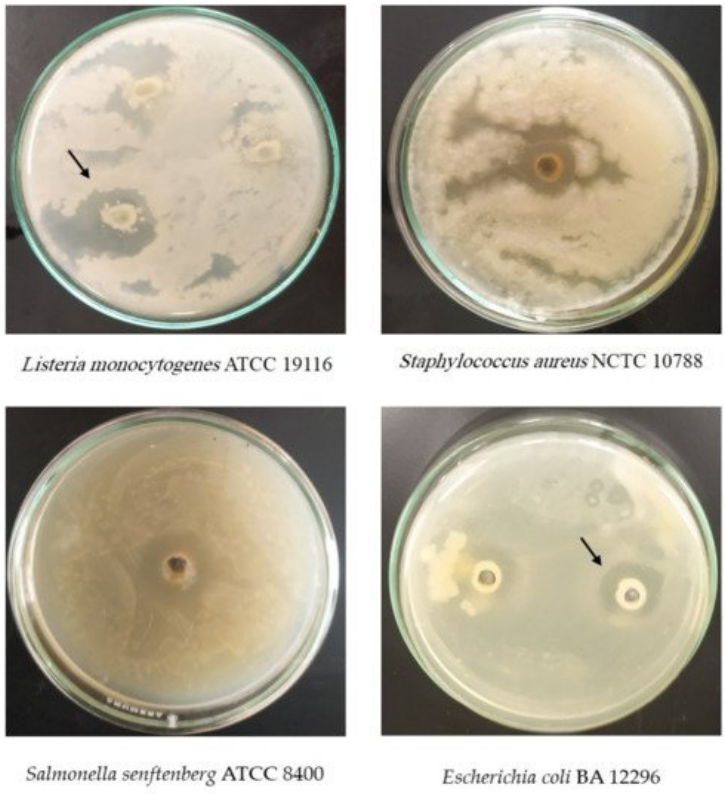

Figure 2. Antimicrobial activity of *Cleome droserifolia* (Forssk.) Del. extract against pathogenic microorganisms.

Table 3. In vitro antimicrobial activity of *Cleome droserifolia* (Forssk.) Del. against pathogenic microorganisms.

Pathogens Microorganisms	Inhibition Zone (mm)
***Staphylococcus aureus* NCTC 10788**	**15.63 ± 1.30 [a]**
***Salmonella senftenberg* ATCC 8400**	**12.70 ± 0.81 [a]**
***Escherichia coli* BA 12296**	**8.06 ± 1.72 [b]**
***Candida albicans* ATCC MYA-2876**	**7.16 ± 2.92 [b]**
***Listeria monocytogenes* ATCC 19116**	**NI**

NI, no inhibitory action.

5. Effect of Treatment on Weight, Feed Intake, and Health Indicators in Rabbits

The treatments with different concentrations of Cd (0, 1.25, or 2.5 g/kg of DM diet) did not affect the overall mean body weight and feed intake of rabbits during the 30-day experimental period (**Table 4**). The treatment with Cdh tended ($p < 0.085$) to decrease the fecal score compared with the other treatments (**Table 4**). Compared with the control, the two concentrations of Cd decreased significantly the overall mean rectal temperature (**Table 4**).

Table 4. Body weight, feed intake, fecal score, and rectal temperature of rabbits treated with different concentrations of *Cleome droserifolia* (Forssk.) Del. (Cd) (0: C, Cdl: 1.25 g/kg of DM diet, or Cdh: 2.5 g/kg of DM diet).

Treatment	Variable (Mean± Standard Error of the Mean, *n* = 10/Treatment)			
	Body Weight, g	Feed Intake, g/day	Fecal Score	Rectal Temperature, °C
C	1454 ± 37.01	99.47 ± 17.36	1.19 ± 0.083	39.07 ± 0.112 [a]
CdI	1413 ± 33.75	100.06 ± 15.88	1.21 ± 0.073	38.80 ± 0.091 [b]
Cdh	1393 ± 37.82	99.14 ± 17.31	1.13 ± 0.063	38.74 ± 0.123 [b]
p-Value	0.478	0.968	0.085	0.007

The mean values indicated in the same columns with different superscripts (a and b) were significantly different ($p < 0.05$).

6. Effect of Treatment on Hemato-Chemistry and Redox Status

The hematological attributes, blood plasma metabolites, and antioxidant activity of rabbits treated with different concentrations of Cd (0, 1.25, or 2.5 g/kg of DM diet) are shown in **Table 5**. No differences were observed for any of the variables at day 0, confirming the homogeneity of the experimental groups before the beginning of the treatment. At day 30 (the end of the experimental period), the treatment had not affected the hematological attributes or blood plasma metabolites. However, both concentrations of Cd significantly increased the levels of the total antioxidant activity and significantly decreased the levels of malondialdehyde in the blood plasma.

Table 5. Hematological attributes, blood plasma metabolites, and antioxidant activity of rabbits treated with different concentrations of *Cleome droserifolia* (Forssk.) Del. (Cd) (0: C, CdI: 1.25 g/kg of DM diet, or Cdh: 2.5 g/kg of DM diet).

Treatment	Variable (Mean± Standard Error of the Mean, *n* = 6)							
	Red Blood Cell Count (10^6/mL)	Packed Cell Volume(%)	Hemoglobin, g/dL	Total Protein, g/dL	Albumin, g/dL	Glucose, mg/dL	Total Antioxidant Capacity, Mm/L	Malondialdehyde, nmol/mL
At day 0								
C	6.31 ± 1.01	32.67 ± 2.45	10.16 ± 0.32	6.34 ± 0.15	4.37 ± 0.11	93.61 ± 2.27	492.75 ± 0.53	5.23 ± 0.43
CdI	5.85 ± 1.09	33.05 ± 3.45	10.79 ± 0.58	6.72 ± 0.19	4.01 ± 0.07	91.51 ± 1.15	425.40 ± 1.64	4.92 ± 0.06
Cdh	6.36 ± 0.97	34.45 ± 3.47	10.58 ± 0.79	6.65 ± 0.27	4.08 ± 0.12	93.31 ± 1.24	430.23 ± 1.59	5.20 ± 0.13
p-Value	0.764	0.947	0.764	0.742	0.641	0.369	0.4752	0.379
At day 30								
C	5.94 ± 1.21	31.35 ± 3.72	10.91 ± 0.67	6.44 ± 0.23	4.37 ± 0.24	91.92 ± 1.01	440.40 ± 0.30 [b]	4.19 ± 0.25 [a]
CdI	5.85 ± 0.98	30.37 ± 1.99	10.91 ± 0.37	6.28 ± 0.24	4.59 ± 0.06	91.56 ± 1.62	444.09 ± 0.95 [a]	3.83 ± 0.04 [b]
Cdh	5.61 ± 1.23	30.01± 2.01	9.86± 0.34	6.14 ± 0.11	4.38 ± 0.15	92.41 ± 0.97	443.37 ± 0.92 [a]	3.73 ± 0.04 [b]
p-Value	0.967	0.641	0.143	0.281	0.287	0.258	0.034	0.002

Mean values indicated in the same columns with different superscripts (a and b) were significantly different ($p < 0.05$).

7. Effect of Treatment on Immune Indicators

7.1. Innate Immune System

The innate immune indicators of rabbits treated with different concentrations of Cd (0, 1.25, or 2.5 g/kg of DM diet) are shown in **Table 6**. No differences were observed for any of the variables at day 0, confirming the homogeneity of the experimental groups before the beginning of the treatment. At day 30 (the end of the experimental period), the treatment had not affected the white blood cell count/differential count, PI, or PA. The treatment with Cdh significantly increased the

blood plasma lysozyme activity compared with the C and Cdl treatments. Moreover, the treatment with Cdh significantly decreased the levels of interleukin-β1 in the blood plasma compared with the C treatment, whereas Cdl yielded an intermediate value.

Table 6. Innate immune indicators of rabbits treated with different concentrations of *Cleome droserifolia* (Forssk.) Del. (Cd) (0: C, Cdl: 1.25 g/kg of DM diet, or Cdh: 2.5 g/kg of DM diet).

Treatment	Variable (Mean± Standard Error of the Mean, *n* = 6)								
	White Blood Cells, $10^3/mm^3$	Lymphocytes, %	Neutrocytes, %	Echinocytes, %	Monocytes, %	Phagocytic Index	Phagocytic Activity, %	Lysozyme Activity, U/mL	Interleukin-β1, pg/mL
At day 0									
C	7.29 ± 1.26	39.90 ±1.28	38.85 ± 2.33	12.49 ± 0.78	13.18 ± 2.00	1.94 ± 0.27	24.90 ± 1.24	0.113 ± 0.37	16.91 ± 0.34
Cdl	6.47 ± 0.88	38.88 ± 1.91	33.75 ± 5.29	10.79 ± 1.18	11.65 ± 2.61	2.04 ± 0.13	19.39 ± 0.80	0.092 ± 0.01	15.21 ± 0.72
Cdh	6.33 ± 1.42	38.71 ± 1.99	37.59 ± 3.32	10.12 ± 1.09	12.91 ± 0.69	1.96 ± 0.41	20.95 ± 0.12	0.101 ± 0.01	15.74 ± 0.82
p-Value	0.560	0.240	0.338	0.327	0.679	0.804	0.258		0.175
At day 30									
C	6.49 ± 0.84	39.56 ± 1.32	32.69 ± 1.35	11.74 ± 0.52	13.22 ± 1.20	2.10 ± 0.35	20.56 ± 1.63	0.104 ± 0.02 ^{b}	18.66 ± 0.22 ^{a}
Cdl	6.33 ± 0.56	42.01 ± 1.68	33.65 ± 3.05	10.22 ± 0.65	11.28 ± 1.53	2.19 ± 0.54	20.63 ± 1.01	0.106 ± 0.12 ^{b}	17.01 ± 0.81 ^{ab}
Cdh	6.02 ± 1.40	44.52 ± 1.21	37.63 ± 1.92	11.51 ± 0.89	10.97 ± 1.37	2.49 ± 0.24	21.2 ± 2.01	0.142 ± 0.01 ^{a}	15.25 ± 0.92 ^{b}
p-Value	0.449	0.123	0.236	0.531	0.195	0.446	0.561	0.046	0.001

Mean values indicated in the same columns with different superscripts (a and b) were significantly different ($p < 0.05$).

7.2. Humoral Immune System

The humoral immune indicators of rabbits treated with different concentrations of Cd (0, 1.25, or 2.5 g/kg of DM diet) are shown in **Table 7**. No differences were observed for any of the variables at day 0, confirming the homogeneity of the experimental groups before the beginning of the treatment. At day 30 (the end of the experimental period), the two concentrations of Cd had significantly increased the levels of IgG in the blood plasma compared with the control. Conversely, the treatments did not affect the levels of IgA and IgE in the blood plasma.

Table 7. Humoral immune indicators (immunoglobulins (Igs)) of rabbits treated with different concentrations of *Cleome droserifolia* (Forssk.) Del. (Cd) (0: C, Cdl: 1.25 g/kg of DM diet, or Cdh: 2.5 g/kg of DM diet).

Treatment	Variable (Mean± Standard Error of the Mean, *n* = 6)		
	IgG, mg/dL	IgA, mg/dL	IgE, mg/dL
At day 0		-	
C	981.32 ± 6.65	84.77 ± 2.68	7.73 ± 1.35
Cdl	989.90 ± 10.41	85.79 ± 4.82	6.69 ± 0.67
Cdh	985.74 ± 8.83	88.47 ± 3.45	7.99 ± 0.49
p-Value	0.516	0.329	0.1602
At day 30		-	
C	974.57 ± 3.84 ^{b}	91.78 ± 2.39	7.99 ± 0.78
Cdl	987.91 ± 6.01 ^{a}	93.86 ± 2.78	6.88 ± 0.38
Cdh	982.99 ± 7.48 ^{a}	93.06 ± 4.26	7.73 ± 0.28
p-Value	0.016	0.647	0.359

Mean values indicated in the same columns with different superscripts (a and b) were significantly different ($p < 0.05$). IgG, immunoglobulin G; IgE, immunoglobulin E; and IgA, immunoglobulin A.

7.3. Intestinal and Cecal Microflora Composition

The gastrointestinal (small intestine and cecum) microflora composition of rabbits treated with different concentrations of Cd (0, 1.25, or 2.5 g/kg of the DM diet) is shown in **Table 8**. At day 30 (the end of the experimental period), the counts of intestine and cecum *Salmonella* and *Coliform* species were significantly reduced in the Cd-treated groups compared with the control group. The two concentrations of Cd significantly increased the counts of intestinal and cecal yeast and Lactobacillus species compared with the control.

Table 8. Small intestinal and cecal microflora composition of rabbits treated with different concentrations of *Cleome droserifolia* (Forssk.) Del. (Cd) (0: C, Cdl: 1.25 g/kg of DM diet, or Cdh: 2.5 g/kg of DM diet).

Treatment	Variable (Mean± Standard Error of the Mean, *n* = 6)			
	Yeast	Lactobacillus	*Salmonella*	*Coliform*
	Intestinal microflora (log cfu/g)			
C	4.83 ± 0.65 ^{b}	6.80 ± 0.91 ^{a}	5.96 ± 0.55 ^{a}	6.30 ± 0.70 ^{a}
Cdl	7.60 ± 0.52 ^{a}	8.10 ± 0.94 ^{a}	3.10 ± 0.65 ^{b}	4.86 ± 0.77 ^{a}
Cdh	8.06 ± 0.66 ^{a}	8.06 ± 0.70 ^{a}	3.13 ± 0.85 ^{b}	4.83 ± 0.85 ^{a}
	Cecal microflora (log cfu/g)			
C	3.56 ± 0.81 ^{b}	5.40 ± 0.55 ^{a}	7.63 ± 0.86 ^{a}	8.13 ± 0.61 ^{a}
Cdl	5.60 ± 1.13 ^{a}	6.57 ± 1.70 ^{a}	5.50 ± 0.45 ^{b}	6.06 ± 1.30 ^{b}
Cdh	5.27 ± 0.83 ^{ab}	6.93 ± 1.53 ^{a}	5.34 ± 0.67 ^{b}	6.20 ± 0.79 ^{b}

Mean values indicated in the same rows with different superscripts (a and b) were significantly different ($p < 0.05$).

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