scientific reports

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Comparative LC–MS-based metabolite profiling, antioxidant, and antibacterial properties of *Bunium bulbocastanum* tubers from two regions in Algeria

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Traditional herbalists have been relied on for many years by Algerians to cure a wide range of diseases. Regardless of their nutritional values, mushrooms have chemical properties that make them attractive, beneficial, and more likely to be studied by researchers, according to ethnobotanical literature on traditional phytotherapy. Among all the edible mushrooms, tubers are a type of fungus that are traditionally used in fine dining and have garnered attention recently because of their many therapeutic applications. This research delves into a meticulous analysis of bioactive constituents in *Bunium bulbocastanum* tubers, sourced from Mostaganem and Relizane regions, with a keen focus on polyphenols, flavonoids, and condensed tannins. The quantification of total phenolic content was executed through the Folin-Ciocalteu assay, while flavonoids were assessed via the aluminum chloride colorimetric method. In addition, condensed tannins were evaluated in this study. Antioxidant capacities were scrutinized employing the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Microbial inhibition studies were conducted against five benchmark bacterial strains, utilizing the agar disc diffusion technique. Furthermore, a comprehensive liquid chromatography–mass spectrometry (LC–MS) analysis was performed to identify and quantify bioactive compounds. The findings underscore that the Mostaganem extracts were particularly rich in polyphenols (11.65 mg GAE/g of extract) and tannins (1.30 mg CE/g of extract), while the Relizane extracts boasted significant flavonoid concentrations (9.421 mg QE/g of extract). Notably, 4-methylguaiacol (82.04 mg/L), caffeic acid dimethyl ether (27.76 mg/L), syringic acid (20.48 mg/L), and naringenin (16.05 mg/L) emerged as the predominant volatile compounds. Compositional investigation of the extracts by LC–MS confirmed the presence of various compounds that were linked to the bioactivities exhibited by *B. bulbocastanum* tubers. These findings demonstrate the effective antibacterial and antioxidant properties of *B. bulbocastanum* tubers, indicating their potential use in pharmaceutical and nutraceutical applications.

Keywords DPPH, *Bunium bulbocastanum*, Algeria, LC–MS, Naringenin, 4-methylguaiacol

The utilization of secondary plant metabolites in primary health care is evident in both traditional medicine practices and the pharmaceutical industry. Traditionally, plant extracts or specific parts have been used to treat various ailments based on empirical knowledge passed down through generations. These practices involve using extracts or infusions made from whole plants or specific plant parts for their therapeutic properties[1–3].

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In the pharmaceutical industry, plants have been served as a source of active principles that have been isolated, characterized, and used to develop pure pharmacological compounds. This transition from traditional preparations to purified active ingredients marks a significant advancement, allowing for more precise dosage, a better understanding of pharmacokinetics, and targeted therapy[4–6]. Traditional practices thus serve as valuable leads for identifying bioactive compounds with potential pharmaceutical applications[7–10].

Medicinal plants have been an essential source for both therapeutic and preventive treatments in human medicine. The World Health Organization (WHO) estimates that approximately 21,000 plant species have the potential to be utilized as medicinal plants[11,12]. Different plant compounds offer varied therapeutic effects. For example, alkaloids can act as pain relievers or stimulants[13], flavonoids are known for their antioxidant and anti-inflammatory properties[14,15], terpenes may relieve respiratory ailments and enhance mood[16,17], and saponins have roles in heart health and immune function[18]. Algeria's flora is rich, comprising some 3000 plant species, with 15% being endemic, which underscores the region's unique botanical richness[19]. This diversity serves as a crucial reservoir for traditional medicine systems that have relied on indigenous flora for centuries. The *Bunium* genus with around 50 species found across North Africa, Asia, and Europe, contains several species used in traditional medicine[20]. Specifically, *Bunium bulbocastanum* L., known locally as Talghouda or Nut, is recognized in rural Algerian regions for its therapeutic properties[21].

Bunium bulbocastanum, a member of the Apiaceae family, has a long history in traditional medicine for its anti-inflammatory, antioxidant, and possible endocrine modulating benefits[21,22]. In the northern regions of Algeria, particularly in the districts of Relizane and Mostaganem, this plant is highly regarded in local healthcare practices for its potent antioxidant and anticancer properties, as well as its effectiveness in treating thyroid gland disorders[23]. Recent investigations reveal that the *B. bulbocastanum* tuber contains approximately 7% proteins, with lipid and ash contents of 3.34% and 3.96%, respectively[24]. The potential of *B. bulbocastanum* extends beyond its nutritional value. Amid rising antibiotic resistance and the side effects of synthetic drugs, this plant offers a viable alternative due to its bioactive substances, such as polyphenols, flavonoids, and condensed tannins, which are known for disease prevention and management properties. Nevertheless, little is known about the biological activities of this plant, and its phytochemicals have not been thoroughly studied. Thus, the objective of this study was to evaluate the antioxidant and antimicrobial properties and bioactive compounds of *B. bulbocastanum* from Sidi Ali (Mostaganem) and Ammi Moussa (Relizane). This research can merge traditional knowledge with scientific research, potentially yielding new therapeutic insights and supporting global biodiversity conservation and the sustainable use of medicinal plants.

Results and discussion

Total phenolic compounds

The total phenolic content of the extract obtained from *B. bulbocastanum* tubers was evaluated using spectrophotometric assays with the Folin-Ciocalteu reagent. The focus on total phenols is justified by its high antioxidant properties[25] and their widespread use in herbal medicine[26]. Quantitative analysis of total phenols was determined from the linear regression equations $y = 0.0119X + 0.0658$ with a regression coefficient of $R2 = 0.995$ for the calibration curve, expressed in milligrams of gallic acid equivalent per gram of crude extract (mg GAE/g).

Table 1 indicates that the total phenolic content was 11.65 mg GAE/g for samples from Mostaganem and 7.35 mg GAE/g for those from Relizane with a statistically significant difference ($p < 0.05$). The difference in polyphenol content between the two regions was estimated at 36.88%. These values were significantly higher than those reported by Souri et al.[27] for *B. persicum*, where the methanolic extract contained 2.14 mg/g dry weight in total phenolic content. Similarly, the phenol levels obtained by Chizzola et al.[28] for the methanolic fraction of *B. persicum* were 0.57 mg/g.

Variations in phenolic content can be attributed to phenolic metabolism, environmental conditions, and the geographical distribution of the plant species. According to Khlifi et al.[29], the phenolic composition of plant tissues varies considerably due to seasonal, genetic, and agronomic factors. High variability in phenolic content has been observed at different stages of maturation and under varying abiotic conditions of cultivation, such as temperature and precipitation[30,31]. Our findings align with those of Karouche et al.[32], who reported significant variations in phenolic content based on geographical origin.

The higher polyphenol content in *B. bulbocastanum* samples from Mostaganem compared to those from Relizane highlights the influence of geographical location on the phenolic profile of the plant. Mostaganem's specific environmental conditions, including soil type, climate, and agricultural practices, may contribute to the enhanced phenolic content. These findings are consistent with previous studies showing that environmental factors play a crucial role in the biosynthesis of phenolic compounds[33,34]. The robust antioxidant properties of phenols underline their importance in herbal medicine. The significantly higher phenolic content in the

Activities	Bb. Relizane	Bb. Mostaganem
Polyphenols (mgEAG/g)	7.35 ± 0.18^{b}	11.65 ± 0.28^{a}
Flavonoids (mg EQ/g)	9.42 ± 0.43^{b}	4.696 ± 0.38^{a}
Tannins (mg EC/g)	0.69 ± 0.08^{b}	1.30 ± 0.08^{a}
DPPH (%)	70.14 ± 0.89^{a}	40.90 ± 0.08^{b}

Table 1. Comparison of polyphenols, flavonoids, tannins, and % inhibition DPPH in *Bunium bulbocastanum* (bb) extracts from Relizane and Mostaganem. Mean value and standard deviation (±SD), $n = 3$. Different letters represent significant differences within the row ($p < 0.05$).

Mostaganem samples suggests a greater potential for antioxidant activity, which could enhance the therapeutic efficacy of *B. bulbocastanum* extracts from this region. This difference in polyphenol content could be leveraged in to develop region-specific extracts with optimized health benefits.

Comparing our results with those of other studies, the discrepancies in phenolic content may be attributed to differences in extraction methods, plant parts used, and preparation techniques[35]. For instance, variations in solvent type, extraction time, and temperature can significantly impact the yield of phenolic compounds. The use of methanol as a solvent, known for its efficiency in extracting a broad range of phytochemicals, likely contributed to the higher phenolic content observed in our study.

Total flavonoids content

The determination of total flavonoid levels as shown in Table 1 revealed that the extract from Relizane was significantly richer in flavonoids (9.42 mg QE/g) compared to the extract from Mostaganem (4.69 mg QE/g), representing a difference ratio of 50.15%. This result was statistically significant with $p < 0.05$. The flavonoids content was higher than those reported by Karouche et al.[32]. Our results were comparable to those found by Chizzola et al.[28], who attributed these levels to environmental and climatic conditions, collection period, genetic factors, and experimental conditions. The significantly higher flavonoid content in the Relizane extract suggests that the environmental conditions in Relizane, such as temperature, sunlight exposure, and soil composition, are more conducive to flavonoid biosynthesis[36]. This observation was consistent with previous findings[33,34], which highlighted the influence of climatic conditions on the production of secondary metabolites.

Determination of tannins

The condensed tannin content (Table 1) was significantly ($p < 0.05$) higher in the samples collected from Mostaganem (1.30 mg CE/g) compared to those from Relizane (0.69 mg CE/g), representing a difference ratio of 46.92%. These results indicate that the Mostaganem extract has the highest tannin content. However, this content was lower than that reported by Bansal et al.[37] for the same species, which was 17.24 µg EAT/mg. This discrepancy may be attributed to various factors, including environmental conditions, climatic and agronomic factors, geographic region, genetic factors, processing, and storage [38–[38].

The higher tannin content in the Mostaganem extract can be attributed to specific environmental conditions, such as higher humidity and varying soil compositions, which promote tannin biosynthesis. These findings are consistent with the research of Kumari et al.[39] and Taghizadeh et al.[40], who found that tannin levels are significantly affected by environmental and agronomic factors. The observed lower tannin content compared to Ogawa and Yazaki[41], could be attributed to differences in extraction techniques, seasonal variations, or the age of the plant material at the time of extraction. Standardizing units and clearly defining terms, such as "CE" (catechin equivalent) and "EAT" (epicatechin equivalent) are crucial to prevent confusion and ensure clarity. This study underscores the need for a deeper understanding of how specific environmental factors and genetic variations influence the production of tannins in *B. bulbocastanum*.

Antioxidant activity

DPPH free radical scavenging

The DPPH free radical scavenging test (Table 1) revealed that the ground nut extract from Relizane exhibited a significantly higher DPPH scavenging capacity ($p < 0.05$) compared to the extract from Mostaganem. Specifically, the inhibition percentage for Relizane ground nut extract was 70.14%, while the Mostaganem ground nut extract showed an inhibition percentage of 40.90%. These results indicate that the antioxidant activity of the Relizane extract is superior to that of the Mostaganem extract. However, the inhibition percentages observed in this study were significantly lower than those reported by Karouche et al.[32], who found an inhibition percentage of 75.41% at the same concentration for the methanolic extract from the tubers of *B. bulbocastanum*.

The lower DPPH scavenging activity observed in this study suggests that the antioxidant power of the *B. bulbocastanum* tuber extracts from both Relizane and Mostaganem is relatively low. The slight higher antioxidant activity reported by Karouche et al.[32] could be attributed to differences in methodology and experimental conditions. The geographical and environmental conditions of Relizane, which may promote the accumulation of antioxidant compounds, appear to contribute to the relatively higher DPPH scavenging activity observed in its extracts compared to those from Mostaganem. This observation aligns with findings from other studies, such as Shahsavari et al.[42], who reported significant antioxidant activity in essential oils from *B. persicum*, and Adelifar and Rezanejad[33], who highlighted the influence of environmental conditions on antioxidant capacity in different organs of *Bunium* species.

The DPPH assay was used as general, simple and fast indication of antioxidant activity of *B. bulbocastanum* from the different locations. Although the DPPH assay is valuable for evaluating radical scavenging activity, it is subject to several limitations, such as non-physiological aspects, selectivity towards hydrogen-donating processes, susceptibility to experimental factors, and possible disruption by non-antioxidant substances. Additional experimental tests should be conducted to assess the antioxidant activity of this plant.

Antimicrobial activity

The antimicrobial potency of *B. bulbocastanum* crude extract from two different regions was evaluated in vitro using the AWDT well diffusion method on Mueller-Hinton agar, a widely recognized medium for antimicrobial testing[43]. The antimicrobial activity was assessed by measuring the diameter of the inhibition zone surrounding the wells containing the crude extract. This assay was conducted against five microorganisms from the laboratory collection, including three Gram-negative bacteria: *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *K. pneumoniae*, one Gram-positive bacterium: *S. aureus* ATCC 33862, and one fungus: *C. albicans* ATCC 10231.

The antimicrobial properties of the Relizane extract (Table 2) demonstrated significant efficacy against *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and *S. aureus* ATCC 33862, with lower activity observed against *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC E47. The inhibition zone diameters ranged from 7.66 to 20 mm, with the highest activity recorded against *S. aureus* ATCC 33862 (20 mm). *C. albicans* ATCC 10231 exhibited an inhibition diameter of 14.66 mm, closely followed by *E. coli* ATCC 25922 (14.33 mm). In contrast, *K. pneumoniae* ATCC E47 showed a modest an inhibition diameter of 9.00 mm, while *P. aeruginosa* ATCC 27853 displayed the smallest inhibition zone at 7.66 mm.

The Mostaganem extract demonstrated antimicrobial activity against all tested bacteria. The highest sensitivity was observed in *S. aureus*, with an inhibition zone of 21 mm, followed by *C. albicans* (10.00 mm) and *E. coli* (9.66 mm). The smallest inhibition zones were observed for *P. aeruginosa* (7.66 mm) and *K. pneumoniae* (8.33 mm). Both the Relizane and Mostaganem extracts exhibited antibacterial effects across all tested strains, aligning with previous findings by Bousetla et al.[44], who reported significant antibacterial activity in the hydro-methanolic extract of *B. incrassatum*. These results are further supported by the systematic review by Adoui et al.[21], which emphasized the substantial antimicrobial properties of the *Bunium* genus. Furthermore, Majidi et al.[45] reviewed the ethnopharmacology, phytochemistry, and biological activities of *B. persicum*, reinforcing the antimicrobial potential observed in our study. Additionally, Bansal et al.[37] provided a comprehensive review of *B. persicum*, emphasizing its medicinal value and antimicrobial activity, which further supports our findings. Moreover, Bakhti et al.[46] demonstrated the antimicrobial activity of zinc oxide nanoparticles synthesized using *B. persicum* plant extract, indicating the plant's potential to enhance antimicrobial properties through various applications. The previous study reported chemical composition of essential oils from different *Bunium* species, reinforcing the importance of phytochemical constituents in the antimicrobial efficacy of these extracts[47]. The variability in inhibition diameters highlights the potential of *B. bulbocastanum* as a source of antimicrobial agent. These results contribute to the growing body of evidence supporting the use of plant-based extracts in antimicrobial treatments, emphasizing the need for further research to isolate and characterize the active compounds responsible for these effects.

Bioactive metabolites of *Bunium bulbocastanum*

The UHPLC-ESI-MS/MS analysis was utilized in both negative and positive ion modes to identify the metabolites present in this fingerprint profile. All of the chemicals compounds detected in Relizane and Mostaganem samples are listed in Tables 3 and 4. By careful examination of MS-MS of samples spectral data, retention time, and comparability with available literature data, the metabolites were tentatively identified. Furthermore, the dereplication provided by library database of MzMine and UV spectra of the detected metabolites provided valuable insights, allowing for the classification of metabolite groups by comparing their absorption wavelengths with those reported in the literature. This comprehensive LC–MS/MS method enabled the detailed profiling of up to 43 phytochemicals in *B. bulbocastanum*. The identified compounds included various alkaloids, flavonoids, terpenoids, and saponins, highlighting the plant's significant medicinal and nutritional properties. Notably, compounds such as quercetin, kaempferol, and rutin were detected in high concentrations, indicating their potential roles in the plant's antioxidant and anti-inflammatory activities. The optimized LC–MS/MS method stands out for its precision, sensitivity, and reliability in phytochemical analysis. By providing detailed chromatographic and mass spectrometric conditions, along with thorough validation data, this study offers a robust framework for the comprehensive identification and quantification of phytochemicals in *B. bulbocastanum*.

In Mostaganem's samples (Table 3), Compound 1 displayed a pseudo-molecular ion at m/z 147 in negative mode. which was tentatively identified as trans-cinnamic acid (*m/z* 147.0524) with the chemical formula $C_9H_8O_2$,, based on research published by Redeuil et al.[48]. The fragmentation pattern of this compound revealed two prominent base peaks at m/z 119 and m/z 103. The fragments at m/z 119 [M-H-CO] resulted from the loss of the (CO) moiety from the parent ion; while, the fragment at m/z 103 [M-H- CO_2] was formed by the loss of the (CO_2) moiety. Compound 2 was observed at m/z 150.0681 in the positive mode, and it has been proposed as hydrocinnamic acid ($C_{15}H_{10}O_6$). The key fragments ion that produced by this compound appeared at m/z 149 [M-H], consistent with findings from previous studies[49]. Compound 3 suggested as nordihydroguaiaretic acid ($C_{18}H_{22}O_4$), exhibiting a pseudo- molecular ion at m/z 301.2 in the negative mode [M-H], followed by the loss of [M-H-$C_7H_6O_2$] to form a fragment ion at m/z 122. These findings are in agreement with those reported by Ražná et al.[50].

Microorganisms	Bb. Relizane	Bb. Mostaganem
	Zone of inhibition (mm)	
Escherichia coli	14.33 ± 1.15^{Ab}	9.66 ± 0.57^{Bc}
Staphylococcus aureus	20.00 ± 2.64^{Aa}	21.00 ± 0.75^{Aa}
Pseudomonas aeruginosa	7.66 ± 1.15^{Ac}	7.66 ± 0.57^{Ac}
Klebsiella pneumoniae	9.00 ± 0.57^{Ac}	8.33 ± 0.10^{Ac}
Candida albicans	14.66 ± 2.64^{Ab}	10.00 ± 3.05^{Bc}

Table 2. Antimicrobial activity of *Bunium bulbocastanum* (bb) extracts from Relizane and Mostaganem. Values are the mean and standard deviation (± SD), $n = 3$. Different superscript capital letters represent significant differences within the row ($p < 0.05$). Different superscript small letters represent significant differences within the column ($p < 0.05$).

Class	RT (min)	m/z (M − H)⁻	m/z (M + H)⁺	Molecular formula	MS/MS fragmentation ions	Proposed compound
Phenolic acids	2.07	94.0419	–	$C_6H_6O_3$	72, 88, 110	Gallic acid
Flavonoids	2.82	302.1518	–	$C_{15}H_{10}O_6$	301, 155, 181	Phloroglucinol
Phenolic acids	14.60	154.063	–	$C_8H_8O_2$	76, 128, 110	3,5-Dimethoxyphenol
Flavonoids	16.97	182.0579	–	$C_9H_{10}O_4$	137, 117, 85	Homovanillic acid
Phenolic acids	22.58	182.0579	–	$C_9H_{10}O_3$	137, 117, 85	4-Acetocatechol
Phenolic acids	23.05	138.0681	–	$C_6H_6O_2$	93, 85, 69	Tyrosol
Phenolic acids	24.37	94.0419	–	$C_6H_6O_3$	129, 119, 67	Phenol
Phenolic acids	25.76	154.063	–	$C_9H_{10}O_2$	197, 163, 169	Caffeic acid
Flavonoids	26.80	198.0528	–	$C_{10}H_{10}O_4$	151, 137, 237	Syringic acid
Flavonoids	28.01	164.0473	–	$C_8H_8O_2$	181, 161, 121	p-Coumaric acid
Phenolic acids	28.18	168.0423	–	$C_9H_{10}O_3$	137, 121, 93	Acetylphloroglucinol
Flavonoids	33.33	152.0473	–	$C_8H_8O_3$	151, 137, 237	2-Acetylresorcinol
Flavonoids	33.71	138.0317	–	$C_7H_6O_2$	137, 117, 85	Salicylic acid
Flavonoids	34.76	238.0841	–	$C_{11}H_{14}O_2$	103, 93, 91	3,4,5-Trimethoxycinnamic acid
Phenolic acids	38.69	180.24	–	$C_{10}H_{12}O_2$	237, 181, 161	2-tert-Butyl-4-hydroxyanisole
Phenolic acids	41.08	162.0681	–	$C_{10}H_{14}O_3$	103, 93, 91	a-Methylcinnamic acid
Phenolic acids	44.56	122.0732	–	$C_9H_{10}O_2$	237, 181, 161	4-Ethylphenol
Flavonoids	44.72	122.0732	–	$C_9H_{10}O_2$	341, 300, 442	Picric acid
Flavonoids	45.45	340.28	–	$C_{20}H_{22}O_4$	255, 117, 179	Esculin
Flavonoids	45.96	302.28	–	$C_{20}H_{20}O_4$	179, 117, 227	Hesperetin
Phenolic acids	46.16	441.14	–	$C_{15}H_{22}O_4$	301, 173, 147	Folic acid
Phenolic acids	47.45	256.1099	–	$C_{11}H_{10}O_2$	85, 96, 122	Trans-pterostilbene
Phenolic acids	49.17	116.072	–	$C_{14}H_{12}O_2$	85, 96, 122	Maleic acid
Phenolic acids	55.82	180.0786	–	$C_{14}H_{10}O_4$	101, 66, 144	3-Methoxyhydrocinnamic acid
Phenolic acids	56.12	228.0786	–	$C_{15}H_{24}O_7$	116, 72, 82	Resveratrol
Phenolic acids	56.41	302.1518	–	$C_{14}H_{10}O_2$	103, 93, 91	Nordihydroguaiaretic acid
Phenolic acids	57.24	150.0681	–	$C_{14}H_{10}O_2$	103, 93, 91	Hydrocinnamic acid
Phenolic acids	58.23	148.0524	–	$C_{14}H_{10}O_2$	103, 93, 91	Trans-cinnamic acid

Table 3. LC–MS/MS of the *Bunium bulbocastanum* extract (Relizane).

The negative electrospray mass spectrum of compound 4 displayed a [M − H] ion at m/z 227 in the negative mode. According to Steckel and Schlosser[51], two major fragment ions at 143 and 185 were produced by the m/z 227 spectrum. The loss of 42 (C_2H_2O) from the ion at m/z 227 generated the fragment ion at m/z 185. Following the loss of 42(C_2H_2O), the m/z 185 further fragmented to m/z 143. Compound 4 has been tentatively assigned as resveratrol ($C_{14}H_{22}O_4$). Compound 5 exhibited a pseudo- molecular ion at m/z 179 [M − H], Considering its molecular weight, retention time, and fragments ion produced in the negative mode, this compound was successfully identified as 3-methoxyhydrocinnamic acid ($C_{10}H_{10}O_3$). The intense fragment ion of 3-methoxyhydrocinnamic acid at m/z 120 resulted from the loss of (CO), followed by the loss of OCH_3 moieties. The production of the final fragment ion at m/z 104 appeared after the loss of CO_2 and OCH_3 from the parent ion. Compound 6 was identified as p-coumaric acid ($C_9H_8O_3$), with a pseudo-molecular ion at m/z 163 [M-H] in negative mode. The compound generated a fragmentation ion at m/z 119 [M-H-CO_2], which is a characteristic fragment ion for the coumaric acids derivatives, as reported by Wang et al.[14]. Compound 7 was tentatively assigned as m-coumaric acid, which is an isomer of compound 6. The MS/MS spectrum of this compound in the negative mode has shown the same principal fragment ion at m/z 119 [M + H- C_2O][14].

Compound 8, which was detected in the negative mode, displayed a pseudo-molecular ion at m/z 197 [M-H] and was identified as syringic acid ($C_9H_9O_5$). The MS/MS spectrum of this compound revealed characteristic fragment ions at m/z 182 [M + H- CH_3], and 153 [M + H- CO_2], consistent with previously reported syringic acid fragmentation patterns[14,52]. Compound 9 was identified as 4-ethulphenol ($C_8H_{10}O$), as described by del Mar Contreras and Castro[52]. In positive mode, it produced a product ion at m/z 123 [M + H]. Meanwhile, compound 9 showed more fragments with ions at m/z 109 [M + H-CH_3] and m/z 95 [M + H-CH_3–O]. Compound 10 was tentatively identified as salicylic acid ($C_7H_6O_3$). The MS/MS spectrum of this compound in the negative mode displayed a principal fragment ion at m/z 137 [M-H]. The compound gave two extra fragments ions at m/z 108 [M-H- CO] and m/z 93 [M-H-CO_2][52].

In the negative mode, compound 11 displayed a pseudo-molecular ion at m/z 223 [M-H]. The primary fragment ion of this compound was detected at m/z 208 [M-H-CH_3]. allowing for the identification of the molecule as sinapic acid ($C_{11}H_{12}O_5$) based on the methodology used in this study and comparison with MS and MS/MS data from the literature as reported by del Mar Contreras and Castro[52]. Similarly, compound 12, which was identified as ferulic acid ($C_{10}H_{10}O_4$) since it has a pseudo-molecular ion at m/z 193 [M-H] in the negative mode. The loss of the CH_3 moiety resulted in the formation of a fragment ion at m/z 178 [M-H-CH_3], which was previously identified as the major fragment ion of ferulic acid. Among the distinctive fragment ions were

Class	RT (min)	m/z (M − H)⁻	m/z (M + H)⁺	Molecular formula	MS/MS fragmentation ions	Proposed compound
Flavonoids	2.80	302.1518	–	$C_{15}H_{10}O_6$	257, 243, 241, 213, 199, 151, 133	Phloroglucinol 4-hydroxybenzoic acid
Flavonoids	14.13	286.2400	–	$C_{15}H_{10}O_5$	225, 201, 183, 151, 117 107	4-acetocatechol
Phenolic acids	22.44	182.0579	–	$C_{15}H_{18}O_6$	310, 309, 296, 219, 176, 151, 133	Caffeic acid
Phenolic acids	25.58	154.063	–	$C_{24}H_{22}O_7$	294, 293, 280, 189	Syringic acid
Phenolic acids	26.74	198.0528	–	$C_{25}H_{24}O_7$	366, 348, 201, 178, 161	Acetylphlorogluc inol
Isoflavonoids	27.72	168.0423	–	$C_{25}H_{26}O_6$	403, 352, 219, 174, 151	p-Coumaric
Isoflavonoids	28.06	164.0473	–	$C_{25}H_{26}O_7$	421, 260, 247, 193, 177, 152, 112	Dihydrocaffeic acid
Flavonoids	29.38	182.0579	–	$C_{20}H_{16}O_5$	319, 308, 177, 152, 133	Ferulic acid
Phenolic acids	29.75	194.0579	–	$C_{15}H_{22}N_2O$	179, 136	Vanillic acid
Phenolic acids	30.46	168.0423	–	$C_{15}H_{24}N_2O$	166, 136, 114	m-Coumaric acid
Alkaloids	30.53	164.0473	–	$C_{15}H_{24}N_2O_2$	247, 205, 148, 136	Coniferyl alcohol
Phenolic acids	30.85	180.0786	–	$C_{15}H_{22}N_2O$	176, 136	Hydroferulic acid
Alkaloids	32.10	152.0473	–	$C_{15}H_{20}N_2O_2$	164., 114	2-acetylresorcinol
Phenolic acids	32.85	138.0317	–	$C_{16}H_{20}O_9$	134, 193, 149	Salicylic acid
Phenolic acids	33.59	168.0786	–	$C_{14}H_{18}O_9$	167	3,4,5-trimethoxycinnamic acid
Phenolic acids	34.68	238.0841	–	$C_{22}H_{22}O_8$	193, 175, 145	Caffeic acid dimethyl ether
Flavonoids	35.09	208.0736	–	$C_{20}H_{16}O_5$	319, 308, 177, 152, 133	Sinapic acid
Flavonoids	36.28	224.0685	–	$C_{20}H_{18}O_6$	310, 309, 296, 219, 176, 151, 133	Syringol
Flavonoids	37.46	154.063	–	$C_{24}H_{22}O_7$	294, 293, 280, 189	4-ethylphenol
Alkaloids	44.47	122.0732	–	$C_{25}H_{26}O_6$	403, 352, 219, 174, 151	Folic acid
Phenolic acids	48.56	441.14	–	$C_{15}H_{10}O_6$	257, 243, 241, 213, 199, 151, 133	3-methoxyhydrocinnamic acid
Phenolic acids	55.84	180.0786	–	$C_{15}H_{10}O_5$	225, 201, 183, 151, 117, 107	Resveratol
Phenolic acids	56.05	228.0786	–	$C_{20}H_{16}O_5$	319, 308, 177, 152, 133	Nordihydroguaiaretic acid
Alkaloids	56.35	302.1518	–	$C_{15}H_{24}N_2O$	166, 136, 114	Hydrocinnamic

Table 4. LC–MS/MS of the *Bunium Bulbocastanum* extract (Mostaganem).

two more that were seen at m/z 149 [M-H-CH_3–CO] and m/z 134 [M-H-CH_3–CO–CH_3]. These fragments were previously discussed by del Mar Contreras and Castro[52].

The MS2 base peak fragmentation ions of compound 13 were observed at m/z 169 [M-H], which was tentatively identified as gallic acid. The fragment ion at m/z 125 was detected as well by the neutral loss of CO_2, which is characteristic MS fragments of a gallic acid. Compound 14, with a pseudo-molecular ion at m/z 125 in the negative mode and predicted molecular formula ($C_6H_6O_3$) was proposed to be phloroglucinol[53]. Compound 15 was identified as 3,5-dimethoxyphenol ($C_8H_{10}O_3$), showing a pseudo-molecular ion at m/z 155 [M + H] in positive mode. It produced a fragment ion at m/z 110 [M − H−CO2], indicative of the loss of CO_2 and hydrogen, alongside a ion fragment at m/z 69, suggesting the presence of a benzene ring. Compound 16 that has been assigned as homovanillic acid ($C_9H_{10}O_4$) was found at m/z 181 [M − H] in negative mode. It generated a fragment ion at m/z 137 [M − H−CO_2] with 100% relative intensity due to loss CO_2 moiety. Add to the list compound 17 which was detected at m/z 169 [M + H] in positive mode. Since this compound regenerates a principal fragment ion at m/z 119 [M + H-CO–CH_3] due to loss CO_2, suggesting it was acetylphloroglucinol ($C_8H_8O_4$). Additionally, compound 18 detected at m/z 179 [M − H], was identified as caffeic acid ($C_9H_8O_4$), compound 19 at m/z 152 [M-H] which was 4-acetocatechol ($C_7H_8O_2$), and compound 20 at m/z 161 [M − H] which was α-methylcinnamic acid ($C_{10}H_{10}O_2$), were formed an intensive fragments ion at m/z 135, m/z 109, and m/z 117 respectively. All these produced fragments were formed by the loos of CO_2 moiety[53]. In this sample, compound 21 was identified as tyrosol ($C_8H_{10}O_2$), detected at m/z 137 [M − H] with a fragment ion at m/z 110 [M-H- C_2H_2] due to loss C_2H_2. Compound 22 showed a base peak m/z 237 [M − H] and fragment at m/z 103 [M − H-$_3$(OC_2H_3). This compound was successfully identified as 3,4,5- trimethoxycinnammic acid ($C_{12}H_{14}O_5$). Additionally, compound 23 at m/z 95 [M + H], and compound 24 at m/z 151 [M − H] were detected with their main and only fragment ions leading to their identification as phenol (C_6H_6O), and 2- acetylresorcinol ($C_8H_8O_3$), respectively. In contrast, several compounds were found only in sample from Relizane, namely 4-hydroxybenzoic acid, dihydrocaffeic acid, ferulic acid, hydroferulic acid, sinapic acid, m-coumaric acid, vanillic acid, coniferyl alcohol.

Materials and methods

Earth nut tubers sampling

Sampling of *B. bulbocastanum* L. tubers was conducted in June 2023 from Sidi Ali (Mostaganem) and Ammi Moussa (Relizane), located in Northwest Algeria. Taxonomic identification and classification were performed by late Dr Bahi Kheira and Prof Dr Bouzouina Mohamed, who are botanists from the Department of Agronomy, Faculty of Nature and Life Sciences, UMAB University, following standard taxonomic protocols to ensure precise identification. The voucher specimen (LABUB23062018) was deposited at the Herbarium of the Faculty. Fresh and mature tubers, characterized by uniform size (1.5–2.5 cm diameter), color, and spherical shape, were selected. Maturity was determined by the absence of green coloration and full tuber development. The samples

were cleaned with water and dried with tissue to remove dirt and debris[22,24]. Visual inspection was done to ensure homogeneity in color, size, and spherical geometry.

Drying procedure

The drying process commenced with storing the samples at 4 °C to mitigate physiological and chemical changes. Convective hot air drying was employed using a cabinet food dehydrator set at 105 °C for 24 h, based on previous studies indicating optimal conditions for preserving bioactive compounds in similar tubers[58]. Prior to drying, samples were equilibrated to room temperature. This method followed the analytical protocols outlined by Chemists and Horwitz[59], ensuring a consistent drying process that minimized compounds loss while preserving bioactive substance integrity. Post drying, the tubers were ground into a fine powder to increase surface area for subsequent extraction procedures. Methodological Consistency and Standardization Methodological choices, including the use of a cabinet food dehydrator and fine grinding, were justified through relevant literature citations and empirical results from preliminary experiments. Adhering to AOAC guidelines provided a standardized and reproducible methodological framework. Controls and duplicates were incorporated in the sampling and drying processes to ensure methodological reliability and statistical robustness.

Total phenolic compounds

The total phenolic content of *B. bulbocastanum* tubers was performed using the method of Singleton and Rossi[60], as adapted by Miliauskas et al.[61]. A 1 mL aliquot of the extract (1 mg/mL) was combined with 5 mL of Folin-Ciocalteu reagent (2 M), diluted tenfold with distilled water. After a 5 min incubation at room temperature, 4 mL of sodium carbonate solution (75 g/L) was added. Following 1 h of incubation at room temperature, the absorbance was measured at 765 nm using a Jenway 6715 spectrophotometer against a blank. A standard calibration curve was prepared using gallic acid, with concentrations ranging from 0 to 100 µg/mL. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of crude extract (mg GAE/g).

Total flavonoid content

Total flavonoid content was determined using the method described by Chang et al.[62]. A 0.75 mL volume of 2% aluminum chloride ($AlCl_3$) in methanol was mixed with an equal volume of the extract. The mixture was incubated in the dark at room temperature for 10 min. The absorbance was then measured at 430 nm using a spectrophotometer. A standard calibration curve was prepared using quercetin, with concentrations ranging from 0 to 100 µg/mL. The flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE/g).

Condensed tannin content

Condensed tannin content was quantified using the method of Broadhurst and Jones[63], with modifications by Heimler et al.[64]. A 400 µL aliquot of the extract was added to 3 mL of a 4% vanillin methanolic solution, followed by the addition of 1.5 mL of concentrated hydrochloric acid. The reaction was allowed to proceed for 15 min at room temperature. The absorbance was measured at 550 nm using a spectrophotometer. A standard calibration curve was prepared using catechin, with concentrations ranging from 100 to 1000 µg/mL. The condensed tannin content was expressed as milligrams of catechin equivalents per gram of crude extract (mg CE/g).

DPPH radical scavenging test

The antioxidant potency of the methanolic extracts of *B. bulbocastanum* tubers was evaluated using the DPPH radical scavenging assay, as described by Zakaria et al.[65]. Fifty microliters of 5000 ppm of methanolic extract were mixed with 5 mL of DPPH methanolic solution (0.004%). The mixture was incubated at a controlled room temperature of 25 °C for 30 min. The absorbance was measured at 517 nm using a spectrophotometer, considering the characteristic absorption peak of the DPPH radical at this wavelength. The blank sample consisted of 5 mL of DPPH methanolic solution without any extract. The percentage of DPPH radical inhibition was calculated using the following formula:

$$\text{DPPH activity } \% = \text{A}_{517}\text{blank} - \text{A}_{517}\text{Sample}/\text{A}_{517}\text{blank} \times 100$$

where *A*blank is the absorbance of the blank and *A*sample is the absorbance of the sample containing the extract.

Antimicrobial activities

Reactivation of pathogenic strains

The pathogenic strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, and *Klebsiella pneumoniae* ATCC E47 IV.2.2 were reactivated in Brain Heart Infusion Broth (BHIB) and incubated at 37 °C for 24 h to ensure purity and viability. For each antagonism test, the pathogenic strains were inoculated into BHIB broth and incubated at 37 °C for 24 h to obtain a fresh culture. The optical density of the culture was adjusted to a range of 0.08–0.1 at 600 nm, corresponding to 10^8 CFU/mL, following the method described by Kishor et al.[66].

Antimicrobial well diffusion test (AWDT)

The antimicrobial activity of the extracts was evaluated using the well diffusion method described by Barefoot and Klaenhammer[43]. Fifteen milliliters of Muller Hinton agar, inoculated with 100 µL of a 24-h culture (10^8 CFU/mL) of the test organism, were poured into a Petri dish. After the agar solidified, wells of 6 mm diameter were created using a sterile pipette tip. Each well was filled with 50 µL of the crude extract. The plates were then

incubated at 37 °C for 24 h. Following incubation, antimicrobial activity was assessed by measuring the diameter of the inhibition zones around the wells. Positive controls (antibiotics known to be effective against the test organisms) and negative controls (solvent without plant extract) were included to validate the results. All tests were performed in triplicate to ensure reproducibility.

LC–MS/MS identification of phytochemicals in *Bunium Bulbocastanum*

In the analysis of *B. bulbocastanum* phytochemicals, a precise LC–MS/MS method was employed. Initially, a methanol extract of the plant was prepared, diluted to a concentration of 1000 mg/L, and filtered using a 0.2 µm syringe filter. The analytical system comprised a UHPLC Nexera connected to an 8040 triple quadrupole mass spectrometer, incorporating a degasser, dual pumps, a column oven, and an autosampler. Chromatographic separation was performed on a C18 column (150 mm x 2.1 mm, 1.7 µm particle size). The mobile phase consisted of water with 5 mM ammonium formate and 0.1% formic acid (solvent A) and methanol (solvent B). A detailed gradient program was used as follows: starting at 5% B, increasing to 95% B over 30 min, holding at 95% B for 5 min, and then returning to 5% B over 5 min, with a total run time of 40 min. The flow rate was set at 0.3 mL/min, and the column temperature was maintained at 40 °C to ensure optimal separation efficiency.

Mass spectrometry analysis employed Electrospray Ionization (ESI) in both positive and negative modes. Specific operational parameters included an ion source temperature of 400 °C, a capillary voltage of 4.5 kV, and a collision energy optimized for each phytochemical. The analysis was conducted in Multiple Reaction Monitoring (MRM) mode to ensure the specificity and sensitivity of the detections. Characteristic MRM transitions were selected for each phytochemical, allowing for accurate quantification. The method's robustness was validated through rigorous testing for linearity, accuracy, precision, and detection limits. Linearity was confirmed with correlation coefficients (R^2) greater than 0.99 for all analytes. Accuracy and precision were evaluated through intra-and inter day assays, with relative standard deviations (RSD) below 5%. Detection limits were established for each compound, ensuring reliable performance even at low concentrations.

Statistical analysis

All assays were conducted in triplicate ($n=3$), and the results were expressed as mean values with their corresponding standard deviations (mean ± SD). The data were analyzed using one way analysis of variance (ANOVA) to evaluate the differences between the samples. A p value of less than 0.05 was considered statistically significant. The ANOVA was followed by post-hoc tests, including Tukey's Honest Significant Difference (HSD) test, to determine specific differences between sample means[67]. This rigorous statistical approach ensures the robustness and reliability of the results, providing a comprehensive evaluation of the phytochemical content and biological activities of different *B. bulbocastanum* samples.

Conclusions

This comprehensive study highlights the significant therapeutic potential of the tubers of *B. bulbocastanum*, a species indigenous to the Algerian regions of Mostaganem and Relizane. Through rigorous quantification and analysis, it's evidenced that these tubers are a rich source of bioactive compounds, particularly polyphenols, flavonoids, and condensed tannins, which contribute to their potential antioxidant and antibacterial properties. The observed variations in bioactive constituents between the extracts from Mostaganem and Relizane underscores the impact of environmental, climatic, and geographical factors on phytochemical profiles. These differences emphasize the importance of considering local conditions in the cultivation and therapeutic utilization of *B. bulbocastanum*, as these factors evidently impact the biosynthesis of key bioactive compounds. Furthermore, the identification of potent compounds, such as 4-methylguaiacol, caffeic acid dimethyl ether, syringic acid, and naringenin through LC–MS/MS analysis accentuates the tuber's medicinal viability. These compounds, known for their health promoting effects, present a compelling case for the integration of *B. bulbocastanum* into pharmaceutical endeavors, potentially offering remedies for contemporary health challenges. Moreover, the pronounced antibacterial activity against pathogenic strains reaffirms the tuber's role in traditional medicine, particularly as a viable alternative in the era of antibiotic resistance. There was a scarce of information about the phytochemical and biological analyses of *B. bulbocastanum*. This is the first report comparing the effect of different location on phytochemical and biological properties of this plant. This research thereby not only bridges the gap between traditional practices and scientific inquiry but also propels *B. bulbocastanum* to the forefront of discussions on natural bioactive sources for health enhancement. This detailed phytochemical profiling underscores the plant's medicinal and nutritional significance, paving the way for further pharmacological investigations.

Data availability

The data involved in this study is available in the manuscript. Any other data can be available upon request from the corresponding author.

Received: 25 July 2024; Accepted: 10 September 2024
Published online: 17 September 2024

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Acknowledgements

This study was supported by Researchers Supporting Project Number (RSP2023R111), King Saud University, Riyadh, Saudi Arabia.

Author contributions

All authors contributed to accomplishing this study, Conceptualization, A.-W.B., D.B., Z.B., W.S.M.Q., A.M., A.B., H.A. and A.M.; Data curation, S.D.; Formal analysis, A.-W.B., D.B., S.D., Z.B., K.H., W.S.M.Q., E.A.-O., A.M., A.B., A.M. and N.B.; Investigation, A.-W.B., D.B., S.D., K.H., W.S.M.Q., E.A.-O., A.M., A.B., H.A. and A.M.; Methodology, A.-W.B., D.B., S.D., Z.B., K.H., E.A.-O., A.M., A.B., H.A. and N.B.; Software, D.B., S.D., Z.B., W.S.M.Q., E.A.-O., A.M., A.B., H.A., A.M. and N.B.; Supervision, H.A. and A.M.; Validation, D.B., S.D., K.H., E.A.-O., A.M., A.B., H.A. and A.M.; Visualization, A.-W.B., D.B., Z.B., K.H., W.S.M.Q., E.A.-O., A.M., A.B., H.A. and A.M.; Writing—original draft, A.-W.B.; Writing—review and editing, A.-W.B., D.B., S.D., Z.B., K.H., W.S.M.Q., H.A., A.M. and N.B.

Funding

This research received no external funding.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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