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GC-MS profiling, anti-inflammatory, antioxidant, anti enzymatic and antimicrobial activities of Algerian *Cléome amblyocarpa* Barr. & Murb

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ABSTRACT: This study evaluated the antioxidant, antidiabetic, anticholinesterase, anti-inflammatory, and antimicrobial effects of Algerian *Cleome amblyocarpa* through its chloroform fraction. Furthermore, phenolic, flavonoids and volatile compounds have been studied. The results indicated high phenols and flavonoids contents, and the gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of various compounds, mainly 2- Tetracosane, Palmitic acid, 2-Aminothiazole, and Linolenic acid. Previous research supports the potential antidiabetic effects of palmitic acid and linolenic acid, while tetracosane has been studied for its anticholinesterase activity, validating the observed biological activities in our research. The fraction has demonstrated a substantial anti-inflammatory activity similar to the reference, antimicrobial actions against *Escherichia coli, Klebsiella pneumonia, Bacillus cereus, Pseudomonas aeruginosa,* and *Candida albicans,* as well as an antioxidant potential, and significant inhibition of key enzymes: alpha-amylase, alpha-glucosidase, acetyl, and butyrylcholinesterase (AChE and BChE), with interesting IC_{50} values. Our result found that *Cléome amblyocarpa* is a rich source of value-enhancing biocompounds, and it presents interesting antioxidant, antidiabetic, and anticholinesterase properties, as well as substantial anti-inflammatory and antimicrobial activities. These findings can be exploited in developing new formulations that may be useful in treating a variety of situations.
KEYWORDS: anti-enzymatic; anti-inflammatory; antimicrobial; antioxidant; *Cléome amblyocarpa* Barr. & Murb. ; GC–MS.

1. INTRODUCTION

Medicinal plants are a crucial source of medicines derived from natural compounds. Various bioactivity assays, separation methods, and spectroscopic techniques have been developed to effectively study and utilize these plants. They contain diverse chemical compounds with beneficial properties, such as antioxidants and antimicrobials. Bioactivity assays evaluate their effectiveness against diseases, while separation methods isolate and purify the active compounds. Spectroscopic techniques aid in their identification and characterization. These advancements have greatly enhanced our understanding and utilization of medicinal plants, enabling the development of effective treatments for various ailments [1].

C. amblyocarpa Bart. and Murb , commonly known as spider flower, is a desert plant, distributed in north and east Africa, Sinai, Sudan, Ethiopia,; Palestine, Saudi Arabia, Iraq, and Iran [2]. It is widely used to treat scabies, abdominal, rheumatic pains, colic, diabetes, and fever. It exhibits anti-inflammatory, sedative, and antihypercholesterolemic activities [3]. Chemistry research was able to isolate many chemical compounds of big structural diversity; amyrin triterpenoids (β-amyrin) responsible for the anti-inflammatory, analgesic, and antipyretic proprieties [4], Dammarane triterpenes, Cleocarpanol lactone, Cabralea lactone, and their derivatives [4], and many flavonoids glycosides and methylated flavonoids in the form of flavones [6]. It is worth mentioning that quercetin, luteolin, kaempferol, isorhamnetin, and phenolic acids were also identified in the plant by high-performance liquid chromatography(HPLC) [7].

In Algeria, there is a lack of research investigating the chemical composition and biological properties of *C. amblyocarpa*. To the best of our knowledge, no prior studies have been published on the chloroform fraction of this plant. Therefore, the primary objective of this research was to analyze the chemical composition of *C. amblyocarpa*'s aerial parts through GC-MS. The study also sought to evaluate its antioxidant potential and ability to inhibit specific enzymes like alpha-amylase, alpha-glucosidase, AChE, and BChE. Moreover, the

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investigation explored the anti-inflammatory and antimicrobial effects of *C. amblyocarpa*. The ultimate goal was to broaden our knowledge regarding the biological characteristics and possible practical uses of this plant.

2. RESULTS AND DISCUSSION

2.1 Chemical composition

A chemical polymorphism characterizes *cleome* species; This chemical composition variation could be attributed to climate change, genetic diversity, and soil composition [8]: thus, the Major components present in *C. gynandra*, *C. rutidosperma*, and *C. viscosa* are Pentanoic acid, E-2-Octadecadecen-1-ol,Ethyl oleate, and 9,12- Octadecadienoyl chloride (Z,Z)- respectively [8]. Results presented in Table 1 shows that the 133 characterized compounds represent 93.651% of our fraction. Characterized primarily by 2-tetracosane (%), followed by palmitic acid (%), 2-aminothiazole (%), and linolenic acid (%). These results confirm the rich and diverse metabolic profile of *Cléom amblyocarpa*, encompassing a broad range of beneficial metabolites, including terpenoids, flavonoids, phenolic acids, alkaloids, and isoprenoids. Previous studies have confirmed the presence of anti-inflammatory, analgesic, and antipyretic triterpenoids such as amyrins. Additional triterpenes such as Cleocarpanol lactone and Cabralealactone and their derivatives have been identified. The plant also contains flavonoid glycosides, methylated flavones, and phenolic compounds, including quercetin, luteolin, kaempferol, isorhamnetin, and phenolic acids [4, 6]. This profile differs slightly from other studies that found a composition dominated by ethyl 3-methylpentanoate, 7-episylphiperfor-5-ene, and α-copaene [2,9,10]. However, other studies on this genus showed the presence of some compounds related to our result, mainly linoleic acid, palmitic acid, stearic acid, and oleic acid [11].

Table 1. Chemical composition, retention indices, and percentage composition of the chloroform fraction of *C. amblyocarpa* Barr. &Murb.

Peak	Compounds	RT	RI[b]	(%)
1	toluene	4.887	755.4	0.043
2	Caproaldehyde	5.949	769	0.147
3	4-Penten-2-OL	6.156	639	0.395
4	ethylbenzene	8.753	854	0.109
5	o-xylène	9.168	862	0.051
6	Methanesulfonic acid, methyl ester	11.894	937	0.057
7	3-Methylpentanoic acid	13.477	968.4	0.084
8	1-Methylpyrrolidine	13.873	673	1.102
9	1-Cyclopropylethanol	14.578	683	0.092
10	Phénol	14.703	959.6	0.114
11	Hexanoic acid	14.838	973	0.064
12	2,4-Heptadienal	15.157	966.6	0.113
13	1,3-Dichloropropane	16.064	747.2	0.045

Table 1 (Continue). Chemical composition, retention indices and percentage composition of the chloroform fraction of *C. amblyocarpa* Barr. &Murb.

Peak	Compounds	TR	RIb	(%)
14	d,l-Limonène	16.310	1020	0.032
15	Thiourea	16.479	531.5	0.078
16	1,1-Dimethoxydecane	16.696	1366	0.053
17	6,11-Dimethyl-2,6,10-dodecatrien-1 -ol	17.333	1634	0.050
18	2-Octenal	17.502	1034	0.035
19	Guaiacol	18.569	1059	0.257
20	Propiophenone	21.107	1140	0.060
21	Octanoic acid	21.484	1154	0.074
22	3'-Methylacetophenone	21.710	1142	0.033
23	Malonamide	22.888	1138	0.088
24	ethyldimethylsilane	23.202	473	0.410
25	Vinyltrimethylsilane	23.602	550	0.060
26	Acetone ethylhydrazone	23.795	801	0.047
27	(2Z)-2-Heptenal	24.061	927	0.054
28	4-Hydrazinopyridine	24.567	1130	0.029
29	1,3,6-Octatriene (CAS)	24.886	1098	0.059
30	2,4-Decadienal	24.978	1270	1.342
31	Ethyl methylcarbamate	25.272	785	0.183
32	2-Aminothiazole	26.010	976	4.213
33	Allyl heptanoate	26.102	1181	0.072
34	5-pentyl-2(5H)-furanone	26.348	1325	0.070
35	1,1,5-Trimethyl-1,2-dihydronaphthalene	26.623	1396	0.065
36	Decoic acid	27.019	1344	0.029
37	Vanillin	27.854	1403	0.040
38	Butyl levulinate	28.766	1225	0.322
39	Geranylacetone	29.244	1426	0.044
40	2-Methylbutyl hexanoate	29.398	1196	0.025
41	Hentriacontane	29.447	472.7	0.018
42	Ethyl 2-(aminooxy) propanoate	29.886	940	0.046
43	β-Ionone	30.108	1423.6	0.058
44	2,4-Di-tert-butylphenol	30.764	1539	0.029
45	(E)-3,7-Dimethylocta-2,6-dienal	31.184	1249	0.071
46	Dihydroactinidiolide	31.300	1426	0.292
47	Lauric acid	32.222	1556	0.104
48	2-Amino-4,6-dihydroxypyrimidine	32.545	1005	0.060
49	Diethyl phthalate	33.380	1543	0.126
50	Linalool	33.496	1081	0.052
51	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, cis-	35.025	1340	0.114
52	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	36.990	1737	0.070
53	2-Butanone, 4-[2-isopropyl-5-methyl-5-(2-methyl-5-oxocyclopentyl)cyclopentenyl]-	37.134	2237	0.064
54	Heptadecane	37.443	282.99	0.086
55	Methyl tetradecanoate	38.326	1713	0.054
56	Carbamic acid, ethylnitroso-, ethyl ester	38.491	1144	0.075

Table 1 (Continue). Chemical composition, retention indices, and percentage composition of the chloroform fraction of *C. amblyocarpa* Barr. &Murb.

Peak	Compounds	TR	RIb	(%)
57	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	38.650	1653	0.155
58	Loliolide	39.552	1792.6	1.146
59	Silane (bromomethyl)-	39.934	563	0.066
60	Tricaproin	40.170	2546	0.041
61	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	40.291	2277	0.245
62	3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one	40.382	1639	0.046
63	Ethyl tetradecanoate	40.484	1778	1.517
64	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	41.521	1811	0.099
65	Neophytadiene	41.714	1827	0.565
66	Hexahydrofarnesyl acetone	41.869	1845	0.190
67	n-Pentadecanoic acid	42.303	1823	0.200
68	Phthalic acid, isobutyl undecyl ester	42.467	2931	0.059
69	Phytol acetate	42.820	2218	0.180
70	2,5-Dimethoxy-2,5-dihydrofuran	43.037	845	0.063
71	Ethyl pentadecanoate	43.153	1878	0.027
72	n-Nonadecane	43.259	312.43	0.274
73	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	43.736	1929	0.283
74	Methyl palmitate	43.920	1908	0.393
76	phytol	44.417	2104	0.493
77	**Palmitic acid**	44.953	1942	**8.044**
78	Ethyl palmitate	45.498	1968	1.176
79	Eicosane	45.585	345.2	0.202
80	Myristic acid	46.178	1748	0.033
81	Heptadecanoic acid	46.917	2038	0.161
82	Methyl linoleate	47.337	2071	0.216
83	Heneicosane	47.689	360.4	1.030
84	Methyl 11-octadecenoate	47.819	2085	0.142
85	1-Pentadecene	48.090	1502	0.053
86	Methyl stearate	48.234	2133	0.112
87	9,12-Octadecadienoic acid (Z,Z)-	48.452	2095	1.914
88	**Linolenic acid**	48.572	2122	**4.141**
89	Stearic acid	49.011	2187	2.930
90	Linoleic acid	49.142	2095	1.083
91	Cycloeicosane	49.368	2398	1
92	Ethyl stearate	49.533	2181	0.447
93	2-Methyl-Z,Z-3,13-octadecadienol	50.001	2104	0.158
94	Methyl palmitoleate	50.589	1885.86	0.179
95	13-Tetradecen-1-ol acetate	51.202	1805	0.360
96	Tricosane	51.410	388.7	0.623
97	Bicyclo[13.1.0]hexadecan-2-one	51.588	1992	0.127
98	Ethyl 9-hexadecenoate	51.767	1955	0.750
99	Oleic Acid	51.926	2142	0.293
100	N-Methylolmaleimide	52.356	2113	0.355
101	Arachidic acid	52.539	2366	0.316

Table 1 (Continue). Chemical composition, retention indices, and percentage composition of the chloroform fraction of *C. amblyocarpa* Barr. &Murb.

Peak	Compounds	TR	RI[b]	(%)
102	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,Z)-	52.887	1658	0.202
103	Ethyl 15-methylheptadecanoate	53.046	2112	0.317
104	**Tetracosane**	**53.113**	**402.5**	**35.553**
105	1,3-Oxathiane, 2-tert-butyl-2,6-di methyl-, stereoisomer (CAS)	53.200	1259	0.352
106	1-Eicosene	53.297	1994	0.138
107	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	53.480	2365.3	0.119
108	6-Octadecenoic acid	53.997	2175	0.046
109	5-Methylindole	54.426	1288	0.127
110	Pentacosane	54.725	394.59	0.484
111	2-Palmitoylglycerol	54.880	2498	0.933
112	Ethyl oleate	55.049	2171	0.311
113	(2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	55.396	1710	0.169
114	Bis(2-ethylhexyl) phthalate	55.522	2499.4	0.138
115	Methyl 17-methyloctadecanoate	56.226	2112	0.285
116	Hexacosyl acetate	56.453	2995.6	0.675
117	N-hydroxy-N'-[2-(trifluoromethyl)phenyl]pyridine-3-carboximidamide	57.051	1939	0.172
118	Heptacosane	57.814	447.8	3.689
119	1,2-Benzisothiazole, 3-(hexahydro- 1H-azepin-1-yl)-, 1,1-dioxide	57.935	2652	0.147
120	Glyceryl monostearate	58.089	2681	0.729
121	Z-8-Methyl-9-tetradecenoic acid	58.263	1813	0.343
122	Stearyl alcohol	58.890	2070	0.084
123	5.beta.-Cholestane-3.alpha.,7.alpha.,12.alpha.,24.alpha.,25-pentol T MS	59.701	3619	0.066
124	1-Docosene	59.822	2192	0.194
125	Farnesol (E), methyl ether	60.193	1643	0.115
126	9-Methyl-Z-10-tetradecen-1-ol acetate	62.336	1822	0.097
127	Methyl 12-oxooctadecanoate	63.644	2213	0.066
128	Tetradecadien-3-one,1,13-	63.731	1529	0.129
129	Triacontane	64.402	488.4	1.245
130	1H-Indole-3-ethanamine, N-methyl-	64.653	1745	0.152
131	1-Hexacosene	64.802	2596	0.134
132	beta-Tocopherol	65.960	3043	1.734
133	Spathulanol	67.355	1569	0.376
	Identified compounds (%)	**Total**		**93.651**

[a]Compounds listed in order of their RT (retention time), [b]RI, theoretical retention index on HP-5 capillary column

2.2 Antioxidant activity

Results shown in Table 2 present significant phenolics and flavonoids contents in our fraction; this is in agreement with another study with slight differences; it was found to be (194.56 ± 1.57 µg equivalent of quercetin per milligram of extract weight (µg EQ / mg extract) and (102.75 ± 0.75µg 194.56 ± 1.57 ug equivalent of gallic acid per milligram of extract weight (µg GAE /mg extract) respectively [15].

Our sample present also a significant antioxidant potential due to a high content of phenolic compounds and flavonoids, which are considered major groups involved in antioxidant activity [12,13]. In the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging test, the fraction has an activity closer to that of α-tocopherol and less than other standards, however in the 2,2'-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) scavenging ability the activity was closer to that of quercetin. For the cupric-reducing antioxidant capacity (CUPRAC) and Phenanthroline assays, the fraction presented effects much closer to ascorbic acid. Nonetheless, regarding the β-carotene-linoleic acid test, antioxidant activity was better than that of quercetin and ascorbic acid. These

differences may be due to different extraction methods, the type of phenol contained in the fraction, the mechanism, and the sensitivity of the reagents used in each method[14]. Our results are in line with several studies demonstrating the strong antioxidant potential of the *Cleome* genus. One study focused on *Cleome viscosa* L, using various metal reduction methods [11]. Additionally, another study evaluated the antioxidant activity of *C. ramosissima* through different tests involving free radicals (DPPH, ABTS·+), metal chelation activity (MCA), and the hydroxyl radical assay (•OH) [15], and those reported by khlifi et al in DPPH essay for stems and leaves parts of C*leom amblyocarpa* (IC_{50}: 0,151 ± 0,01 and 0.732 ± 0.13 µg/mL), and (IC50: 0.356 ± 0.03 0.952 ± 0.02 µg/mL, respectively [16]. These slight variations might be due to genotype–environment interactions, geographical habitats of the plants, ecological conditions, and phonological stage, which causes some adaptation responses [15].

Table 2. Antioxidants activity of chloroform fraction of *C. amblyocarpa* Barr. &Murb by DPPH•, ABTS•+, CUPRAC, Phenanthroline, and β-carotene- linoleic acid assays and Total phenolics and Flavonoïdes contents

samples	ABTS·+ assay	DPPH· assay	CUPRAC assay	Phenanthroline assay	β-carotene-linoleic acid assay	Total phenolics (µg GAE/mg)**	Flavonoïdes (µg QE/ mg)***
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$A_{0.50}$ (µg/mL)	$A_{0.50}$ (µg/mL)	IC_{50} (µg/mL)		
Chloroform fraction	94.36±0.92^{e}	43.80±2.2^{d}	127.28±0.40^{e}	71.00±1.73^{d}	107.28±0.40^{b}	141.19±0.44	71.59±0.19
BHA*	2.98±0.11^{b}	9.01± 0,38^{b}	2.22±0.15^{a}	1.49±0.08^{a}	9.82±0.22^{a}	NT	NT
BHT*	1.31 ±0.06^{a}	1.31± 0.06^{a}	5.53±0.03^{b}	2.20±0.04^{a}	6.26±0.17^{a}	NT	NT
α-Tocopherol*	2.50±0.06^{d}	12.43±1.05^{c}	17.56±0.17^{d}	5.78±0.30^{b}	1.76±0.06^{a}	NT	NT
Quercitine*	10.54±0.07^{b}	2.30±0.34^{a}	2.42±0.1^{a}	0.65±0.04^{a}	NT	NT	NT
Ascorbic acid*	4.04±0.02^{c}	2.69±0.22^{a}	10.98±0.14^{c}	8.30±0.76^{c}	NT	NT	NT

The concentration at 50% inhibition and 0.50 absorbance are called IC50 and A0.50 values using linear regression analysis; the IC50 and A0.50 values were determined and expressed as mean _ SD (n = 3).*: Reference compounds. BHA: Butylatedhydroxyanisole, BHT: Butylatedhydroxytoluene, Results of Total phenolics and flavonoids re-expressed as means ± standard deviation of three measures (Tukey test, $p \leq 0.05$). The values with different superscripts ((a, b, c,d, e or f) in the same columns are significantly different ($p < 0.05$); **Total phenolics are expressed as µg Gallic acid equivalents/mg of fraction; ***Total flavonoids are expressed as µg Quercetin equivalents/ mg of fraction NT: not tested

2.3 Anti-enzymatic

2.3.1 Anti-anticholinesterases activity

According to the results represented in Table 3, our sample showed moderate inhibitory activity against the AChE enzyme compared to the standard (Galantamine). However, this fraction inhibited BChE better, with IC_{50} value closer to that of galantamine, due to the presence of molecules ensuring this inhibition, such as phenolic compounds, particularly the flavonoids [17], or other natural molecules involved in the inhibition of acetylcholinesterase belonging to alkaloids, terpenes, sterols, and coumarins [18] . Furthermore, literature data indicated that tetracosane, the major identified compound, demonstrated a good AChE inhibitory activity (14.8 ± 0.7% of inhibition at 100 µg/mL) [19] .

2.3.2 Antidiabetic activity

Results of Table 4 indicated that chloroform fraction exhibited a better α-amylase inhibition activity than acarbose. Regarding the anti-α-glucosidase propriety, our sample presents an IC_{50} value comparable to the standard. The inhibitory effects are due to certain molecules, such as flavonoids and tannin [20] or certain unsaturated fatty acids, which have an unsaturated double bond related to the ability of this inhibitory activity [21], principally palmitic acid, which showed high effectiveness against these enzymes (66.5% and 53.2%, for α-amylase and α -glucosidase respectively), and linoleic acid; with an effect of 60.1% and 38.8% for the two enzymes respectively. These findings are constant with other study showing the excellent inhibitory

effects of alpha-amylase and glucosidase of the *Cleome* genus focused on *Cleome amblyocarpa* Barr. And Murb. And *Cleome arabica* L [16]

Table 3. Acetylcholinesterase and Butyrylcholinesterase inhibitory activities of various extracts of *Cléome amblyocarp*Barr. &Murb

Acetylcholinesterase inhibitory activity								
Samples	6.25µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	IC_{50} (µg/mL)
Chloroform fraction	1.50± 0.25	1.50± 0.25	1.50± 0.25	1.50± 0.25	1.50± 0.25	4.31± 0.83	13.45± 3.87	>400[b]
Galantamine*	25.02 ± 0.77	35,93 ± 2,28	43,77 ± 0.00	68,50 ± 0,31	85,78 ± 1,63	91,80 ± 0,20	94,77 ± 0,34	6.27±1.15[a]
Butyrylcholinesterase inhibitory activity								
Samples	6.25µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	IC_{50} (µg/mL)
Chloroform fraction	0.98± 0.07	1.0.2± 0.09	1.41± 0.11	1.50± 0.25	43.73± 0.24	63.53± 0,77	75.83± 1.77	75.21±1.40[b]
Galantamine*	3,26± 0,62	6,93± 0,62	24,03± 2,94	45,13± 2,60	63,87± 2,85	73,57± 0,77	78,95± 0,58	34.75±1.99[a]

IC_{50} values are defined as the concentration of 50% inhibition percentages, calculated by linear regression analysis, and expressed as Mean ± SD (n=3). The IC_{50} values with different superscripts (a or b) in the same column are significantly different ($p < 0.05$). *: reference compounds.

Table 4. α-amylase and α-glycosidase inhibitory activities of chloroform extract of *Cléome amblyocarpa* Barr. &Murb

α-glycosidase inhibitory activity								
Samples	25 µg/mL	50 µg/mL	100 µg/mL	200µg/mL	400 µg/mL	800 µg/mL	1600 µg/mL	IC_{50} (µg/mL)
Chloroform fraction	13.90±1.01	16.23±0.84	19.22±0.77	21.65.±0,91	27.87±0.56	46.08±1.03	77.98.±0,72	903.21±4.29[b]
Acarbose*	78.125 µg/mL	156.25 µg/mL	312.5 µg/mL	2500 µg/mL	625 µg/mL	1250 µg/mL	2500 µg/mL	IC_{50} (µg/ml)
	21,19±0,73	27,43±2,18	38,91±3,20	54,86±1,79	67,29±2,63	80,19±1,66	85,54±0,45	275,43±1,59[a]
α-amylase inhibitory activity								
Samples	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL	1600 µg/mL	3200 µg/mL	IC_{50} (µg/mL)
Chloroform fraction	47.42±0.24	48.00±0.10	50.72±1,22	2545.50±78.17[a]	47.42±0.24	48.00±0.10	50.72±1,22	2545.50±78.17[a]
Acarbose*	62,5 µg	125 µg	250 µg	500 µg	1000 µg	2000 µg	4000 µg	IC_{50} (µg/mL)
	7,76±0,17	8,08±0,30	9,46±0,11	10,70±0,96	31,81±2,89	37,21±3,54	53,05±1,59	3650,93±10,70[b]

IC_{50} values are defined as the concentration of 50% inhibition percentages, calculated by linear regression analysis, and expressed as Mean ± SD (n=3). The IC_{50} values with different superscripts (a or b) in the same column are significantly different ($p < 0.05$), *: reference compound.

2.4 The anti-inflammatory activity

According to the results represented in Figure 1, our sample has given substantial anti-inflammatory activity. Ibuprofen obtained maximum inhibition at a concentration of 1000 µg/mL. Tukey's multiple range test showed five homogenous groups and classified our fraction at 1000 µg/mL in the same group with Ibuprofen at 250 µg/mL, exhibiting a high percentage of inhibition (90.04 ±1.27 %). These results demonstrated the good anti-inflammatory activity of our fraction extract and its ability to maintain protein conformation and control self-antigen production. This agrees with Khlifi et al., which revealed the substantial anti-inflammatory activity of the Tunisian *C. amblyocarpa* [16]. This activity could be attributed to phenolic compounds, flavonoids, or tannins, as well as the several identified and isolated molecules such as amyrin triterpenoids (β-amyrin), quercetin, luteolin, kaempferol [4, 22]. This study's results further validate this genus's previously established anti-inflammatory activity, notably emphasizing the highly significant anti-inflammatory effect of *C. arabica* as a natural source of anti-inflammatory agents [16].

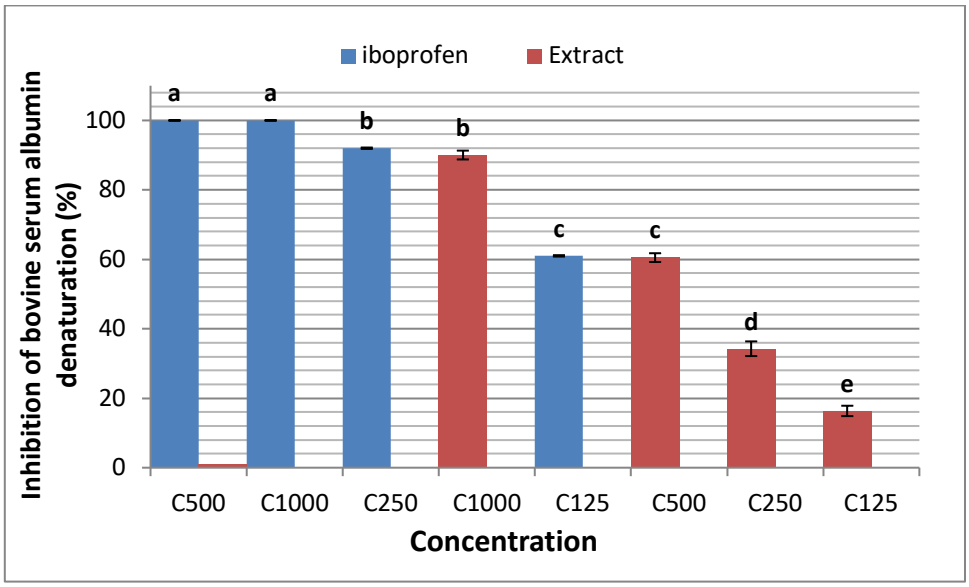

Figure 1. Anti-inflammatory activity of chloroform fraction of *C. amblyocarpa* Barr. &Murb in comparison with Ibuprofen: C125-C1000 are concentration from 125 to 1000 µg/mL, vertical bars are standard deviation, and the different letters indicate significant differences according to Tukey's range test at $p < 0.05$.

2.5 The antimicrobial activity

Zones of inhibition of bacterial growth were assessed as antimicrobial potency of our fraction against four bacterial strains; *Bacillus cereus* ATCC 11778, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas pneumonia* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922 in addition to *Candida albicans* ATCC 2019 as fungal strain. The results show that the inhibition of microbial growth varied between the fraction and the standard, according to the strains (figure 2). Our fraction showed similar antimicrobial activity as gentamicin against *E.coli* and *K. pneumonia*. However, the fraction activity was less than that provided by the standard against the other strains. Despite this difference, the activity of our fraction remains significant, noting inhibition zones superior to 10 mm for all the bacterial strains tested. In the same context, Khlifi et al. reported the significant antibacterial potential of some *Cleome* species (*C amblyocarpa, C. droserifolia*, *C. gynandra*, and *C. viscosa*) against some of these bacterial and fungal strains [10]. This antibacterial activity could be due to phenolic compounds such as Gallic acid, flavonoids, saponins, triterpenoids, tannins, and sterols [23,24].

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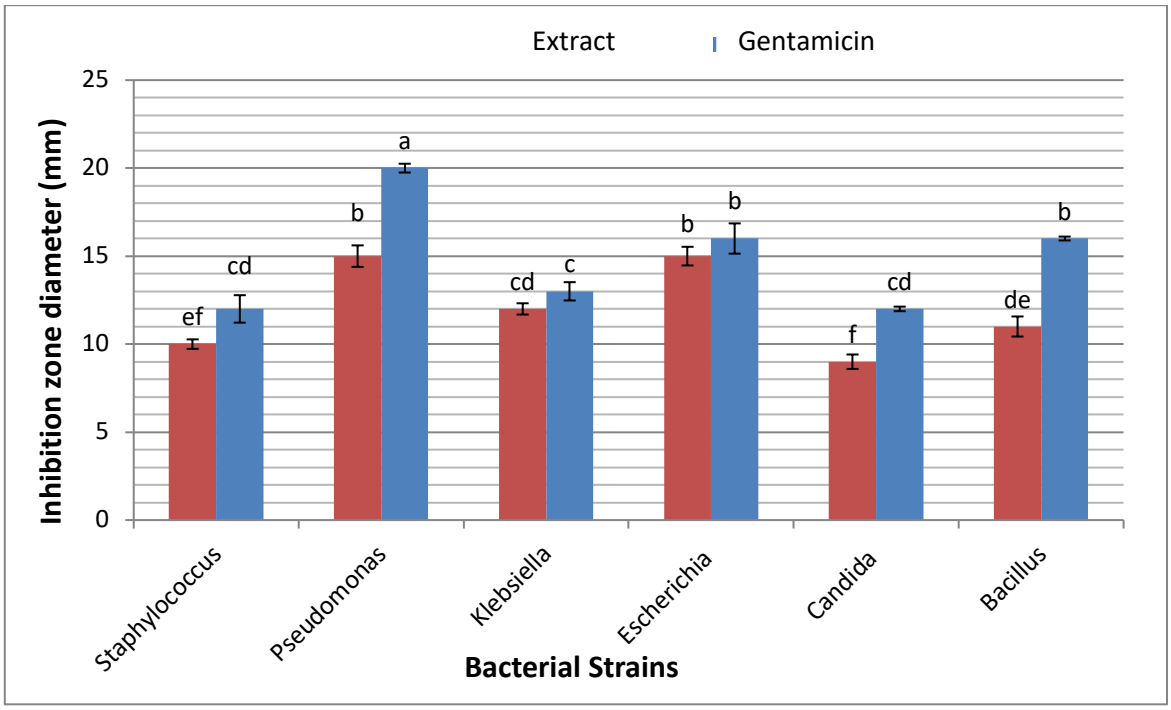

Figure 2. Antimicrobial activity of chloroform fraction of *C. amblyocarpa* Barr. &Murb compared with gentamicin on six strains: Vertical bars are standard deviation, and the letters indicate significant differences according to Tukey's range test at $p < 0.05$.

3. CONCLUSION

The present work is the first evaluation of GC-*MS* profiling, antioxidant, anti-enzymatic, antimicrobial, and anti-inflammatory properties of the Algerian *C. amblyocarpa* Barr. & Murb using the chloroform fraction. GC-*MS* profiling of this fraction indicates its richness in 2-Tetracosane, Palmitic acid, 2-Aminothiazole, and Linolenic acid. The result also showed a height antioxidant potential related to a high Phenol and flavonoid content. Furthermore, this fraction demonstrated substantial anti-inflammatory, anti-enzymatic activities and interesting antimicrobial properties. The observed pharmacological activity of the extracts obtained from *Cleome* spp. can be exploited in various formulations as therapeutic agents for a wide range of human diseases without harmful side effects.

4. MATERIALS AND METHODS

4.1 Plant material and extraction method

Aerial part of *Cleom amblyocarpa* collected at Djbal Antar, Bechar (southwestern Algeria) (sud-ouest algérien/31° 56′ 34″ nord, 1° 55′ 52″ ouest) in April 2021. The plant was authenticated by Mr. Mohamed Kaabeche, a botanist at Ferhat Abbas University, Setif-Algeria, and a voucher specimen was stored in the departmental Herbarium of the Biotechnology Research Center's Health division (CRbt/05/2021). Air-dried and ground aerial parts of *C. amblyocarpa* (100 g) were added to methanol/water ratio: (80: 20, v/v) 24 h with mechanical stirring at ambient temperature in the dark. The residue was dissolved in water and has undergone liquid with chloroform, and stored in the dark at 4° C until their analysis.

4.2 GC-MS profiling

Chloroformic fraction examination was assessed on an Agilent gas chromatography model Agilent's GC7090 B associated with an Agilent 5977, a mass selective detector. The separation has been made on an HP 5MS capillary column (30m*0.25mm*0.25mm) by this temperature program: 40 °C for 8 min, then 150 °C for 5 min at the rate of 5 °C/min to 260 °C for 15 min at a rate of 5 °C/min. Then 0.5 uL of the sample was injected for analysis using a splitless mode, the carrier gas is helium, and the helium gas flow rate was 1mL/min. The

injector and mass transfer line temperatures were set at 150 and 230 C°. The mass detector conditions are as follows: Electron impact (EI) mode at 70 eV, source temperature 280 °C, and mass scan range m/z 34-450.

4.3 Total phenolics and flavonoids content determination

4.3.1 Measurement of total polyphenol content (TPC)

The Folin determined the total phenolic content–Ciocalteu method [25] with slight modifications. The mixture contains 20 µL of the fraction, 100 µL Folin–Ciocalteu reagent, and 75 µL (7.5%) sodium carbonate. The absorbance was measured at 740 nm in the microplate reader after two h incubation in darkness at room temperature using a 96-well microplate reader (Perkin Elmer, Enspire). A blank was prepared similarly by replacing the fraction with the used solvent. The experiment was performed in triplicate, and the total phenolic compounds were expressed as gallic acid equivalents/mg DE (µg GAE/mg DE) using the calibration equation of the gallic acid standard: y =0.003x+0.104 (R2= 0.997).

4.3.2 Measurement of total flavonoids content (TPC)

The total flavonoids were determined according to Moreno et al. [26]. The protocol was based on mixing 50 µL of the diluted fraction solution with 30 µL of methanol, 10 µL of 1 M potassium acetate, and 10 µL of 10% aluminum nitrate; a blank was prepared in the same way by replacing the fraction with the used solvent. The absorbance at 415 nm was measured after incubation at room temperature in darkness for 40 min. The experiment was performed in triplicate, and the total flavonoid content was expressed as µg of quercetin equivalents per mg of fraction (µg QE/mg DE) using the flowing calibration equation: y=0.004x, R 2=0.997.

4.4 Colorimetric Antioxidant potential

The antioxidant capacity of the fraction was assessed using five methods: DPPH phenanthroline, $ABTS^{\bullet+}$, CUPRAC, and β-carotene-linoleic acid test, compared with five positive standards; BHT, BHA, quercetin, ascorbic acid, and alpha-tocopherol.

4.4.1 DPPH scavenging activity

The DPPH scavenging activity was established according to Blois [27]. 40 µL of each fraction concentration was added to 160 µL of a methanolic DPPH solution (0.6 $mg.L^{-1}$). The plate was kept in darkness at room temperature for 30 min. The absorbance was determined at 517 nm, and the results were given as 50% inhibition concentration (IC_{50}). The scavenging activity of the fraction was calculated using the following formula:

$I(\%) = \frac{A_C - A_S}{A_C} \times 100$ (1), where I (%) is the percentage of inhibition, and A_C and A_S are the absorbance of the control and the test sample, respectively. The results were given as IC_{50} (µg/mL).

4.4.2 Colorimetric ABTS activity

The $ABTS^{\bullet+}$ scavenging activity was evaluated according to Re et al. [28]. The mixture (40 mL of the sample solution + 160 µL of $ABTS^{\bullet+}$ solution) was stored for 10 min, and then the absorbance was measured at 734 nm. The scavenging ability of $ABTS^{\bullet+}$ was calculated using the previous formula (1). The results were given as IC_{50} (µg/mL).

4.4.3 Phenanthroline Assay

The Phenanthroline activity was determined according to Szydłowska-Czerniak et al. [29]. For this, 10 µL of the sample solution was mixed with 50 µL of $FeCl_3$ (0.2%), 30 µL of phenanthroline (0.5%), and 110 µL of methanol. The mixture was incubated for 20 min at 30 °C, and the absorbance was measured at 517nm. The results were given as $A_{0.50}$ (µg/mL).

4.4.4 β-carotene/linoleic acid bleaching test

The β-carotene bleaching activity was evaluated using the β-carotene-linoleic acid system [30]. Thus, a solution of β-carotene (0.5 mg/ mL of chloroform) is added to 25 Ml of linoleic acid and 200 µL of Tween 40. After evaporation under the vacuum of the chloroform, 50 mL of Hydrogen peroxide is added under vigorous agitation. The solution absorbance is adjusted to 0.8-09 nm. In a 96-well plate, 160 µL of this solution is added to 40 µL of the sample solution at different concentrations. The absorbance was measured at 470 nm after two h at 50 °C. The results were given as IC_{50} (µg/mL) calculated using formula 1.

4.4.5 Cupric reducing antioxidant capacity test

Evaluation of Cu^{2+} ions reduction was determined according to Apak et al. [31]. The mixture contains 40 µL of the sample solution, 60 µL of ammonium acetate buffer solution (1 M, pH 7.0), 50 µL of copper (II) chloride solution (0.01 M), and 50 µL of neocuproine solution (0.0075 M). The plate was incubated for 60 min at room temperature, and the absorbance was measured at 450 nm. The results were given as $A_{0.50}$ (µg/mL).

4.5 Bioassays

4.5.1 Anti-cholinesterase activity

Both anti-acetyl and butyrylcholinesterase (AChE and BChE) inhibitory potentials were established by the Ellman method [32]. The total reaction mixture (200 µL) [150 µL (0.1 mol/L) of sodium phosphate buffer (pH 8.0), 20 µL of AChE or BuChE (0.45 U/mL), and 10 µL of the test solution] was incubated at 25 °C for 15 minutes, then 10 µL of DTNB (0.03 mmol/L) and 10 µL of acetylthiocholine iodide or butyrylthiocholine iodide (0.68 mmol/L) were added. The absorbance values were read at 405 nm, galantamine hydrobromide was used as the positive control, and the percentage of inhibition was calculated using the following formula: $I(\%) = \frac{E-S}{E} \times 100$. (2), Where E is the enzyme's activity without the tested sample, and S is the enzyme's activity in the presence of the tested sample. The enzyme inhibitory activities were expressed as IC_{50} (µg/mL).

4.5.2 Antidiabetic activity

The study of the antidiabetic activity was assessed in vitro by inhibition of two key enzymes (α-amylase and α-glucosidase). Acarbose was used as a positive standard.

A. Alpha-amylase Inhibition activity

The anti-α-Amylase effect was investigated using the Caraway-Somogyi iodine/potassium iodide method with slight modifications [33]. 25 µL of the sample at different concentrations were mixed with α-amylase solution (50 µL) and incubated at 37 °C for 10 min. Then, the reaction was started by adding 50 µL of starch solution (0.1%). Similarly, a blank was prepared by adding the sample solution to all the reaction reagents without the enzyme solution. After incubation (10 min at 37°C), the reaction was stopped by adding 25 µL of HCl (1M) and 100 µL of iodine potassium iodide solution. Acarbose was used as standard. The absorbances were read at 630 nm, and the results were given as IC_{50} value (µg/mL) using formula (2).

B. Alpha -Glucosidase inhibitory activity

The α-glucosidase inhibitory assay was performed according to Lordan et al. [34]. 20 µL of the fraction at different concentrations were added to 100 µL of the enzyme solution (0.1U/mL) and 80 µL of the substrate solution (using 5 mM p-nitrophenyl- α-Dglucopyranoside in 100 mM sodium phosphate buffer, pH 6.9). The mixture was incubated for 5 min at 37°C; the absorbance was measured at 405 nm. Acarbose was used as a positive control, and the results were given as IC_{50} (µg/mL) using formula (2).

4.6 Anti-inflammatory Test

Anti-inflammatory propriety was estimated by the bovine serum albumin denaturation method [35]. For this, different concentrations of our sample or standard (Ibuprofen) were prepared, and 500 µL of each sample was mixed with 500 µL of bovine serum albumin (0.2%) dissolved in Tris Buffer Saline (pH 6.6), then incubated for 10 minutes at 37°C followed by 5 minutes at 72°C. After the cooldown, the absorbance at 660 nm was measured.

4.7 Antimicrobial activity

Antimicrobial activities were established according to Vijayakumar et al. [36] on five common bacteria strains; *Bacillus cereus* ATCC11778, *Klebsiella pneumonia* ATCC700603, *Pseudomonas pneumonia* ATCC 27853, *Staphylococcus aureus* ATCC25923, and *Escherichia coli* ATCC25922. In addition to *Candida albicans* ATCC 2019, a fungal strain. The antibacterial efficacy of our fraction was estimated *in vitro* by the disk-diffusion method in triplicate. Bacterial strains were grown in Mueller-Hinton agar (MHA), and *Candida albicans* strain in Potato dextrose agar (PDA).
Discs (6 mm diameter) containing plant fraction (100 mg/mL) were placed on MHA plates for bacterial and PDA for *Candida* strains with 10^8 microorganism cells. MHA and PDA Plates were then incubated for 16-20 h (at 37°C) and 42-48 h (at 32°C), respectively. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (mm). Discs (6 mm in diameter) containing gentamycin were used as a positive control.

4.8 Statistical analysis

One Way ANOVA Analysis was performed on all estimated parameters with three replicates. ANOVA test was considered statistically significant if *P-Value* ≤ 0.05. Tukey's multiple range test assessed the differences in the means between treatments. These analyses were performed by Genstat Release 11.1 software (VSN International Ltd., Hemel Hempstead, UK).

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