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Original Research Article

Phenolic Profile, Antioxidant Activity, and Litholytic Cholesterol Properties of Algerian *Bunium incrassatum* L. Extracts

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ABSTRACT

Bunium incrassatum (Apiaceae), widely cultivated along Africa's Mediterranean frontier. It has antioxidant, anti-inflammatory, anti-bronchitis, antibacterial, and anti-cancer impacts. This investigation estimates the chemical composition of methanolic extracts from the roots (MERO), seeds (MESE), and stems (MEST) of *B. incrassatum* using high-performance liquid chromatography/ultraviolet (HPLC/UV), antioxidant and litholytic effects *in vitro*. The Fourier Transform Attenuated Total Reflectance (FTIR-ATR) identified cholesterol gallstones. These gallstones were classified into low, medium, and high weight groups and subjected to agitation with *B. incrassatum* extracts for 9 weeks. The control was sodium chloride (NaCl, 9 g/L). Weekly, gallstones were weighed after 18 h of drying at 40 °C, and their gallstones-dissolving capacity percentage; (% GDC) and the experimental medium pH were assessed. HPLC/UV analysis shows MESE has 25 phenolic compounds, MERO 24, and MEST 21. Furthermore, MESE exhibited the highest phenolic levels (70.80 mg GAE/g DE, 18.44 mg QE/g DE) and the highest antiradicalaire activity among the extracts tested, with a median inhibitory concentration (IC_{50}) of 0.23 mg/mL for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and 0.66 mg/mL for ferric-reducing antioxidant power (FRAP). Highly substantial ($P < 0.001$) rise in % GDC was noted, with decreasing effectiveness in the following order: MERO, MESE, and MEST compared to the control throughout the trial, associated with phenolic composition, gallstone weight, and research duration. This study showed the importance of the phytochemical, antioxidant, and litholytic characteristics of *Bunium incrassatum* extract, affirming its possibilities in pharmacological approaches to treating cholelithiasis.

Keywords: *Bunium incrassatum*, HPLC/UV, Antioxidant activity, Cholesterol gallstones, Gallstones-dissolving capacity percentage, Cholelithiasis.

Introduction

Traditional medicine has used an array of plants with therapeutic potential for managing a wide variety of diseases. These plants are known for their intriguing bioactive profiles, which include anti-oxidative, anti-inflammatory, anti-diabetic, anti-cancer, and more.[1,2,3] Preclinical and clinical trials have shown that scientists are developing an interest in herbal remedies treatments as prospective sources of novel pharmaceuticals that can improve human health with minimal or no adverse impacts, unlike the current synthetic chemical compounds.[4,5,6]*Bunium incrassatum* L., also known commonly as Talghouda, is extensively distributed in northern Algeria. This medicinal plant possesses a powerful antioxidant capabilities and has long been used to treat thyroid problems, diarrhoea, inflammatory haemorrhoids, bronchitis, and other complications.[7]

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Cholelithiasis is one of the world's most frequent and complicated diseases, affecting around 10-20% of the population in Europe, and is increasingly common among children and adolescents.[8] It is the result of defects in hepatic and biliary cholesterol metabolism, gallbladder stasis, and abnormal nucleation. Phospholipids, cholesterol, and supersaturated bile acids, which cause cholesterol deposition, contributed to this condition. Gallstones are classified based on their cholesterol concentration: cholesterol (≥70%), pigment (≤30%),[9,10] or mixed (30-70%).[11] Genetic predisposition,[12] female sex, advanced age, hypercholesterolaemia, alcohol consumption, type 2 diabetes, a high-fat diet, obesity, metabolic syndrome, and a sedentary lifestyle all contribute to cholelithiasis development,[13] condition that frequently necessitates immediate surgical intervention.[14] Medicinal plants offer an effective natural option for the prevention and treatment of cholelithiasis.[15] This paper aims to identify the phytochemical compounds by HPLC/UV, to screen the antioxidant's effectiveness, and then to evaluate the litholytic impact of *B. incrassatum* extracts.

Materials and Methods

Chemicals and reagents

Sigma-Aldrich (St. Louis, MO, USA) supplied all chemicals used, including absolute methanol (≥99.7%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), formic acid (CH_2O_2, 98–100%), trifluoroacetic acid (TFA, $C_2HF_3O_2$, 99%), acetonitrile (C_2H_3N), authentic phenolic standards (trans-cinnamic, gallic, syringic, sinapic, quinic, protocatechic, ferrulic,

p-coumaric, caffeic, carnosic, chlorogenic, vanillic, and ellagic acids, catechin, epicatechin, catechol, carnosol, thymol, galangin, pinocembrin, rutin, chrysin, luteolin, fisetin, myricetin, naringin, acacetin, kaempferol, quercetin, tannic acid, ascorbic acid, menthol, and bis- methylated quercetin), folin-ciocalteu's reagent (FCR), aluminum chloride ($AlCl_3$), iron (III) chloride ($FeCl_3$), sodium bicarbonate (Na_2CO_3), potassium ferricyanide (III) ($K_3Fe(CN)_6$), trichloroacetic acid (TCA, $C_2HCl_3O_2$), and sodium chloride (NaCl). The analytical grade of all chemicals and reagents employed in the current study.

Gallstones
The General Surgical Service of Hospital Bourdj Bounaama (Tissemsilt, Algeria) willingly removed massive gallstones from the lithiasis patient approved by our local ethics committee authorised the experimental gallstones use (Institutional Review Board) of food department (Abdelhamid Ibn Badis University, Mostaganem, Algeria). The FTIR-ATR (IR Affinity-1S, Shimadzu, Japan) identified them chemically.

Plant materials and identification
Bunium incrassatum (*Bunium pachypodum*) was collected in June 2021 from Labiod Medjadja, Chlef (Algeria) (36°15'0"N, 1°24'0"E, an altitude of 176 m). The plant received a voucher specimen (H.S. 01998.1) from the Ecology Vegetal Laboratory, Department of Biology, University of Ahmed Ben Bella (Oran 1, Algeria). After cleaning with distilled water, air-drying, and finely powdering, root, stem, and seed were sealed in bottles until extraction.

Bunium incrassatum extraction
After separately macerating 10 g of *B. incrassatum* root, stem, and seed powder in 100 mL of methanol (MeOH, 99.7%) at room temperature for 72 h. Methanol was removed using rotary evaporator (BOECO, type RVO 400 SD, Germany) at 40 °C to obtain the roots (MERO), seeds (MESE), and stems (MEST) extracts of *B. incrassatum*. It was preserved at +4 °C up until used.[16] Formula (1) computed extract yield percentage (% Y).[17]

$$\% Y = \frac{M0}{M1} \times 100 \qquad (1)$$

Where:
M1: mass in grams of the dry extract obtained.
M0: mass in grams of initial dry plant material.

Analysis of B. incrassatum extracts by HPLC/UV
Methanolic *B. incrassatum* extracts (MESE, EMST, and MERO) were analysed using an Agilent 1100 high-performance liquid chromatography system (LC-2040C, Shimadzu, Japan). The separation process used an Agilent 120 EC poroshell column (100 mm × 2.1 mm, 2.7 µm) with water/TFA/formic acid (99:0.25:0.75) (A) and acetonitrile (B). The elution was performed at 55 °C at 0.6 mL/min with a 10 µL aliquot. The solvent gradient was (0, 0), (1, 10), (2, 12.5), (3, 15), (9, 80), (10, 100), (11, 100), and (14, 0) with post-5 min. UV detection at 270 and 320 nm.[18] Methanol was diluted 1:100 (volume for volume, V/V) with each extract to make the sample.
Using retention times (Rt) and spectral matching with 33 phenolic standards (trans-cinnamic, gallic, syringic, sinapic, quinic, protocatechic, ferrulic, *p*-coumaric, caffeic, carnosic, chlorogenic, vanillic, and ellagic acids), flavonoids (catechin, epicatechin, catechol, carnosol, thymol, galangin, pinocembrin, rutin, chrysin, luteolin, fisetin, myricetin, naringin, acacetin, kaempferol, and quercetin), tannins (tannic acid), and other compounds (ascorbic acid, menthol, bis-methylated quercetin). Stock solutions at 1 mg/mL were made from standards dissolved in methanol. Phenol components were revealed through calibrated graphs of multiple references in mg/1g of crude extract.

Total phenol content (TPC) estimation
The total of phenolic amount was quantified through Folin-Ciocalteu's method.[19] A mix consisting of 200 µL extract or standard and 1500 µL of 10% Folin-Ciocalteu's reagent (FCR) was incubated in darkness for 5 min, and 1500 µL of a 60% sodium bicarbonate solution (Na_2CO_3) was added. A UV-V spectrophotometer (Optizen Pop, Korea) measured absorbance at 725 nm after 90 min of reaction time against a blank. Gallic acid was used to calibrate the curve. The total phenol content was reported as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DE). Triplicate tests were performed.

Total flavonoid content (TFC) estimation
The aluminum chloride technique.[20] was applied for determining the total of flavonoid level. Briefly, a combined volume of 1000 µL of 2% aluminum chloride ($AlCl_3$) solution and 1000 µL of extract or standard was used. The colorimetric measurements of the absorbance at 517 nm were performed as opposed to a blank, after 60 min of incubation at ambient temperature in darkness. As a reference, quercetin was employed. The data were given as milligrams of quercetin equivalent per gram of dry extract (mg QE/g DE). The measurements were conducted three separate times.

Antioxidant activity in vitro

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay
The DPPH radical-scavenging effect of the *B. incrassatum* extracts (MERO, MESE, and MEST) was determined using the previously reported method.[21] The combination included 250 µL of every extract at different amounts and 1000 µL of 0.004% DPPH. The absorbance was obtained at 517 nm after 30 min of exposure to ambient temperature and darkness. The references employed for the experiment were trolox and ascorbic acid, which were tested under similar experimental circumstances. The free radical-scavenging activity (% RSA) was estimated throught the formula (2).[22,23] Thereafter, the inhibitory concentration median (IC_{50}) was estimated through a linear regression method. Every measurement was tripled.

$$\% RSA = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100 \qquad (2)$$

Ferric-reducing antioxidant power (FRAP) assay
The FRAP was assessed through the already explained technique.[24] For this test, 1000 µL of extract at various contents was combined with 2500 µL of phosphate buffer solution (0.2 M, pH 6.6) and 2500 µL of 1% potassium ferricyanide solution ($K_3Fe(CN)_6$). After 20 min in a water bath maintained at 50 °C, mixture cooled to ambient temperature in dark conditions. The process was ceased by adding 2500 µL of 10% trichloroacetic acid (TCA, $C_2HCl_3O_2$). Then, 2500 µL of supernatant was added to 2500 µL of distilled water and 500 µL of 0.1% iron (III) chloride ($FeCl_3$) after centrifugation at 3000 rpm/10 min. The measurement of absorbance was detected at 700 nm following 10 min of incubation. Trolox and ascorbic acid served as standards, and the inhibition concentrations (IC_{50}) were calculated using the model of linear regression. Triplicate measurements were taken.

Litholytic activity in vitro
The litholytic effect of *B. incrassatum* extracts on cholesterol gallstones was evaluated *in vitro* using the procedure outlined previously. [25]

Gallstones-dissolving capacity
Gallstones were separately covered in sterile porous tissue to prevent mechanical stress, then immersed in 100 mL of each extract in glass Erlenmeyer flasks, hermetically sealed, and shaken at 130 rpm at room temperature for 9 weeks (the period of traditional medical therapy). Weekly, the gallstone was retrieved from the reaction medium and dried at 40°C for 18 h before being weighed using a precision balance (Ohaus, PA213, China) to determine mass loss. Sodium chloride (NaCl, 9 g/L) served as a control. The gallstones-dissolving capacity percentage (% GDC) was determined according to the formula (3). All measures were tripled.

$$\% GDC = \left[\frac{M_i - M_f}{M_i}\right] \times 100 \qquad (3)$$

Where:

M_i: initial mass of the gallstones (mg).
M_f: final incubation mass of the gallstones (mg).

pH evolution
The pH of the experimental mixture was measured at the beginning and weekly thereafter to record its evolution using a pH meter (HANNA HI 2211, HANNA instruments, Romania). Triplicate measurements were performed.

Statistical analysis
Findings from three tests are shown as mean ± standard deviation, processed by using IBM SPSS Statistics version 22, 2023, and subjected to a one-factor ANOVA (analysis of variance) with Tukey's posthoc test and Past 4.03, 2020 software. All tests were carried out three times. The difference is significant at $P<0.05$, very significant at $P<0.01$, highly significant at $P<0.001$, or not significant at $P>0.05$. * Compared with NaCl control. Graphs were represented using GraphPad Prism 10.3.1 (509).

Results and Discussion

Bunium incrassatum extracts composition
The HPLC/UV profile detecting at 270 and 320 nm of seed, root, and stem methanolic extracts of *Bunium incrassatum* permitted the separation and identification of 25, 24, and 21 phenolic components, respectively (Table 1 and Figures 1-3). Besides this finding, the methanolic extract of seeds demonstrates a notably higher phenolic concentration than the others. In contrast, the root and stem extracts exhibit distinct compounds. Polyphenolic acids, including gallic, ferulic, and ellagic acid, show variations across the extracts, with MESE typically exhibiting higher amounts (7.13, 2.17, and 2.02 mg/g of seed extract, respectively).

Table 1: Composition of the methanolic extracts of *Bunium incrassatum* (MESE, MERO, MEST) by HPLC/UV (mg/g)

Peak number	Compounds	Amount (mg/g MESE)	Rt $_{MESE}$ (min)	Amount (mg/g MERO)	Rt $_{MERO}$ (min)	Amount (mg/g MEST)	Rt $_{MEST}$ (min)
1	Quercetin	10.38	1.72	8.36	1.72	9.41	1.72
2	Kaempferol	4.26	2.19	3.12	2.19	1.85	2.19
3	Luteolin	0.03	3.20	0.89	3.24	-	-
4	Acacetin	0.11	5.35	-	-	-	-
5	Naringin	6.47	6.74	-	-	8.06	6.75
6	Bis-methylated quercetin	2.10	6.99	0.96	7.00	-	-
7	Chrysin	2.03	7.14	0.94	1.15	1.74	7.12
8	Catechin	0.44	9.12	0.81	9.13	0.06	9.11
9	Epicatechin	0.40	9.27	0.85	9.26	0.05	9.24
10	Galangin	0.65	10.03	2.86	10.04	0.52	10.02
11	Pinocembrin	0.13	10.28	0.99	10.27	-	-
12	Carnosol	0.16	10.74	1.12	10.73	-	-
13	Thymol	0.11	11.15	1.19	11.17	0.03	11.15
14	Menthol	0.12	11.55	1.22	11.55	0.04	11.54
15	Myricetin	0.68	13.71	1.23	13.72	0.80	13.71
16	Ascorbic acid	0.01	0.45	0.06	0.36	-	-
17	Gallic acid	7.13	1.73	5.11	1.73	3.62	1.73
18	Caffeic acid	0.73	2.03	0.84	2.04	0.54	2.04
19	*P*-coumaric acid	0.58	2.21	0.70	2.19	0.71	2.19
20	Chlorogenic acid	0.56	5.41	-	-	-	-
21	Quinic acid	0.94	6.47	-	-	-	-
22	Vanillic acid	0.98	6.75	-	-	3.05	6.76
23	Ferrulic acid	2.17	6.99	1.38	6.99	1.02	6.99
24	Ellagic acid	2.02	7.14	1.87	7.15	0.74	7.14
25	Trans-cinnamic	0.01	13.56	1.42	13.57	0.45	13.57
26	Sinapic acid	-	-	0.01	9.13	-	-
27	Tannic acid	-	-	0.01	10.33	-	-
28	Carnosic acid	-	-	0.02	11.27	-	-
29	Syringic acid	-	-	0.97	12.60	-	-
30	Rutin	-	-	-	-	3.12	6.40
31	Fisetin	-	-	-	-	3.25	6.62
32	Catechol	-	-	-	-	1.26	6.63
33	Protocatechic acid	-	-	-	-	0.20	9.93

MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, -: absent.

Furthermore, quercetin, naringin, and kaempferol, as major flavonoids, are consistently identified with the highest concentration in seed (10.38, 6.47, and 4.26 mg/g of extract, respectively). As well as, luteolin is primarily found in root and seed extracts, with negligible amounts in stem. These secondary metabolites are noted for wide spectral activities, including antioxidant, anti-inflammatory, analgesic, antimicrobial, and neuroprotective.[26, 27,28,29, 30]
These findings align with several studies that have shown coumarin in the roots of *B. incrassatum*,[31] and naringin, catechin, kaempferol, quercetin, caffeic, ferulic, chlorogenic, gallic, *p*-coumaric, and ellagic acid in the seeds of the same species.[7] The presence of these bioactive compounds highlights scientific validation for both traditional and

modern uses of this plant species among North African populations and supports its promising potential for various pharmacological applications.

Extraction yields, total polyphenols, and flavonoids of B. incrassatum
Table 2 shows the extraction yield and phenolic component amounts of the *B. incrassatum* extracts. The MERO had the best yield (12.19%), followed by the MESE with 4.46% and the MEST with 1.77%.

Despite these findings, in M'sila, Algeria**,** the preparation of *B. incrassatum* methanolic root extract by maceration revealed the lowest extraction yield of 3.36%,[32] In addition, collected seed from the region of Tlemcen, Algeria, of the same space, which was extracted under reflux for 1 h, yielded 7.1%.[7]

Table 2: Extraction yields and phenolic contents in extracts of *Bunium incrassatum* parts

Extracts of ***B. incrassatum***	**% Y**	**Phenolic compounds**	
		TPC (mg GAE/g DE)	**TFC (mg QE/g DE)**
MESE	4.46 ± 0.20	70.80 ± 0.47	18.44 ± 0.08
MEST	1.77 ± 0.36	48.75 ± 0.64	6.49 ± 0.07
MERO	12.19 ± 0.41	34.43 ± 0.38	0.54 ± 0.02

Findings from three tests are shown as mean ± standard deviation. MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, % Y: extract yield percentage, TPC: total phenol content, TFC: total flavonoid content, mg GAE/g of DE: milligrams of gallic acid equivalent per gram of dry extract, mg QE of DE: milligrams of quercetin equivalent per gram of dry extract.

As highlighted in various studies, both the solvent type, extraction conditions, and geographical origin of the species can influence the extraction yield.[33] It has been demonstrated that methods including maceration, Soxhlet, and ultrasound-assisted extraction have an impact on the effectiveness of bioactive compound extraction.[34,35]
According to this study, total phenol and flavonoid content in MESE is found to be relatively high when compared to MEST and MERO, with a range of 70.80 mg GAE/g and 18.44 mg QE/g DE, respectively. Compared to these data, a recent researcher estimated a lower level of polyphenol content, around 13.00 g GAE/mg DE, and an important level of flavonoid with a value of 16.32 g QE/mg DE in the roots methanolic extract in the same species.[32]

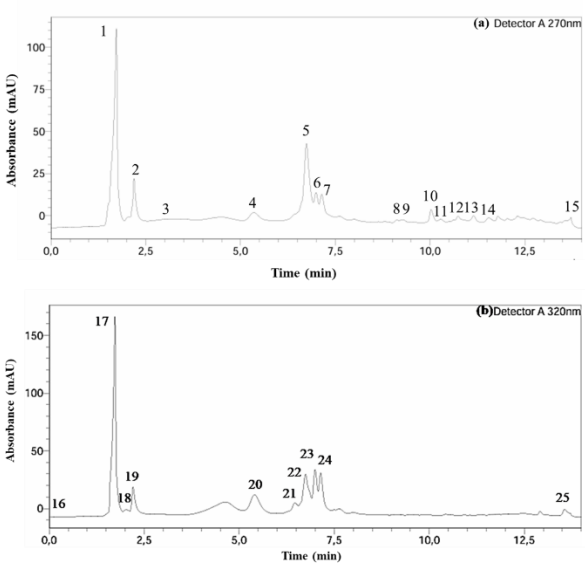

Figure 1: HPLC/UV chromatograms of MESE at 270 nm (a) and 320 nm (b): peaks:1) quercetin, 2) kaempferol, 3) luteolin, 4) acacetin, 5) naringin, 6) bis-methylated quercetin, 7) chrysin, 8) catechin, 9) epicatechin, 10) galangin, 11) pinocembrin, 12) carnosol, 13) thymol, 14) menthol, 15) myricetin, 16) ascorbic acid, 17) gallic acid, 18) caffeic acid, 19) *p*-coumaric acid, 20) chlorogenic acid, 21) quinic acid, 22) vanillic acid, 23) ferrulic acid, 24) ellagic acid, 25) trans-cinnamic.

A similar quantity of these compounds was shown in other investigations (37.37 mg GAE/g DE and 2.36 mg QE/g DE).[36] In contrast, it was noted that there were higher total phenolic and flavonoid amounts in *B. incrassatum* seed methanolic extract of 185.04 mg GAE/g DE and 72.07 mg CE/g DE (catechin equivalent per gram of dry extract) in comparison to these data.[7]
This marked quantitative variation in phytochimecal analysis is dependent on a number of factors, including harvest season, plant geographical origin, selection for specific genus and species, morphological and ecological characteristics, drying mode and duration, storage, plant parts used, developmental stage, circumstances, solvent used, temperature, period, and extraction procedures.[37]

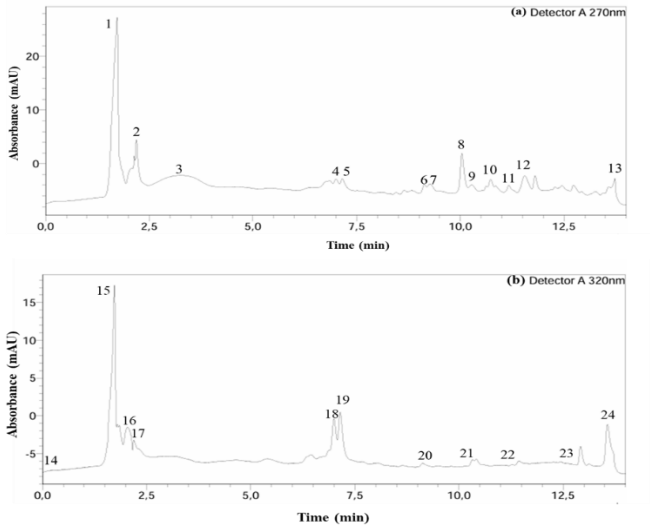

Figure 2: HPLC/UV chromatograms of MERO at 270 nm (a) and 320 nm (b): peaks:1) quercetin, 2) kaempferol, 3) luteolin, 4) bis-methylated quercetin, 5) chrysin, 6) catechin, 7) Epicatechin, 8) galangin, 9) pinocembrin, 10) carnosol, 11) thymol; 12) menthol, 13) myricetin, 14) ascorbic acid, 15) gallic acid, 16) caffeic acid, 17) *p*-coumaric acid, 18) ferrulic acid, 19) ellagic acid, 20) sinapic acid, 21) tannic acid, 22) carnosic acid, 23) syringic acid, 24) trans-cinnamic.

Antioxidant activity in vitro
The DPPH activity of *B. incrassatum* extracts is illustrated in Figures 4 and 5. At 1 mg/mL, MESE displayed 97.46% of RSA and an IC_{50} of 0.23 mg/mL, following MEST (96.04%, IC_{50} of 0.30 mg/mL) and MERO (40.16%, IC_{50} of 1.68 mg/mL), respectively. It is observed that

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extracts from seeds and stems have a lower IC_{50} than those from roots, while their activity is not as high as that of the reference compounds (Trolox and ascorbic acid) (Figures 4 and 5). Figures 6 and 7 show the ferric-reducing inhibition potential of *B. incrassatum* methanolic extracts.

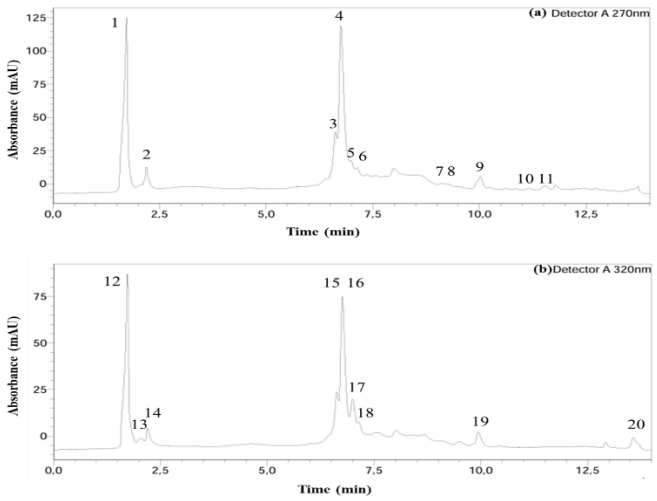

Figure 3: HPLC/UV chromatograms of MEST at 270 nm (a) and 320 nm (b): peaks: 1) quercetin, 2) kaempferol, 3) rutin, 4) fisetin, 5) naringin, 6) chrysin, 7) catechin, 8) epicatechin, 9) galangin, 10) thymol, 11) menthol, 12) gallic acid, 13) caffeic acid, 14) *p*-coumaric acid, 15) catechol, 16) vanillic acid, 17) ferrulic acid, 18) ellagic acid, 19) protocatechic acid, 20) trans-cinamic.

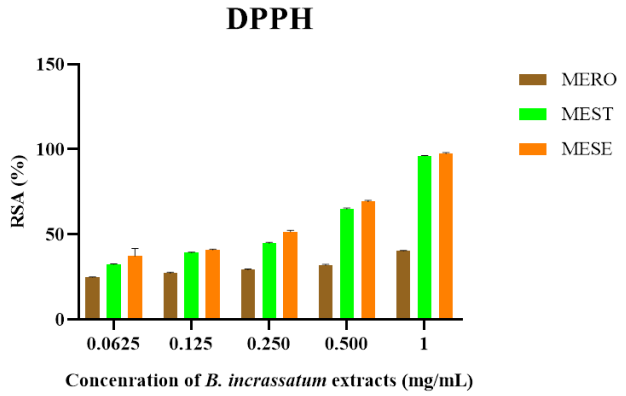

Figure 4: DPPH assay of *B. incrassatum* extracts. MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, DPPH: 2,2-diphenyl-1-picrylhydrazyl, % RSA: radical scavenging activity percentage. Findings from three tests are shown as mean ± standard deviation.

Seed and stem extracts had lower IC_{50}, 0.66 mg/mL and 0.73 mg/mL, respectively, than root extracts (1.09 mg/mL). Their reducing power (RP) is less than that of the references.

The same DPPH potency was appeared in *B. incrassatum* root methanolic extract (IC_{50} = 1.60 mg/mL) when compared to our result,[36] and lower than other findings with IC_{50} = 21.18 mg/mL.[32] Whereas it was discovered a slightly greater anti- free radical impact (IC_{50} = 0.15 mg/mL) than ours in the seed methanolic extract of the same species.[7]

In the FRAP assay, antioxidants donate an electron to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), modifying the reaction medium's colour from yellow to blue around 700 nm, whereas the absorbance intensity correlates to the antioxidant power. These plant-based phenolic compounds have redox properties that neutralise free radicals while also reducing iron ions. This conclusion aligns with previous reports.[38]

Gallstones analysis

Figure 8 depicts the FTIR-ATR spectroscopic analysis of cholesterol gallstones. Massive gallstones are classified into three groups: low-weight cholesterol gallstones (LWCG) of 47.25 mg, medium-weight gallstones (MWCG) of 106.58 mg, and high-weight gallstones (HWCG) of 193.67 mg. Each weight group has 12 gallstones.

Litholytic activity in vitro

The litholytic activity of *B. incrassatum* extracts was evaluated *in vitro* in the presence of cholesterol gallstone groups (LWCG, MWCG, and HWCG) over 9 weeks (Figures. 9-11).

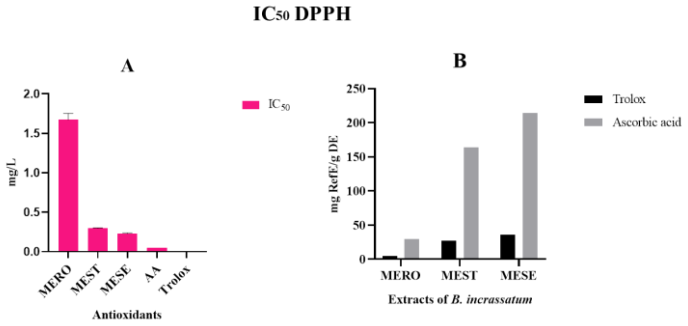

Figure 5: IC_{50} DPPH assay: A, IC_{50} (mg/mL) of *B. incrassatum* extracts and references, B, IC_{50} (mg RefE/g DE) of *B. incrassatum* extracts. MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: inhibitory concentration at 50%, mg RefE/g DE: milligrams of reference equivalent per gram of dry extract. Findings from three tests are shown as mean ± standard deviation.

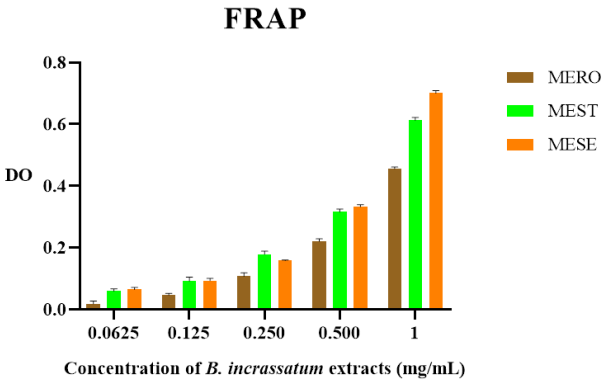

Figure 6: FRAP assay of *B. incrassatum* extracts. MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, FRAP: ferric-reducing antiodidant power, % RSA: radical scavenging activity percentage. Findings from three tests are shown as mean ± standard deviation.

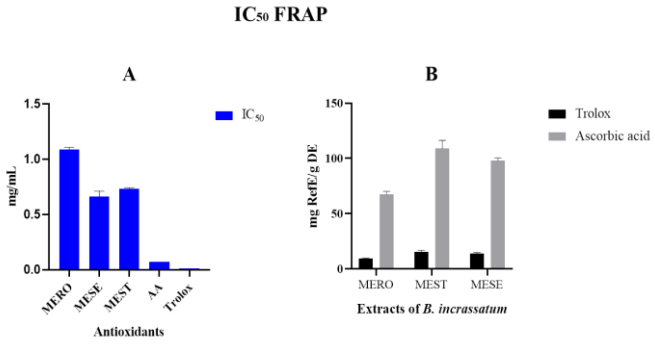

Figure 7: IC_{50} FRAP assay: A, IC_{50} (mg/mL) of *B. incrassatum*

extracts and references, B, IC_{50} (mg RefE/g DE) of *B. incrassatum* extracts. MERO: methanolic extract of roots, MESE: methanolic extract of seeds, MEST: methanolic extract of stems, AA: ascorbic acid, FRAP: ferric-reducing antiodidant power, IC_{50}: inhibitory concentration at 50%, mg RefE/g DE: milligrams of reference equivalent per gram of dry extract. Findings from three tests are shown as mean ± standard deviation.

Effect of B. incrassatum extracts on the low-weight cholesterol gallstones group

The initial LWCG mass was 53 mg for MERO, 46.33 mg for MESE, 42.33 mg for MEST, and 47.33 mg for NaCl control. Complete dissolution of LWCG gallstones was observed in MERO in the first week (W1), MESE in the second week (W2), and MEST in the fourth week (W4). However, the NaCl control lost them at 6.33 mg, and gallstones-dissolving capacity was 13.29% at W4. Despite treatment settings, the LWCG noted greater mass loss over time (*P<0.001*). *B. incrassatum* extracts significantly (*P<0.001*) increased mass loss in the LWCG compared to the NaCl control (Figures. 9A, B, and 10A).

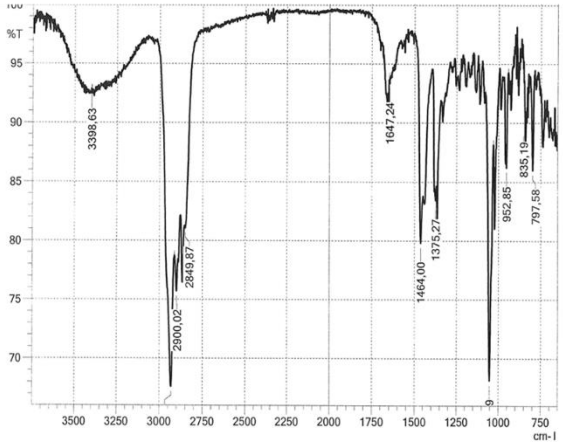

Figure 8: ATR-FTIR spectrum from cholesterol gallstones, acquired from 4000 to 725 cm^{-1}

Effect of B. incrassatum extracts on the medium-weight cholesterol gallstones group

The MWCG had initial masses of root, seed, and stem extracts, and NaCl control, with respective values of 97.33, 113.33, 112.33, and 103.33 mg. The MERO had a fully reduced weight and total dissolution of the MWCG in W4, MESE in W5, and MEST in W8, whereas the control exhibited a weight loss of 18.00 mg and 17.46% GDC in the final trial (W9). Across all treatment conditions, the MWCG saw substantial rises (*P<0.001*) in mass loss during the course of treatment. The extracts significantly (*P<0.001*) enhanced the MWCG mass loss more than NaCl (Figures. 9C, D, and 10B).

Effect of B. incrassatum extracts on the high-weight cholesterol gallstones group

According to Figures 9E, F, and 10C, the starting masses of MERO, MESE, MEST, and control for the HWCG were 192.33, 190.67, 192.67, and 199.00 mg, respectively. HWCG found total dissolution in W5 with MERO and W9 with MESE. The mass loss and gallstones-dissolving capacity for MEST and NaCl solutions were 188.00 mg, 97.58%, and 11.33 mg, 5.70%, respectively. The MWCG exhibited significantly higher mass reduction results (*P<0.001*) in all of the treatment circumstances over the experimental period. The extracts tested elevated significantly (*P<0.001*) HWCG mass losses versus the control throughout the study. The experiment displayed a highly substantial (*P<0.001*) GDC increase than NaCl, with decreasing efficacy observed in the following order: root, seed, and stem extracts of this plant.

The dissolution of cholesterol gallstones occurs earlier in the presence of root extract, followed by seed and finally stem extracts, compared to the control one during the treatment period. This variation may be attributed to the quality, amount, and synergy of phenolic components in cholesterol gallstone dissolution. These findings suggested that the weight of cholesterol gallstones may impact mass loss.[25] While it was demonstrated that the GDC for high-weight cholesterol gallstones in *Citrus limon* juice and *Herniaria hirsuta* extracts was 72% and 26%,

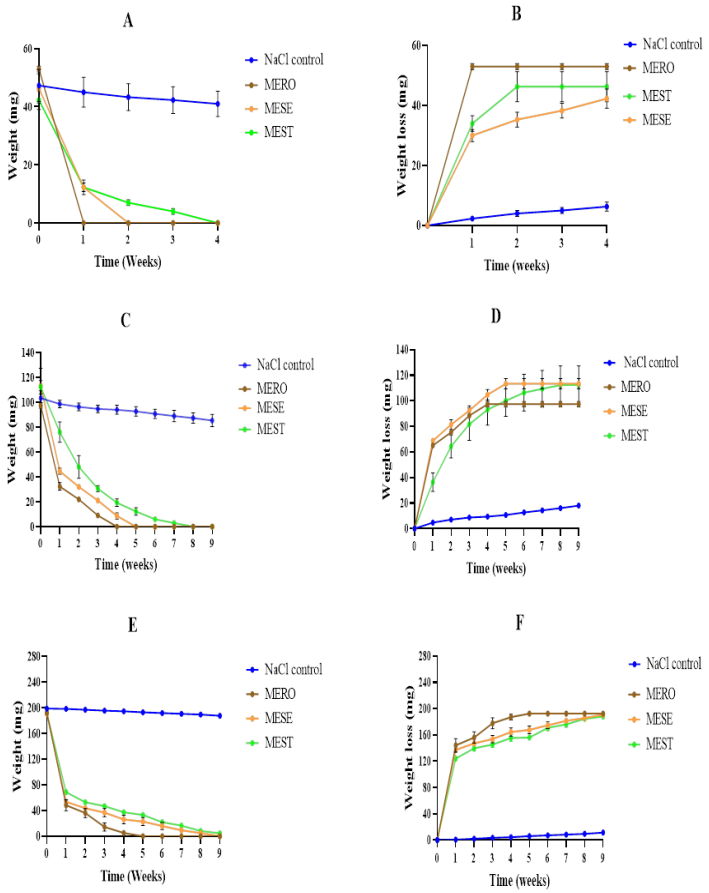

Figure 9: Evolution of cholesterol gallstone weight groups by *B. incrassatum* extracts as a time function: A, weight of low-weight cholesterol gallstones (LWCG), B, weight loss of low-weight cholesterol gallstones (LWCG), C, weight of medium-weight cholesterol gallstones (MWCG), D, weight loss of medium-weight cholesterol gallstones (MWCG), E, weight of high-weight cholesterol gallstones (HWCG), F, weight loss of high-weight cholesterol gallstones (HWCG). MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, NaCl control: sodium chloride control. Findings from three tests are shown as mean ± standard deviation.**P<0.05*, ***P<0.01*, ****P<0.001* than the control.

respectively, after two weeks of exposure.[39] In contrast to these results, the *B. incrassatum* methanolic extracts tested have a more potent gallstone-dissolving capacity in the same experimental conditions.

pH progress

The pH evolution of litholytic activity is apparent in Figure 11. It was proved the initial pH values for root, seed, stem extracts, and NaCl control were 6.86, 6.41, 6.32, and 6.50, respectively. After four weeks, LWCG pH dropped to 4.63 for MERO, 4.81 for MESE, 5.10 for MEST, and 4.07 for control. MWCG pH lowers to 4.12 for MERO, 4.04 for MESE, 4.31 for MEST, and 2.92 for control. All these samples reduced HWCG pH values to 5.03, 4.64, 4.86, and 3.36, respectively, at the end

of the experiment (W9). A substantial decline (*P<0.05*) in pH was marked in all treated groups over time. Nonetheless, the pH values for the control and the plant extracts assessed were almost similar. Recent research revealed that the pH of the studied samples had no influence on the dissolution of cholesterol gallstones.[25] This result confirmed those recorded.

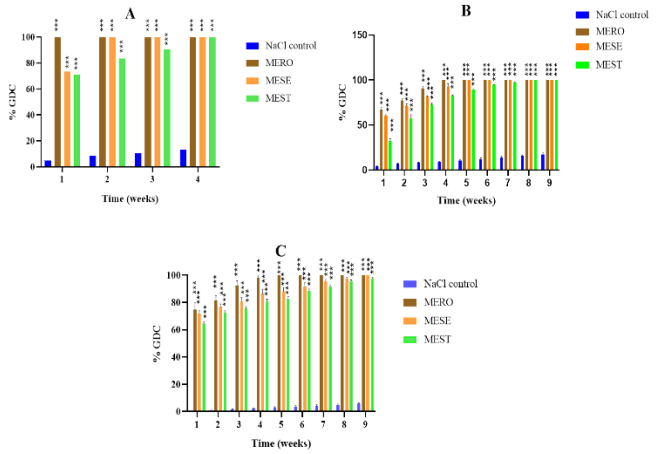

Figure 10: Kinetic evolution of the gallstones-dissolving capacity percentage (% GDC) of cholesterol gallstone weight groups by the extracts of *B. incrassatum* as a time function: A, low-weight cholesterol gallstones (LWCG), B, medium-weight cholesterol gallstones (MWCG), C, high-weight cholesterol gallstones (HWCG). MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, NaCl control: sodium chloride control, % GDC, gallstones-dissolving capacity percentage. Findings from three tests are shown as mean ± standard deviation. **P<0.05*, ***P<0.01*, ****P<0.001* than the control.

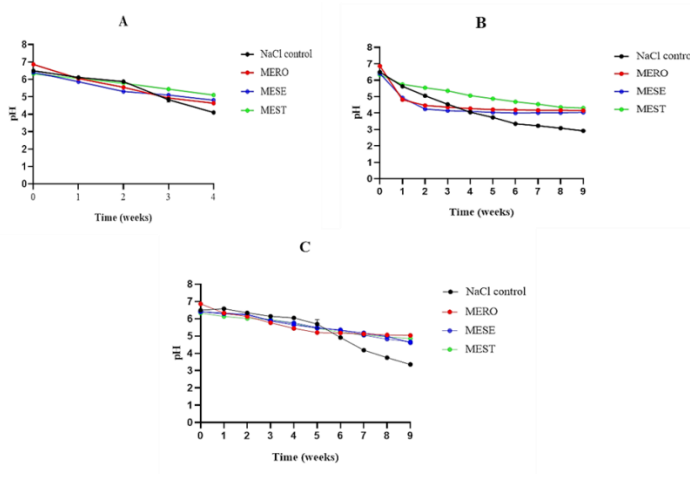

Figure 11: Progress of pH in *B. incrassatum* extracts in the presence of cholesterol gallstone weight groups as a time function: A, low-weight cholesterol gallstones (LWCG), B, medium-weight cholesterol gallstones (MWCG), C, high-weight cholesterol gallstones (HWCG). MESE: methanolic extract of seeds, MEST: methanolic extract of stems, MERO: methanolic extract of roots, NaCl control: sodium chloride control. Findings from three tests are shown as mean ± standard deviation. **P<0.05*, ***P<0.01*, ****P<0.001* than the control.

Conclusion

This research revealed that Algerian *Bunium incrassatum* extracts possessed a high phenolic content and demonstrated a powerful antioxidant capacity, serving as radical scavengers and ferric ion reducers. Furthermore, these extracts showed a strong litholytic effect on cholesterol gallstones *in vitro*, conferring an important pharmacological potential for *B. incrassatum* is a natural source of bioactive chemicals for the pharmaceutical sector.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

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