

The Therapeutic Potential of *Cleome amblyocarpa* from Al-Yutamah Dome Area, El Madina El Menawara: Phytochemical Analysis and Biological Activity

Ameina S. Almoshadak

Department of Biological Sciences, Faculty of Science
King Abdulaziz University
Jeddah, Kingdom of Saudi Arabia

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Abstract

This study aimed to analyze the phytochemical constituents of *Cleome amblyocarpa* aerial parts using LC-ESI-MS/MS methodology, determine the content of fat-soluble and water-soluble vitamins, mineral composition, ABA and polyphenols levels, and investigate the antioxidant and antimicrobial activities of the plant extract. LC-ESI-MS/MS analysis revealed the presence of eleven identified compounds in *C. amblyocarpa* aerial parts, with naringenin being the major component. The plant exhibited a rich composition of fat-soluble vitamins (vitamin D2) and water-soluble vitamins (vitamin C and B complex). The mineral composition included significant levels of sulphate, bicarbonate, nitrogen, and potassium. ABA and polyphenols were detected to be present in significant amounts in *C. amblyocarpa*. The methanolic extract displayed notable antioxidant activity and exhibited antimicrobial activity against the investigated microbial pathogens. Overall, the aerial parts of *C. amblyocarpa* contain a diverse range of phytochemicals, vitamins, minerals, ABA, and polyphenols. These findings highlight the potential health benefits and nutritional value of *C. amblyocarpa*, supporting its potential as a natural therapeutic agent. Further research is needed to explore its mechanisms of action, bioavailability, and safety for human consumption.

Keywords: Al-Yutamah area; Antimicrobial activity; *Cleome amblyocarpa*; DPPH scavenging; LC-ESI-MS/MS; polyphenols; vitamins

Introduction

The utilization of herbal medicines for primary healthcare has shown tremendous potential in both developed and developing nations. These remedies offer numerous advantages, including minimal adverse effects and cost-effectiveness. Among the various sources of valuable secondary metabolites that can contribute significantly to healthcare advancements, the *Cleome* species stands out. Wild plants, often referred to as "green factories," have the capacity to produce numerous bioactive substances that find applications in the treatment of diverse diseases, weed control as biocides, and pharmaceutical, industrial, and agricultural sectors (1 &2) .

The employment of plants for pharmaceutical reasons has a deeply rooted and enduring history. The *Cleome* genus, belonging to the *Cleomaceae* family, comprises approximately 200 species (3). Several species within this genus, such as *C. arabica*, *C. rutidosperma*, *C. gynandra*, *C. droserifolia*, *C. viscosa*, and *C. amblyocarpa*, have been identified. Notably, *C. amblyocarpa* has properties that can help reduce fever and alleviate diarrhea and has been traditionally used in addressing scabies (4), inflammations, blood disorders, rheumatic pains, diabetic hyperglycemia, paralysis, convulsions, spasms, pain, uterine ailments, malaria, anthelmintic problems, epilepsy, and skin diseases (5). The traditional applications of *Cleome* encompass the use of various plant parts, including leaves, roots, and seeds, as stimulants, antiscorbutic agents, anthelmintics, rubefacients, vesicants, and carminatives (6).

The *Cleome* species contains several key therapeutic compounds such as alkaloids, flavonoids, triterpenoids, phytosterols, saponins, tannins, glycosides, and essential oils with diverse medicinal applications (7). The main identified alkaloids in *Cleome* species are cleomiscosins with antipyretic, anti-inflammatory, analgesic, and antitumor properties (8). Flavonoids that have been identified to be abundant in *Cleome* species included calycopterin, pinocembrin, rutin, luteolin and kaempferol (1). These compounds possess antioxidant, anti-inflammatory, antimicrobial, anticancer, and hepatoprotective activities. They also contribute to the vasorelaxant effects and have potential remediating of cardiovascular ailments (9). Moreover, *Cleome* species contain triterpenoids such as lupeol, α -amyirin, and β -amyirin (10). These compounds exhibit significant anti-inflammatory, analgesic, wound healing, hepatoprotective, and antitumor activities (11). Saponins, identified in *Cleome* species demonstrate diverse pharmaceutical characteristics, like antinociceptive, anti-inflammatory, antimicrobial, and antitumor effects. They also contribute to hepatoprotection and have potential as immunomodulatory agents (12). The essential oils abundant in *Cleome* species were reported to possess antimicrobial, antifungal, insecticidal, and larvicidal activities (13). Henceforth, the therapeutic compounds found in *Cleome* species offer a wide range of potential

medicinal applications, making *Cleome* species promising source for the development of novel therapeutic agents.

C. amblyocarpa is commonly distributed in arid desert regions characterized by sandy or rocky terrain (14). This species has long been utilized in folk medicine as a treatment for various disorders, including diabetes, colic, stomach disorders, rheumatic fever, scabies, and inflammation (15). Extensive investigations have revealed that *C. amblyocarpa* harbors a diverse array of beneficial bioactive compounds, notably flavonoids and glucosinolates, saponins, and triterpenoids (14). As a result, this species has scientifically documented to exhibit multiple therapeutic properties, comprising anti-inflammatory, antiviral, genotoxic, antidiabetic, and antioxidant, as well as antibacterial activities (15, 16; 17). The geographical distribution of *C. amblyocarpa* includes North and East Africa, Iran, Sudan, Iraq, Palestine, Ethiopia, and Saudi Arabia (18).

The existing knowledge concerning the phytochemical composition and associated activities of *C. amblyocarpa* extracts is limited. Consequently, the principal objective of this study was to comprehensively assess the phytochemical profile of *C. amblyocarpa* using LC-ESI-MS/MS approach. In addition, the investigation aims to explore the occurrence of vitamins, minerals, as well as abscisic acid, in *C. amblyocarpa* aerial parts. Additionally, the study aimed to evaluate the overall phenolic profile, antioxidant properties, and antimicrobial effects of the methanolic extract of *C. amblyocarpa*.

Material and methods

Plant material collection and taxonomy

The collection and taxonomic identification of plant materials involved the procurement of fresh aerial parts of *C. amblyocarpa* from Al-Yutamah Dome area, El Madina El Menawara, located at coordinates (23.787786,39.651276) in Saudi Arabia. The collection took place during the flowering period in March 2020. An image of the plant can be found in Picture 1. To ensure accuracy, the botanical survey division of Dr. Ghalia Aljeddani, College of Science, Jeddah University, Jeddah, KSA authenticated and taxonomically identified the collected plant materials.

Preparation of *C. amblyocarpa* samples

The fresh aerial parts were first destalked and then washed with clean cold tap water. Subsequently, the aerial parts were dried naturally at the ambient temperature in a shaded area for four weeks. This drying process was conducted to facilitate subsequent biological activities and chemical analyses. Once dried, the aerial parts were finely ground using a mortar and pestle, followed by sieving through 0.2 mm sieve. The resulting fine powder was stored in air-tight containers under low temperature conditions for further analysis. These powders were utilized for nutritional assessment as well as to acquire methanolic extracts for further evaluations, as described by (19).

Preparation of methanolic extract from *C. amblyocarpa*

To prepare the methanolic extract of *C. amblyocarpa*, 100 grams of the aerial parts dry powder were subjected to extraction with 80% methanol for of five days (14). Subsequently, the resulting solutions underwent filtering three times to obtain the crude extract. The removal of the solvent from the extract was accomplished at reduced pressure by a rotary evaporator set at 40 °C. The resulting residue was resuspended in distilled water and then utilized for both HPLC-ESI-MS/MS chromatographic and biological investigations.

Identification of active constituents of *C. amblyocarpa* through LC-ESI-MS/MS

The exploration of the phytochemical active ingredients of *C. amblyocarpa* aerial parts was examined using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) methodology. For separation, an Exion LC AC system was employed, while detection was accomplished using the SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionization (ESI), as described by (20).



Picture 1: A photo of *C. amblyocarpa* taken at Al-Yutamah Dom area, South of Al Madinah El Menawara, KSA. The photo was captured using the iPhone 7 (A1778 model).

Nutritional analyses of *C. amblyocarpa***Assaying of fat-soluble vitamins**

The extraction of fat-soluble vitamins (A, D₂, D₃, and E) from *C. amblyocarpa* aerial parts was conducted following the methodology outlined in (21). In summary, a solution was prepared by combining 10 g of *C. amblyocarpa* powder, 1 g pyrogalllic acid, 70 ml ethanol, and 30 ml 50% KOH. The mixture was then agitated and heated using a water bath set at a temperature of 50 ± 2 °C for 40 min. This extraction process was repeated three times using different ether concentrations (50, 30, and 20 ml). The resulting extract was neutralized with deionized water and dehydrated with anhydrous sodium sulfate. Subsequently, the

extract was reduced to about 5 ml in a water bath set at 50 ± 2 °C. It was then mixed with methanol to reach a volume of 10 ml, filtered through a 0.45 µm membrane, and ultimately analyzed using HPLC (Agilent technologies, Germany 1200 series).

Quantification of vitamin C concentration

The quantification of vitamin C level in the *C. amblyocarpa* aerial parts was performed by means of a high-performance liquid chromatography system (Agilent 1260 series, Germany 1200) according to (22). The separation was conducted utilizing an Eclipse C8 column (4.6 mm x 150 mm i.d., 5 µm). The mobile phase was composed of a mixture containing 0.01% TFA: MeOH (70:30) at a flow rate of 1 ml/min., with the mobile phase programmed as isocratic. The MWD detector was set to monitor at 262 nm. The sample solution was injected with a volume of 10 µl. The temperature of the column was held constant at 40 °C.

Determination of vitamin B content

The water-soluble vitamin B was extracted from the above-ground portions of *C. amblyocarpa* using the procedure outlined in (23). Briefly, 2 g of *C. amblyocarpa* powder was mixed with 25 ml of 0.1 N H₂SO₄ solution and incubated at 121°C for 30 min. The contents were subsequently cooled and pH 4.5 was achieved by adding 2.5 M sodium acetate. An amount of 50 mg of Taka-diestase enzyme was added, and the mixture was kept at a temperature of 35 °C overnight. The mixture was passed through a Whatman No. 4 filter paper before being mixed with 50 ml of distilled water and filtered through a microspore filter with a pore size of 0.45 µm. The HPLC apparatus was fed 20 µl of the filtrate. An Agilent HPLC system (1260 series, Germany) was used to quantify the vitamin B concentration by comparing it to vitamin B standards.

Mineral content determination

To determine the mineral content of *C. amblyocarpa* aerial parts, a sample of 0.5 g of the powdered material was mixed with 5 ml of nitric acid and 0.5 ml of H₂O₂ (30%). The digestion process followed the American Public Health Association (APHA) guidelines (APHA 3500-K B and APHA 3500-Na B aqua-regia digestion flame photometric method for potassium and sodium determination, respectively). The elements calcium, magnesium, manganese, copper, iron, and zinc were determined using the PG-990 Atomic Absorption Spectrophotometer (AAS) coupled with the APHA 3111 B Aqua-regia Digestive Direct Air Acetylene Flame Method. An APHA 4500-P C aqua-regia digestion vanado-molybdo-phosphoric acid colorimetric technique was used with a T-80 UV/Vis Spectrophotometer to ascertain the phosphorus level (24).

Determination of abscisic acid

For the determination of abscisic acid (ABA) in *C. amblyocarpa* aerial parts, the plant material was carefully processed and mixed with a solution of methanol, water, and acetic acid (90:9:1, v/v/v) along with 2,6-di-tert-butyl-4-methylphenol (200 mg/l). As part of the extraction process, ¹³C₂-ABA as an internal standard was

introduced at the start, following the procedure outlined by (25). Following the addition of 17.5 ml of water, supernatants were purified by centrifugation and then submitted into Oasis HLB cartridges (Waters). ABA was eluted by implementing a 1 ml mixture of methanol, water, and acetic acid (90:9:1, v/v/v). A 5 ml aliquot of the sample was analyzed using HPLC with a Capcell Pac C18 column (150 mm × 2 mm; Shiseido). The HPLC analysis employed a binary solvent system of methanol and water (1:1, v/v) containing 0.1% formic acid, with a flow rate of 0.2 ml/min. Tandem mass spectrometry with multiple reaction monitoring in negative-ion mode was used for compound analysis. The precursor ion (m/z) and product ion (m/z) sets for ABA and the $^{13}\text{C}_2$ -ABA internal standard were 263 and 153, and 265 and 153, respectively.

Assessment of total polyphenols level

The total polyphenol content (TPC) in the methanolic extract of *C. amblyocarpa* aerial parts was estimated using the Folin–Ciocalteu method, as described by (26) and (27). In this method, 0.5 ml of Folin–Ciocalteu reagent was combined with 0.5 ml of the methanolic extract, followed by the addition of 4 ml of a 1 M sodium carbonate solution. The mixture was immersed in a water bath at 45 °C for 5 min., after which the measurement of absorbance was accomplished at 765 nm. A calibration curve created using gallic acid (GA) was utilized to calculate the TPC of *C. amblyocarpa* as mg GAE/g DM.

Determination of DPPH free-radical scavenging activity

The DPPH radical scavenging assay was employed to evaluate the free-radical scavenging activity of the methanolic extract of *C. amblyocarpa* aerial parts according to the method of (26). The assay involved incubating 100 µl of the extract with a DPPH solution (3.9 ml) in methanol (0.36 mg/l) in dark place at the ambient temperature for 30 min. The optical density was recorded at 490 nm, with BHT serving as the reference antioxidant. The radical scavenging activity (%) was evaluated from the following formula:

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

Antimicrobial activity of *C. amblyocarpa* extract

The antimicrobial activity of the extract from *C. amblyocarpa* was evaluated against pathogenic bacteria and fungi in this study. The tested microorganisms included human pathogenic strains, namely *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* NRRL B-313 as Gram-positive bacteria, and *Escherichia coli* NRRL B-210 and *Pseudomonas aeruginosa* NRRL B23 27853 as Gram-negative bacteria. Additionally, *Candida albicans* NRRL Y-477 and *Aspergillus niger* NRRL-3 represented the pathogenic yeast and fungi, respectively. These strains were acquired from the Natural Research Center, Department of Chemistry of Natural and Microbial Products in Cairo, Egypt. The obtained microorganisms were cultured on nutrient agar medium for bacteria and Sabouraud nutrient agar

medium for fungi. To compare the antibacterial efficacy of the *C. amblyocarpa* extract, tetracycline (TE) at a concentration of 30 µg/ml was used as the positive control for bacteria, while neomycin (30 µg/ml) served as the positive control for fungi. To ensure optimal microbial suspension density, the concentration was set at 10^5 CFU/ml at 600 nm using saline (27). The well diffusion technique, as described by (28), was employed to screen the antimicrobial activity. Nutrient agar medium (NA) and potato dextrose agar (PDA) were used for pathogenic bacteria and fungi, respectively. The zone of inhibition produced by the *C. amblyocarpa* extract was compared with the control antimicrobial agents utilized (19).

Statistical analysis

The obtained results were reported as the mean \pm the standard deviation of at least three independent replicates. Data means were separated by conducting a one-way analysis of variance (ANOVA) utilizing the CoStat software (v 6.311, CoHort). The significance of the mean differences was assessed using least significant difference (LSD) at 5% level.

Results and discussion

HPLC-ESI-MS/MS analysis of phytochemical active constituents of *C. amblyocarpa*

The aerial parts of *C. amblyocarpa* were subjected to HPLC/MS analysis, and the results are displayed in Table 1 and Fig. 1. The analysis revealed the presence of twenty-one peaks in the standard profile, out of which eleven peaks were successfully identified and characterized in the extract of the aerial parts of *C. amblyocarpa*. These identified peaks were tentatively assigned as follows: peak 1 as caffeic acid, peak 2 as coumaric acid, peak 3 as vanillin, peak 4 as naringenin, peak 5 as quercetin, peak 6 as 3,4-dihydroxybenzoic acid, peak 7 as kaempferol, peak 8 as ferulic acid, peak 9 as syringic acid, peak 10 as apigenin, and peak 11 as luteolin.

Among these compounds, naringenin was found to be the major component in *C. amblyocarpa* aerial parts, with a concentration of 140 µg/g. α -ferulic acid was also present in moderate amount at 7.48 µg/g. The remaining identified constituents, namely apigenin, coumaric acid, 3,4-dihydroxybenzoic acid, syringic acid, kaempferol, caffeic acid, vanillin, quercetin, and luteolin, were present in lesser quantities, ranging from 2.95 to 0.02 µg/g (2.95, 1.64, 1.54, 1.28, 0.8, 0.58, 0.47, 0.06 and 0.02 µg/g, respectively).

These findings align with earlier studies on various *Cleome* species, including *C. droserifolia*, *C. amblyocarpa*, *C. brachycarpa*, and *C. chrysantha*, as described by (29). They reported the existence of flavonoid glycosides in these *Cleome* species. The occurrence of these compounds in the *Cleomaceae* family has been widely reported (30), and some studies have suggested their potential role in prostate cancer prevention (31). These phytochemicals have attracted considerable attention because of their wide range of biological activities, such as inhibiting V-ATPase, having anti-cancer effects, and possessing antiviral potential (32; 33).

Naringenin, the main constituent in *C. amblyocarpa*, extract, is a flavanon compound, possesses a range of health beneficial properties such as antidiabetic, anticancer, antimicrobial, antiobesity, gastroprotective, immunomodulatory, cardioprotective, nephroprotective, and neuroprotective effects. These diverse properties can primarily be attributed to its antioxidative and anti-inflammatory activities (34).

Naringenin exhibits notable antioxidant actions, including the eradication of free radicals and prevention of lipid-mediated peroxidation. Furthermore, it has been shown to enhance the levels of enzymatic antioxidants while inhibiting metal chelation and various pro-oxidant enzymes. In terms of anti-inflammatory activities, naringenin is associated with the suppression of mitogen-activated protein kinase activities and nuclear factor kappa B (35; 34). This is achieved through the modulation of both the expression and release of proinflammatory cytokines and enzymes. Ferulic acid is a derivative from 4-hydroxycinnamic acid. It is present in a variety of food products, fruits, and drinks. It possesses clinically validated antioxidant, anti-inflammatory, anti-aging, antiangiogenic, anticancer, antibacterial, and antioxidant characteristics (36; 37). The therapeutic properties of naringenin and ferulic acid make *C. amblyocarpa* an excellent candidate for multiple pharmaceutical industries.

Table 1: Identification of the bioactive compounds in the aerial parts of *C. amblyocarpa* using HPLC-ESI-MS/MS approach.

Compound	Area x 10 ²		RT		Concentration	
	STD	<i>C.amblyoc</i>	ST	<i>C.</i>	STD	<i>C.</i>
Chlorogenic acid	24600	ND	7.35	ND	0.2	ND ^g
Daidzein	35300	ND	12.8	ND	0.2	ND ^g
Gallic acid	33500	ND	3.91	ND	0.2	ND ^g
Caffeic acid	182500	2132000	8.02	8.03	0.2	0.58±0.01
Rutin	222100	ND	9.7	ND	0.2	ND ^g
Coumaric acid	274500	90290	9.49	9.48	0.2	1.64±0.02
Vanillin	6758	631	9.45	9.46	0.2	0.47 ^{fg}
Naringenin	956.1	26770	14.9	14.89	0.2	140.0±1.5
Quercetin	125800	1488	13.5	13.51	0.2	0.06 ^g
Ellagic acid	3510	ND	9.92	ND	0.2	ND ^g
3,4-Hesperetin	16360	5029	5.73	5.74	0.2	1.54±0.02
Myricetin	32940	ND	15.5	ND	0.2	ND ^g
Cinnamic acid	5122	ND	11.6	ND	0.2	ND ^g
Methyl gallate	73.53	ND	14.2	ND	0.2	ND ^g
Kaempferol	215600	ND	7.41	ND	0.2	ND ^g
Ferulic acid	7400	1181	15.2	15.27	0.2	0.80±0.01
Syringic acid	10610	15880	10.1	10.16	0.2	7.48±0.28
Apigenin	3402	874	8.35	8.36	0.2	1.28±0.01
Catechin	698.9	412.2	14.9	14.97	0.2	2.95±0.01
Luteolin	5182	ND	7.34	ND	0.2	ND ^g
	45640	163.9	13.4	13.46	0.2	0.02±0.00

Statistics	
F	19686.98
P	0.0000
LSD at 5%	0.642

RT = retention time (min.), STD = standard, ND = not detected, P = probability, LSD = least significant difference. Different letters within the same column indicate significant differences at 5% level.

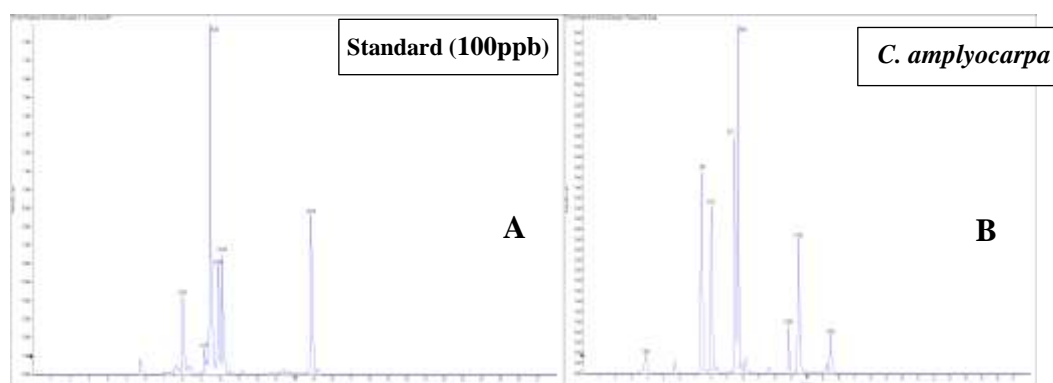


Fig. 1: HPLC-ESI-MS/MS profile of stander (A) and *C. amblyocarpa* extract (B).

Nutritional value of *C. amblyocarpa* extract

Vitamins

The findings of the current investigation demonstrate that *C. amblyocarpa* possesses a diverse of both fat-soluble and water-soluble vitamins, as indicated in Table 2. The analysis detected the occurrence of vitamin D2 (5.30 µg/g) and vitamin C (167.38 µg/g). Additionally, the aerial parts of *C. amblyocarpa* exhibited significant concentrations of vitamin B complex, encompassing B1 (2312.76 µg/g), B2 (76.58 µg/g), B6 (4126.56 µg/g), B9 (630.60 µg/g), and B12 (2421.61 µg/g). Consequently, the abundance of vitamins in *C. amblyocarpa* aerial parts can be ranked in the following order: B6 > B12 > B1 > B9 > C > B2 > D2. These findings shed light on the remarkable nutritional value of *C. amblyocarpa*, particularly in terms of its vitamin content.

Plants are essential to human nourishment, in especially; leafy green vegetables are a great provider of vitamins. Previous research studies have indicated that *C. amblyocarpa*, *C. viscosa*, and *C. burmanni* provide significant levels of dietary fibers, protein, fat, vitamins, and minerals (16; 18). The existence of vitamin C in these plants demonstrates potent antioxidant properties. In addition to maintaining healthy connective tissues and promoting wound healing, vitamin C boosts the uptake of iron from food within the digestive tract (38). Vitamin C is essential for several metabolic processes, such as the triggering of folic acid, the transformation of cholesterol into bile acids, and the transformation of tryptophan (an amino acid) into serotonin (a neurotransmitter). It acts as an antioxidant, safeguarding the body

Table 2: Vitamin composition of *C. amblyocarpa* aerial parts. Data are the means of three replicates \pm standard deviation (SD).

Vitamin	Area		Conc. ($\mu\text{g/ml}$)	
	STD	C. <i>amblyocarpa</i>	STD	C. <i>amblyocarpa</i>
VIT D3	330.48	ND	10.00	ND ^h
VIT D2	248.81	30.33	7.50	5.30+0.02 ^g
VIT E	307.62	ND	35.00	ND ^h
VIT A	118.43	ND	9.80	ND ^h
Vit C	1579.6	26.44	100	167.38+1.34 ^e
Vit. B1	426.29	123.24	80.00	2312.76+3.14 ^c
Vit. B2	468.04	10.75	33.33	76.58+1.39 ^f
Vit. B6	130.49	107.70	50.00	4126.56+2.48 ^a
Vit. B9	92.74	17.55	33.33	630.60+1.67 ^d
Vit. B12	239.65	38.69	150.00	2421.61+3.17 ^b
Statistics				
F				1534194.70
P				0.0000
LSD at				3.714
5%				

ND = not detected, P = probability, LSD = least significant difference. Different letters within the same column indicate significant differences at 5% level.

against damage caused by free radicals. Moreover, vitamin C safeguards the immune system, mitigates the intensity of allergic responses, and assists in combating infections. Nonetheless, there is still debate on the exact role that vitamin C plays and how useful it is for disorders including cancer, coronary artery disease diabetes, neurological disorders, and metal poisoning in humans (38; 39; 40).

Vitamin D, unlike other essential vitamins for health, stands out due to the diverse sources from which it can be obtained. Ergocalciferol (vitamin D2) is derived from the UV irradiation of ergosterol, a steroid found predominantly in fungi but also in some plants (41). Both vitamin D2 and vitamin D3 function as prohormones, lacking biological effects themselves, with the only difference being the structure of their side chains. Consequently, the body utilizes them in an equivalent manner (42). After being consumed, both vitamin D2 and vitamin D3 experience metabolism in the liver into 25-hydroxyvitamin D [25(OH)D; D represents either D2 or D3], and subsequently in the kidneys to produce 1,25-dihydroxyvitamin D (43). To the best of the authors' information, based on an extensive review of the literature, this study is the first to demonstrate the presence of vitamin D2 in the above-ground parts of *C. amblyocarpa*. Further comprehensive and specific investigations are necessary in the future to explore the existence of vitamin D in this plant.

The aerial parts of *C. amblyocarpa* were found to contain significant amounts of vitamin B complex, including B1 (2312.76 µg/g), B2 (76.58 µg/g), B6 (4126.56 µg/g), B9 (630.60 µg/g), and B12 (2421.61 µg/g). This is consistent with previous studies that reported the presence of dietary fibers, proteins, fats, vitamins, and minerals in the aerial parts of *C. viscosa* and *C. burmanni* (Moghaddam et al., 2021). Although *Cleome* species are known for their bitter taste attributed to high phenolic compounds, chemical analysis has revealed unique features related to mineral nutrition, crude fibers, and vitamins B and C, as well as Zn and Fe (44). B-group vitamins, which encompass water-soluble organic compounds acting as coenzymes in various metabolic processes, play essential functional roles. These roles encompass a wide range of important functions within the cell, such as energy production, methyl donor production, neurotransmitter synthesis, immune system functioning, 1-carbon metabolism, cellular cross-talk, and nucleic acid biosynthesis (45). Moreover, there is emerging evidence indicating that B-group vitamins have an impact on the composition and function of the gut microbial community, highlighting their additional importance (46). Insufficient levels of vitamin B can heighten the likelihood of experiencing anxiety, depressive disorders, dementia, and Alzheimer's disease. In addition, a lack of vitamin B can interfere with the methylation process of homocysteine, which may result in hyperhomocysteinemia. In addition, a lack of vitamin B can weaken the immune system, increase the production of pro-inflammatory cytokines, and activate NF-κB (47). Therefore, considering the significant content of vitamin B complex in *C. amblyocarpa*, it holds promise as a valuable natural source of these vitamins for individuals with vitamin B deficiency, potentially offering a range of health benefits. Nonetheless, additional future research is necessary to fully explore the potential utilization of *C. amblyocarpa* as a vitamin B source.

Mineral contents

The evaluation of mineral composition of *C. amblyocarpa* aerial parts revealed the presence of several minerals including bicarbonate, chlorides, sulphate, nitrogen, phosphate, sodium, calcium, magnesium, potassium, and selenium (24.0, 12.5, 35.0, 21.2, 5.2, 1.5, 9.2, 3.3, 14.5, and 0.05 mg/g DM, respectively). Notably, the data demonstrated significantly high levels of sulphate, bicarbonate, nitrogen, and potassium (35.0, 24.0, 21.2, 14.5, and 12.5 mg/g DM, respectively), while calcium, phosphate, manganese, and sodium exhibited moderate values (9.2, 5.2, 3.3, and 1.5 mg/g DM, respectively), with selenium exhibiting the lowermost mineral content (0.05 mg/g DM) among the analyzed minerals in the aerial parts of *C. amblyocarpa* (Table 3).

These findings are consistent with previous research reported by (48). A comparison of the present study's data with previous investigations suggests that *C. gynandra* collected in Burkina Faso contains similar quantities of calcium, magnesium, and iron elements to those grown in South Africa, Ghana, and Kenya (48). It is worth mentioning that the levels of sodium and phosphorus in the present investigation were higher compared to those reported by and (49).

The minerals found in *C. amblyocarpa* can contribute to various potential health benefits. For example, calcium is essential for the maintenance of robust skeletal structures, including bones and teeth. Additionally, it contributes to muscle function, neuronal transmission, and coagulation of the blood (50). Magnesium plays a crucial role in several biochemical events inside the body and is necessary for maintaining proper muscle and nerve function, controlling blood pressure, bolstering the immune system, and fostering robust bones (51). Potassium is an electrolyte that helps maintain optimal fluid equilibrium, facilitates neural and muscular activity, controls blood pressure, and enhances cardiovascular overall health (52). As well, sulphate is involved in the detoxification processes of the body and is necessary for the synthesis of proteins, enzymes, and other important molecules (53). Though its low concentration in *C. amblyocarpa*, selenium plays a crucial role as an antioxidant, defending cells against oxidative damage. Additionally, it plays a crucial role in supporting a profound immune system and is essential for proper thyroid hormone metabolism (54). It is worth noting that while these minerals have potential health benefits, the presence of vitamins and minerals in *C. amblyocarpa* indicates that the aerial parts of this plant could be recommended for inclusion in a nutritious diet.

Table 3: Mineral composition of *C. amblyocarpa* aerial parts. Data are the means of three replicates \pm standard deviation (SD).

No	Minerals	(mg/g DM)
1	Bicarbonate	24.0 \pm 0.47 ^b
2	Carbonate	ND ^j
3	Chlorides	12.5 \pm 0.57 ^e
4	Sulphate	35.0 \pm 1.75 ^a
5	Nitrogen	21.2 \pm 0.87 ^c
6	Phosphate	5.2 \pm 0.09 ^g
7	Sodium	1.5 \pm 0.01 ⁱ
8	Calcium	9.2 \pm 0.67 ^f
9	Manganese	3.3 \pm 0.06 ^h
10	Potassium	14.5 \pm 1.08 ^d
11	Selenium	0.05 \pm 0.00 ^j
Statistics		
F		2202.20
P		0.0000
LSD at 0.05		0.764

DM = dry mass, ND = not detected, P = probability, LSD = least significant difference. Different letters within the same column indicate significant differences at 5% level.

Absciscic acid (ABA)

Fig. 2 presents the chromatogram illustrating the abscisic acid (ABA) concentration in the aerial parts of *C. amblyocarpa*. The obtained data indicates that the overall ABA content in the aerial parts of *C. amblyocarpa* amounted to 276.24 mg/g FM. As a means to enhance their defense mechanisms, plants have evolved diverse mechanisms at various levels, including the accumulation of antioxidants such as ABA. ABA is an important phytohormone that plants use in a wide range of physiological processes, particularly during stress responses. While ABA is primarily associated with plant functions, there has been growing interest in exploring its potential health benefits when present in high concentrations within plant tissues. Some potential health benefits associated with high concentrations of ABA in plant tissues include its antioxidant properties, which can assist in the neutralization of detrimental free radicals in the body, thereby defending cells and tissues against oxidative injury and promoting the good health of cells (55). ABA has been found to possess anti-inflammatory properties. The anti-inflammatory effects of ABA may help reduce inflammation and potentially alleviate related health issues (56). Furthermore, ABA has been implicated in the regulation of various metabolic processes, involving carbohydrate and lipid metabolism. It may help regulate blood sugar levels, lipid profiles, and energy metabolism, thus potentially contributing to the prevention or management of metabolic disorders such as diabetes and obesity (57). It is prominent to state that though these potential health benefits are supported by scientific research, further studies are needed to better understand the mechanisms and evaluate the efficacy of ABA in human health. Additionally, the bioavailability and potential side effects of ABA when consumed from plant sources require further investigation.

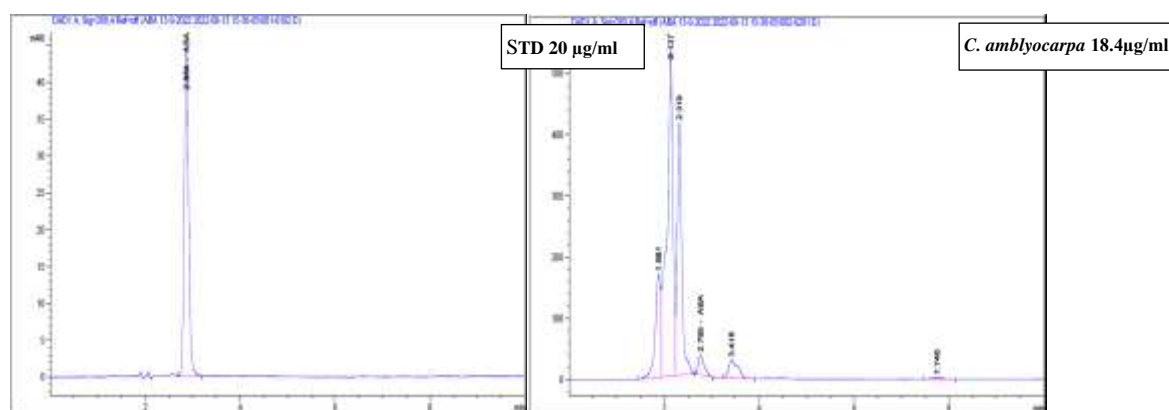


Fig. 2. HPLC chromatogram of ABA detection in *C. amblyocarpa* aerial parts.

Total polyphenolic content

Fig. 3 displays the total polyphenolic compounds content in the aerial parts of *C. amblyocarpa*. Total polyphenolic compounds of the methanolic extract of *C.*

amblyocarpa showed relatively high (28.8 GAE/g DM) when contrasted to the used standard phenols (ruten and gallic acid with concentrations of 40.0 and 36.8 mg, respectively). Thus, the existence of superior levels of polyphenols in the aerial parts of *C. amblyocarpa* may contribute to its potential as a potent antioxidant, antimicrobial, and anticancer agent. These results align well with the research conducted by (58). (59) also documented the presence of various polyphenolic compounds, including flavonoids, in leaf extracts of certain *Cleome* species. Aicha et al. (60) investigated the antioxidant potential and phenolic compounds content of *C. arabica*, demonstrating that, among the extracts from the roots and seeds, the leaf extract had the greatest total phenolic and flavonoid contents. Likewise, (61) reported on the antioxidative characteristics, phenols, and flavonoid of the aerial parts of *C. viscosa*. Furthermore, the findings of this current study align with the findings reported by (62). They observed significant quantities of phenols and flavonoids in the aerial parts, along with notable chelating activity. One possible rationalization for *C. amblyocarpa*'s strong radical scavenging activity is the presence of these phytochemicals.

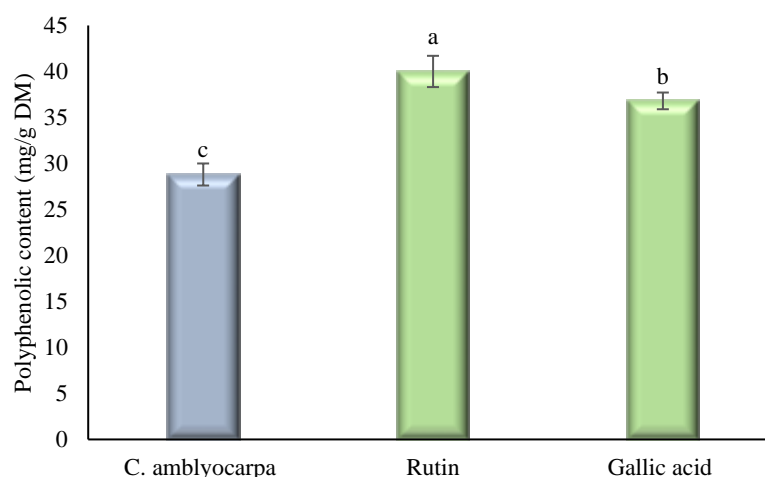


Fig. 3. Total polyphenolics contents of *C. amblyocarpa* aerial parts. Data are the means of three replicates \pm standard deviation (SD). Different letters indicate significant differences at 5% level.

DPPH antioxidant activity of *C. amblyocarpa*

Using the DPPH test, the antioxidant capacity of the *C. amblyocarpa* methanolic extract was evaluated, and the corresponding findings are presented in Fig. 4. The reported capacity to scavenge the DPPH radical scavenging activity of the methanol-based extract of *C. amblyocarpa* was 70.1%, whereas ascorbic acid, serving as the standard, exhibited a scavenging activity of 76.34%. Consequently, the extract from *C. amblyocarpa* exhibits remarkable antioxidant capabilities and can be regarded as a valuable source of antioxidants in the human diet, contributing to the mitigation of health issues associated with oxidants and prooxidants. The significance of antioxidant activity lies in its potential to protect food products against the detrimental impacts of oxidants. Research has increasingly suggested

that cellular damage induced by free radicals can lead to the occurrence of prominent ailments (63). The findings of the present study are consistent with those obtained by (62), who observed potent free radical scavenging efficacy in extracts derived from the aerial parts of *C. amblyocarpa* and *C. ramosissima*, potentially attributed to the presence of polyphenolic compounds, specifically flavonoids and phenolic acids. Many investigations have confirmed a significant correlation between anti-oxidative and anti-radical properties and the total polyphenols concentration (64; 65;).

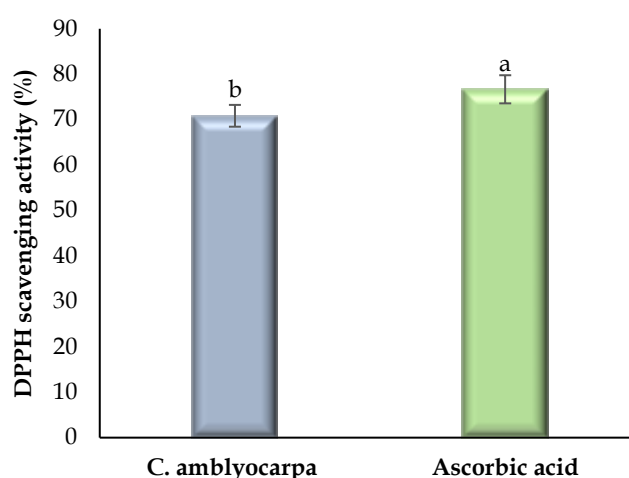


Fig. 4. The antioxidant activity of *C. amblyocarpa* aerial parts. Data are the means of three replicates \pm standard deviation (SD). Different letters indicate significant differences at 5% level.

Antimicrobial activity of *C. amblyocarpa* methanolic extract

The antimicrobial activity of the methanolic extract derived from the aerial parts of *C. amblyocarpa* was examined, and the findings are illustrated in Fig. 5, which highlights the inhibition zone diameters against the antimicrobial agents novobiocin and tetracycline. In most cases, the extract of *C. amblyocarpa* exhibited larger inhibition zone diameters compared to the antimicrobial agents. The outcomes demonstrated that *C. amblyocarpa* displayed the most potent antibacterial activity, as evidenced by an inhibition zone diameter of 26.0 mm against *B. subtilis*, *E. coli*, and *C. albicans*. As well, *S. aureus* occupied the second position with an inhibition zone diameter of 24.0 mm. However, *P. aeruginosa* and *A. niger* exhibited the least response to the methanolic extract of *C. amblyocarpa*, with an inhibition zone diameter of 18.0 mm. Overall, the antimicrobial activity of the methanolic extract of *C. amblyocarpa* holds promise as a potential treatment against various microbial pathogens.

C. amblyocarpa is widely utilized in the folk medicine for managing diabetes and colic (16). Cleome species are commonly employed in traditional medicine as stomachic and rubefacient remedies, as well as for treating scabies,

rheumatic fever, inflammation, and demonstrating antimicrobial, antioxidant, and cytotoxic activities (66). The presence of phenolic compounds, particularly polyphenols, flavonoids, and tannins in the extracts of *C. amblyocarpa* may contribute to its antimicrobial activity (57). These findings align with our research and indicate the potential antimicrobial and antioxidant properties of the *Cleome* genus. Therefore, further investigations on *C. amblyocarpa* are advantageous to determine and delineate the major compounds, either individually or in combination, as potential antioxidants or antimicrobial agents, while also evaluating their biosafety.

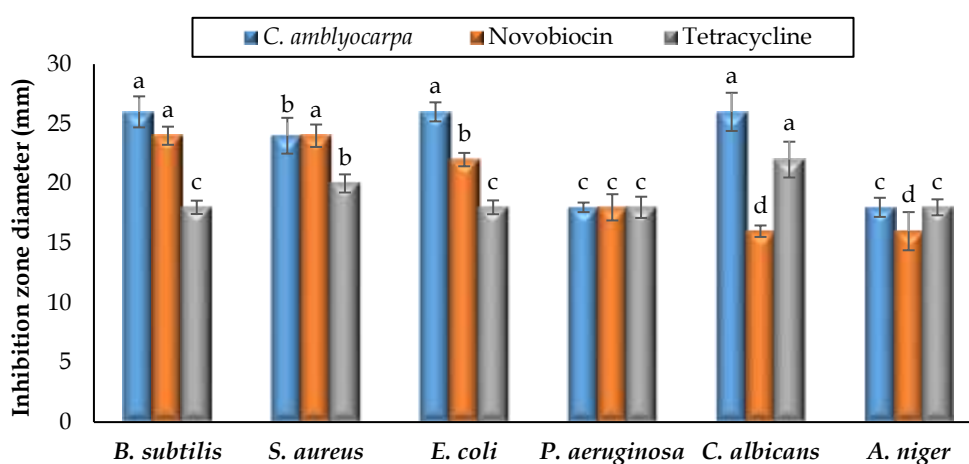


Fig. 5. The antimicrobial activity of the methalolic extract *C. amblyocarpa* aerial parts. Data are the means of three replicates \pm standard deviation (SD). Different letters within the same series indicate significant differences at 5% level.

Conclusion

In conclusion, the analysis of the aerial parts of *C. amblyocarpa* using HPLC/MS revealed the presence of numerous bioactive compounds with potential health benefits. Among the identified compounds, naringenin was found to be the major component. The abundance of vitamins, including vitamin D2, vitamin C, and various B complex vitamins, highlighted the remarkable nutritional value of *C. amblyocarpa*. Additionally, the evaluation of mineral composition showed the presence of several minerals, with high levels of sulphate, bicarbonate, nitrogen, and potassium, which can contribute to various health benefits.

Furthermore, the aerial parts of *C. amblyocarpa* exhibited a significant concentration of abscisic acid (ABA). Moreover, the methanolic extract derived from the aerial parts of *C. amblyocarpa* displayed significant antimicrobial activity against the investigated bacterial and fungal pathogens. Overall, the findings from this study emphasize the potential health benefits of *C. amblyocarpa*. The plant

possesses a rich composition of bioactive compounds, including antioxidants, vitamins, minerals, ABA, and polyphenolic compounds, which collectively contribute to its nutritional and medicinal value. The antioxidant, antimicrobial, and anticancer properties of *C. amblyocarpa* make it an attractive candidate for further research and development in the field of natural medicine.

Conflict of interest.

The authors declare that they do not have any conflict of interest with the data contained in the manuscript.

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