Comparative analysis of phenolics, flavonoids, antioxidant, anti-inflammatory and antimecrobial potential of methanolic and aqueous extracts from bunium incrassatum tubers

Análise comparativa dos fenólicos, flavonoides, potencial antioxidante, anti-inflamatório e antimecânico dos extratos metanólicos e aquosos dos tubérculos de bunium incrassatum

Análisis comparativo de los fenólicos, flavonoides, potencial antioxidante, antiinflamatorio y anticancerígeno de extractos metanólicos y acuosos de tubérculos de bunium incrassatum

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ABSTRACT

This study investigates the comparative biological activity and chemical composition of aqueous and hydromethanolic extracts of *Bunium incrassatum* tubers, a plant with traditional medicinal uses. Both extracts were evaluated for their antioxidant, anti-inflammatory and antimicrobial activities. Antioxidant activity was measured by DPPH scavenging assays. *In vitro* anti-inflammatory activity using bovine serum albumin technique. While the antimicrobial activity was investigated using the disc diffusion assay against four Gram-positive and Gram-negative bacteria and three fungi.
The total phenol and flavonoid content showed that the methanolic extract had a significantly higher total phenolic content (167.4±3. 53 µg GAE/mg E) and flavonoid content (36.27± 2.18 µg QE/mg E) of than aqueous extracts. The antioxidant activity revealed that methanolic extract of *B. incrassatum* demonstrated higher antioxidant activity (IC50=1.15 ± 0.02 mg/mL). In contrast, it is the aqueous extract that has shown the most important anti-inflammatory activities to those of standard (sodium diclofenac).The study of antimicrobial activity demonstrated that the extracts showed a modest antibacterial and antifungal activity. Both extracts showed antibacterial activity, but the methanolic extract exhibited a higher zone of inhibition, especially against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This comparative analysis provides insights into the effectiveness of different solvent-based extractions for harnessing the therapeutic properties of *Bunium incrassatum*.

Keywords: *Bunium incrassatum,* antioxidant activity, anti-inflammatory activity, antibacterial activity.

RESUMO

Este estudo investiga a atividade biológica comparativa e a composição química de extratos aquosos e hidrometanólicos de tubérculos de *Bunium incrassatum*, uma planta com usos medicinais tradicionais. Ambos os extratos foram avaliados quanto às suas atividades antioxidantes, anti-inflamatórias e antimicrobianas. A atividade antioxidante foi medida por ensaios d'eliminação de DPPH. Atividade anti-inflamatória in vitro usando a técnica d'albumina sérica bovina. Enquanto a atividade antimicrobiana foi investigada usando o ensaio de difusão em disco contra quatro bactérias Gram-positivas e Gram-negativas e três fungos.

O conteúdo total de fenóis e flavonoides mostrou que o extrato metanólico tinha um conteúdo fenólico total significativamente maior (167,4 ± 3,53 µg GAE/mg E) e conteúdo de flavonoides (36,27 ± 2,18 µg QE/mg E) do que os extratos aquosos. A atividade antioxidante revelou que o extrato metanólico de *B. incrassatum* demonstrou maior atividade antioxidante (IC50=1,15 ± 0,02 mg/mL). Em contraste, é o extrato aquoso que demonstrou as atividades anti-inflamatórias mais importantes do que as do padrão (diclofenaco de sódio). O estudo da atividade antimicrobiana demonstrou que os extratos apresentaram uma modesta atividade antibacteriana e antifúngica. Ambos os extratos apresentaram atividade antibacteriana, mas o extrato metanólico exibiu uma zona de inibição mais alta, especialmente contra *Staphylococcus aureus* e *Pseudomonas aeruginosa*. Esta análise comparativa fornece insights sobre a eficácia de diferentes extrações à base de solvente para aproveitar as propriedades terapêuticas de *Bunium incrassatum.*

Palavras-chave: *Bunium incrassatum*, atividade antioxidante, atividade anti-inflamatória, atividade antibacteriana.

RESUMEN

Este estudio investiga la actividad biológica comparativa y la composición química de extractos acuosos e hidrometanólicos de tubérculos de Bunium incrassatum, una planta con usos medicinales tradicionales. Ambos extractos fueron evaluados por sus actividades antioxidantes, antiinflamatorias y antimicrobianas. La actividad antioxidante se midió mediante ensayos de eliminación de DPPH. La actividad antiinflamatoria in vitro utilizando la técnica de albúmina de suero bovino. Mientras que la actividad antimicrobiana se investigó utilizando el ensayo de difusión de disco contra cuatro bacterias Gram-positivas y Gram-negativas y tres hongos.

El contenido total de fenol y flavonoides mostró que el extracto metanólico tenía un contenido fenólico total significativamente mayor (167,4 ± 3,53 µg GAE/mg E) y contenido de flavonoides (36,27 ± 2,18 µg QE/mg E) que los extractos acuosos. La actividad antioxidante reveló que el extracto metanólico de *B. incrassatum* demostró una mayor actividad antioxidante (IC50=1,15 ± 0,02 mg/mL). Por el contrario, es el extracto acuoso el que ha mostrado las actividades antiinflamatorias más importantes que las del estándar (diclofenaco sódico). El estudio de la actividad antimicrobiana demostró que los extractos mostraron una modesta actividad antibacteriana y antifúngica. Ambos extractos mostraron actividad antibacteriana, pero el extracto metanólico exhibió una zona de inhibición más alta, especialmente contra *Staphylococcus aureus* y *Pseudomonas aeruginosa*. Este análisis comparativo proporciona información sobre la eficacia de diferentes extracciones basadas en disolventes para aprovechar las propiedades terapéuticas de *Bunium incrassatum*.

Palabras clave: *Bunium incrassatum*, actividad antioxidante, actividad antiinflamatoria, actividad antibacteriana.

1 INTRODUCTION

Algeria is among the nations with a wealth of medicinal plants that need to be used for a variety of purposes. Numerous studies have attempted to further our understanding of medicinal plants' historic applications and their effects on the health of humans and animals. The genus *Bunium* which belongs to the Apiaceae family consists of 50 accepted species. In the Algerian flora, this genus includes seven species, four of which are endemic (Quezel, 1963). Due to their medicinal and nutriment benefits, several species of this genus are often used in folk medicine. The species *Bunium incrassatum,* has shown promising medicinal properties. Its seeds and tubers contain various bioactive compounds, including phenolics and flavonoids, which contribute to its antioxidant and anti-hemolytic activities (Berroukeche et al., 2021; Toul et al., 2022). In addition, dried and powdered tubers are regarded as astringent, antidiarrheic, anti-hemorrhoidal, for bronchitis, and cough treatment (Sharififar et al., 2010). B. incrassatum has shown promise in improving thyroid tissue damage in rats with hyper- and hypothyroidism (Aiouaz & Bitam, 2022). Additionally, the aqueous root extract demonstrates analgesic and anti-inflammatory effects in various animal models (Amraoui *et al.*, 2023). When used as a dietary supplement, *B. incrassatum* can significantly increase body weight and affect biochemical parameters in rats (Berroukeche *et al.*, 2021). These findings support the traditional use of *B. incrassatum* in Algerian medicine and cuisine, highlighting its potential as a source of bioactive compounds for therapeutic applications. The present study was planned to investigate and compare antioxidant, antiinfalmmatory and antimicrobial activities of methanolic, aqueous extracts from the seeds of *Bunium incrassatum* as well as the impacts of solvents' polarities on phenols and flavonoids continents.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND EXTRACTION

The tubers of *Bunium incrassatum* were collected from kais, Khenchela, Algeria and identified by a botanist, Department of Biology, Faculty of Nature and Life Sciences, University of Khenchela, Algeria. The plant was washed, air-dried, and ground into a fine powder.

The extraction used was 10 g of powdered seeds suspended in 100 ml of hot water, or 80% (v/v) methanol. Extracts were stirred mechanically for 12 h at room temperature (25°C), the hot water extract (80°C) that was made in 30 min. After filtration, the extracts were dried in a rotary evaporator at 40°C to obtain the concentrated extract and stored at 4°C for further process.

2.2 PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening was conducted to identify the presence of major bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, and saponins using standard qualitative methods.

2.3 THE TOTAL PHENOLIC CONTENT

The amounts of total phenolic compounds in methanolic and aqueous extracts were determined by FolinCiocalteu reagent assay. 1 ml of Folin reagent (diluted x10) is added to 200 µl of sample or standard (prepared in methanol) with suitable dilutions. After 4 min, 800 µl of sodium carbonate solution (75 mg/ml) are added. After 2 h of incubation at room temperature, the absorbance is measured at 760 nm. The concentration of total polyphenols is calculated from the regression equation of the calibration range established with gallic acid and is expressed in µg of gallic acid equivalent per milligram of extract (µg EAG/mg of extract).

2.4 THE TOTAL FLAVONOID CONTENTS (TFC)

The aluminum trichloride method is used to quantify flavonoids in *B. incrassatum* extracts, as described by Bahorun *et al*. (1996). Briefly, 1 ml of sample or standard (prepared in methanol) is added 1 ml of AlCl3 solution (2% in methanol). After 10 minutes of reaction, the absorbance is read at 415 nm. The concentration of flavonoids is deduced from a calibration range established with quercetin and is expressed in microgram of quercetin equivalent per milligram of extract (µg EQ/mg of extract).

2.5 DPPH RADICAL SCAVENGING ASSAY

The antioxidant potential of the extracts was evaluated using the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay (Mansouri *et al*., 2005). In this test, 50 microliters of various concentrations of extracts were mixed with 1950 µL of a 0.025 g/L methanolic DPPH solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. The positive control is represented by a solution of a standard antioxidant; ascorbic acid whose absorbance was measured under the same conditions as the samples and for each concentration, the test is repeated 3 times. The percentage inhibition was calculated to measure antioxidant capacity using the following equation:

$$\% \text{ antiradical activity} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Where A blank is the absorbance negative control and A sample is the absorbance of the tested compound. The concentration providing 50% of inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentages against extract concentrations.

2.6 ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity was determined using the protein denaturation assay described by Habibur *et al*. (2015), where the ability of the extracts to prevent protein denaturation was measured. Briefly, 0.45 ml of bovine albumin serum was combined with 0.05 mL different concentrations of extracts, or standard drug diclofenac sodium (100, 200, and 400 µg/ml). After 20 minutes of incubation at 37 °C, the sample extracts were heated to 57 °C for thirty minutes. After cooling the samples, 2.5 ml of phosphate buffer with a pH of 6.4 was added. The UV visible spectrophotometer was used to measure the absorbance at 660 nm.

2.7 ANTIMICROBIAL ACTIVITY

The antimicrobial properties were evaluated using the disc diffusion assay against Gram-positive and Gram-negative bacteria and fungi. The zone of inhibition was measured to determine the antibacterial and antifungal efficacy. For bacterial assays, Sterile Mueller-Hinton agar plates were used and Sabouraud dextrose agar plates for fungal assays.

Twenty microliters of each extract at a concentration of ranging from 6.25 mg/mL to 25 mg/mL were deposited onto sterile paper disks, each measuring 6 mm in diameter. After that, these disks were put on the agar plates that had been infected. The plates were incubated for 24 hours for bacteria and 48 hours for fungi at 37°C and 28°C, respectively. The zones of inhibition surrounding the disks were measured following incubation. An index of antibacterial activity was the zones' diameter, which was measured in millimeters. The positive controls were standard antibiotics, such as ampicillin (50 µg/mL) for bacteria and fluconazole (50 µg/mL) for fungi, whereas the negative controls were distilled water.

2.8 STATISTICAL ANALYSIS

Triplicate studies were performed, and the results were expressed as the mean ± standard deviation. $P < 0.05$ was considered statistically significant.

3 RESULTS

3.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The qualitative phytochemical screening of *B. incrassatum* extracts revealed the presence of several bioactive compounds. The analysis revealed the presence of terpenoids, coumarins, tannins, reduced sugars, and amines in both extracts Table 1. The presence of alkaloids is recorded in the aqueous extract, and a large amount of phenolic compounds in the methanolic extract. This indicates that both extracts contain significant amounts of these bioactive compounds, which contribute to their biological properties.

Table 1. Results of qualitative phytochemical analysis of *B. incrassatum* tubers. AqBI: Aqueous extract MeBI Methanolic extract

Bioactive compound	Test used	Positive reaction	AqBI	MeBI
Alkaloids	Dragendorff's test	Positive (reddish-brown)	+++	+
	Mayer's test	Positive (cream-colored)		
Flavonoids	$AlCl_3$ test	Positive (jaune)	+	+
Terpenoids	*Salkowski test*	Positive (reddish brown)	++	+++
Tannins	Ferric chloride test	Positive(blue-black/greenish-black)	++	+++
Coumarins	NaOH chloroform	Positive (yellow)	++	++
Saponins	Foam test	Positive (persistent foam)	+	-
Anthocyanins	NAOH test	Blue-gren	–	-
Phenolic compounds	Ferric chloride test	Positive (deep blue/black)	+	+++
Quinones	Hcl	Green	+	+
Proteins/amino acids	Ninhydrin test	Positive (Tache violette)	+++	+++
Reducing sugars	Fehling's test	red-brick precipitate	+++	+++

Source: Prepared by the authors (2024).

3.2 TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT

The total phenolic content (TPC) of hydroethanolic and aqueous extracts from *Bunium incrassatum* were determined in terms of µg GAE/g Extract, and The total flavonoid content (TFC) were determined in terms of µg QE/g Extract. The results are displayed in Figure1.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of different extracts of *Bunium incrassatum* expressed as µg GAE/g DW and µg QE/g DW, respectively (n = 3).

	AqBI	MoBI
TPC µg GAE/mg E	61.2+3.64	167.4+4.00
TFC µg EQ/mg E	12.51+0.31	36.27+3.8

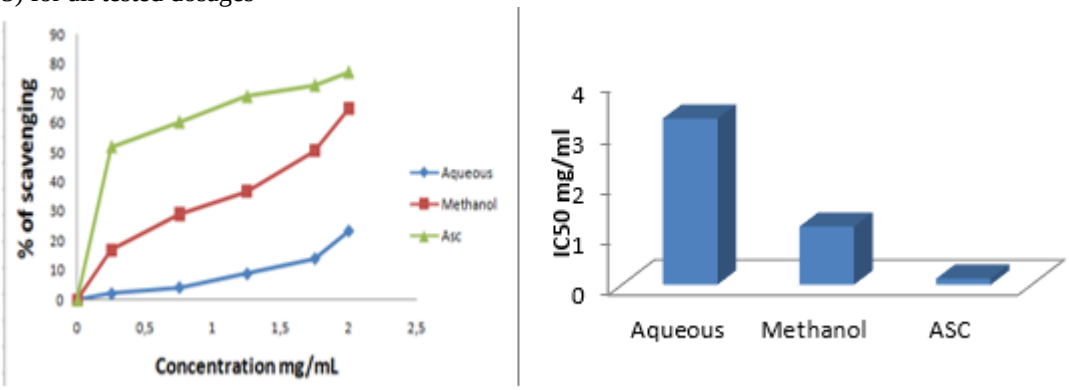
Source: Prepared by the authors (2024).

Hydroalcoholic extracts showing higher phenolic content than aqueous extracts. The differences were statistically significant ($p < 0.05$). The hydroethanolic extracts of *Bunium* had higher flavonoid content compared to the aqueous extracts. These results provide a comprehensive profile of the bioactive constituents present in *Bunium incrassatum*, these compounds have been reported to possess strong antioxidant and free radicals scavenging activity.

3.3 ANTIOXIDANT ACTIVITY

The ability of the extract to decolourize the purple colour of initial DPPH radical was determined by measuring the absorbance at 517 nm. The antioxidant potential of the different solvent extracts is inversely proportional to IC_{50} values.

Figure 1. Determination of **a** DPPH radical scavenging activity and **b** IC_{50} of methanolic and aqueous extracts of *Bunium incrassatum*. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, $p < 0.05$) for all tested dosages

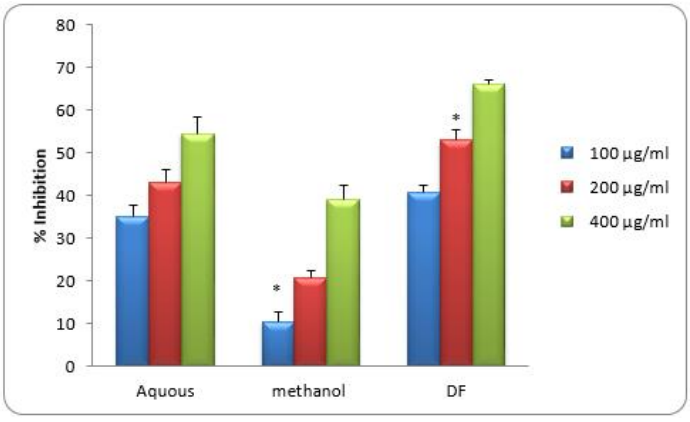


Source: Prepared by the authors (2024).

According to the results shown in figure 1, of antioxidant activity through the DPPH assay, the percentage inhibition of DPPH radical by the methanolic, aqueous extracts and ascorbic acid are 61. 6, 23.72 and 80.42 % respectively at a concentration of 2 mg/mL. Methanol extract exhibited the highest scavenging of the DPPH radica (IC50=1.15±0.12 mg/mL), they showed significant results compared to the standards (0.12±0.00mg/mL). However, aqueous extract gave the lowest values (3.29±0.23 mg/mL).

3.4 ANTI-INFLAMMATORY ACTIVITY

Figure 2. Protein denaturation inhibitory of *B. incrassatum* aquous and methanolic extracts compared to Diclofenac Sodium.



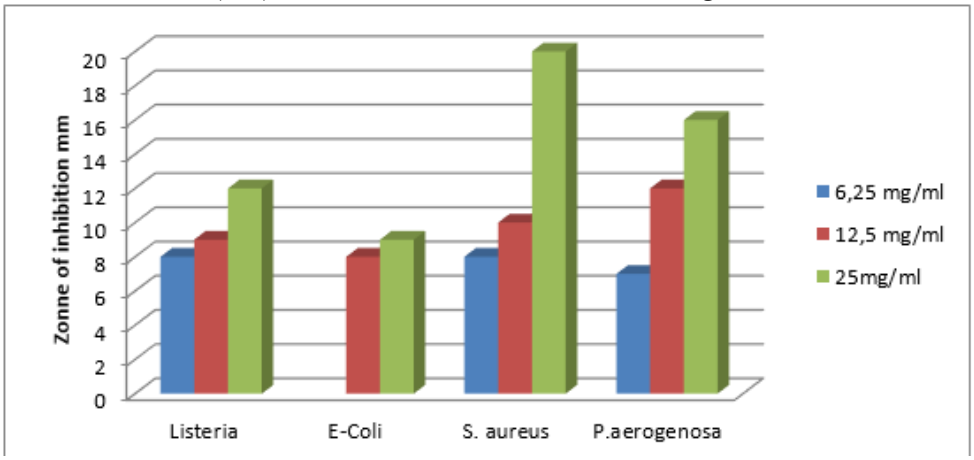
Source: Prepared by the authors (2024).

The anti-inflammatory activity of Bunium extracts was evaluated using the bovine serum albumin denaturation assay. The extract was tested at different concentrations, and its inhibitory effects were compared to standard values (Diclofenac Sodium). The results showed that both extracts demonstrated significant anti-inflammatory activity by inhibiting protein denaturation. Diclofenac Sodium showed a maximum inhibition between 40 and 68% observed in the different concentrations (100, 200, 400 µg/ml). The aqueous extract inhibits albumin denaturation in a range of 35–56%; while for the methanolic extract, the inhibition rate is between 10–37%.

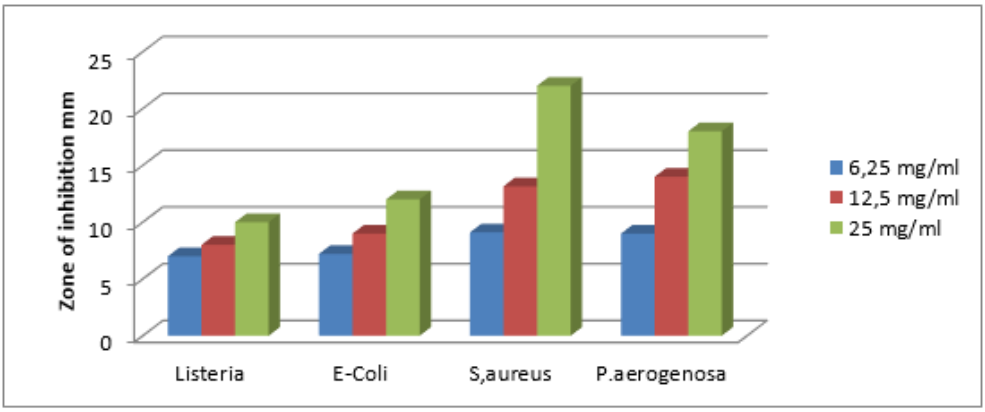
3.5 ANTIMICROBIAL ACTIVITY

The Antimicrobial activity tests were carried out on hydromethanolic and aqueous decocted extract using disk diffusion method against four human pathogenic bacteria, including Gram positive i.e. *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aerogenosa*, Gram-negative bacteria *Staphylococcus aureus* and three *fungi*; *Candida albicans*, *Aspergillus flavus*, and *Penicilium candidum*.

Figure 3. Zones of inhibition (mm) of different extracts of B. incrassatum against four bacterial strains (n = 3).



Source: Prepared by the authors (2024).



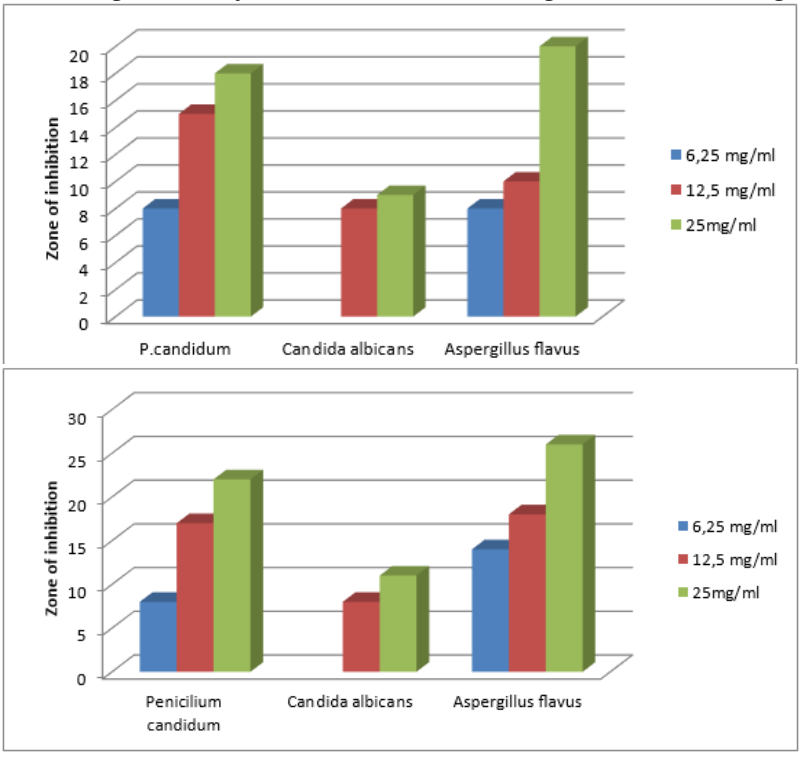
Source: Prepared by the authors (2024).

The antimicrobial activity of the extracts was quantitatively assessed by the presence of zone diameter of inhibition (mm) at three different concentrations of 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml methanol and aqueous extract against four standard bacteria *Listeria monocytogenes* (Listeria), *Escherichia coli* (E. coli), *Pseudomonas aeruginosa* (P. *aeruginosa*) and *Staphylococcus aureus* (S. aureus), the results are displayed in figure 3.

Both extracts showed antimicrobial activity, but the methanolic extract exhibited a higher zone of inhibition, especially against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The aqueous extract also displayed moderate antibacterial activity, but its antifungal activity was less pronounced compared to the methanolic extract. *Aspergillus flavus* was the most susceptible, and the aqueous and methanolic extracts exhibited a slightly higher efficiency against this pathogen with zones of inhibition ranging from 17 to 25 mm, followed by *Penicilium candidum* and *Candida albicans* with zones of inhibition ranging from 9 to 17.3 mm. When comparing the two extraction methods, there was no significant difference between the hydromethanolic and aqueous decoction extracts. However, both extracts demonstrated lower antimicrobial activity compared to the standard antimicrobial drugs (ampicillin for bacteria and fluconazole for fungi), which showed larger zones of inhibition ranging from 17 to 25 mm for ampicillin and from 6 to 20.8 mm for fluconazole. In general antibacterial activity increases with increase in concentration of extract as evident by the zone of inhibition.

Figure 4: Antifungical activity of *B. incrassatum* extract against the various fungi strains.



Source: Prepared by the authors (2024).

4 DISCUSSION

Medicinal plants are widely recognized as a significant source of bioactive compounds. In the present study, *B. incrassatum* are investigated as a potential source of active compounds with the aim of the valorization of these plants. For this purpose, a simple chemical screening was performed and the results confirmed the presence of active molecules including tannins, coumarins, flavonoids, and terpenoids in the extracts. These results are consistent with earlier research on *B. incrassatum* which reported the presence of phenolic compounds, flavonoids, and sesquiterpenes (El Kolli *et al*., 2017; Toul & Djendar, 2023).

In a study on the chemical composition of *B. incrassatum* tubers, Bousetla et al. (2011) extracted two coumarins, sucrose, oleic acid, and β-sitosterol. The fruits contain about 2% (v/w) essential oil, and caryophyllene, γ-terpinene and cuminyl acetate are the major components (Shahsavari *et al*., 2008). Phytochemical analysis identified 13 phenolic compounds in B. incrassatum seeds, including flavonoids (catechin, kaempferol, quercetin, hesperetin, naringenin) and phenolic acids (caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, p-coumaric acid, sinapic acid, syringic acid (Toul *et al*., 2022).

Furthermore, the studied fractions were found to contain considerable amounts of phenols and flavonoids; the results for methanol extracts are comparable to those described in a study realized by Toul *et al*. (2022), in which methanolic fraction contained 185.04 µg GAE/mg E. Benhammou *et al*. (2009) and Dehimi *et al*. (2021) reported even lower phenolic contents in different solvent extracts of B. incrassatum tubers. The total flavonoid content was found to be significantly ($p < 0.05$) higher in methanolic extracts than in the aqueous decocted extracts. These results provide a comprehensive profile of the bioactive constituents present in *B. incrassatum*, supporting their potential use as antimicrobial agents.

According to researchs, phenolic content and antioxidant activity in plants are directly correlated (Molehin and Adefegh, 2014; Bouhalit & Brahmi, 2021). It has been established that the high capacity of flavonoids and phenols to donate hydrogen is the cause of their antioxidant properties (Shimanda *et al*., 1992). In this study, methanolic fraction which is the richest in phenols gave the best activity antioxidant (IC_{50}= 1.15±0.03 mg/mL). The obtained results for this assay are in accordance with those mentioned by Toul *et al*. (2022) (IC50=0.15±0.02mg/mL), this activity increased with the increasing concentrations of phenolics in a dose dependent manner and were much higher than those reported by Dehimi *et al.* (2020) and Adelifar *et al*. (2021) in which four different species of *Bunium* from Turkey showed less potent effects against DPPH radical for *B. incrassatum*, acetone and methanol extractions seem to be more active and contained the highest

amounts of total phenols, besides the aqueous extraction which showed a good effect in the β-carotene bleaching assay.

The studies investigated the phytochemical profile and antioxidant activity of *Bunium incrassatum* extracts using various solvents. Methanol extracts consistently showed the highest DPPH scavenging potential and inhibitory effect against β-carotene bleaching. RP-HPLC analysis identified numerous phenolic compounds, including phenolic acids and flavonoids, with slight variations between studies (Toul *et al.*, 2022; Toul & Djendar, 2023). Methanol extracts yielded the highest total phenolic content (26.57±0.15 mg GAE/g E), while chloroform extracts were richest in flavonoids. These findings support the potential of *B. incrassatum* as a source of bioactive compounds with antioxidant properties, warranting further investigation into its medicinal and culinary applications (Toul *et al.*, 2022).

Some studies showed that the chemical composition of *Bunium* has an impact on the antioxidant activity. However the presence of some molecules, even in minority, like, carvacrol and thymol were found to attribute a high degree of anti-oxidant property by synergy against free radicals and they were shown to be as strong reductors of DPPH and lipidic peroxidation inhibitors. The presence of hydroxyl groups in the chemical structure, such as phenolic compounds were present in methanolic extracts (gallic acid, syringic acid, ellagic acid, and sinapic acid) flavonol (quercitine) and flavones (luteolin), which are widely known to be effective free radical scavengers, was shown to be the cause of the antioxidant activity (Tool and Djendar, 2023; Senigra *et al*., 2024)

Protein denaturation has been identified as the cause of inflammation (Williams *et al*., 2012).The production of autoantigens in certain arthritic disease may be due to protein denaturation and membrane lysis action. The possible mechanism of denaturation is the alteration of electrostatic, hydrogen, hydrophobic and disulfide bonds that maintain the three-dimensional structure of proteins (Barros *et al*., 2008). In addition, a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair, occur, causing the protein to lose its molecular conformation and functions or become denatured. It is therefore deduced that, substances which are able to avoid these alterations and inhibit thermally or heat induced protein denaturation, have potential therapeutic value as anti-inflammatory agents (Sweetha *et al.*, 2013; Nivetha *et al*., 2022). The ethical concerns surrounding the use of humans or animals were avoided in this study by using the BSA protein denaturation assay to assess the anti-inflammatory potential of bunium extracts in vitro. Furthermore, it has been stated that protein denaturation is a pathological process that results in the loss of configuration and, consequently, functionality. This makes the reduction in protein denaturation, and by extension the BSA protein denaturation assay, ideal for the determination of anti-inflammatory potential.

In our present study, extracts of *Bunium* inhibited heat induced protein denaturation and may be one of the reason of possessing anti-inflammatory and anti-arthritic activity. The denaturation inhibitory activity of BSA may be attributed to the presence of different bioactive compounds such as alkaloids, flavonoids, glycosides, tannins, steroids and sesquiterpens in the composition of the extracts of *B. incrassatum* can be responsible for a significant antiinflammatory response (Sachin *et al.,* 2010; Alhakmani *et al*., 2013).

Few studies have explored the anti-inflammatory properties of Bunium species native to Algeria. *Bunium incrassatum* aqueous root extract demonstrated significant anti-inflammatory effects in various edema models and exhibited analgesic activity in pain tests (Nacer Amraoui *et al*., 2023). The essential oils and methanolic extracts of *B. incrassatum* and *B. alpinum* showed antibacterial, antioxidant, anti-hemolytic, and anti-inflammatory activities (El Kolli *et al*., 2017). The aqueous extract of *Bunium pachypodum* also demonstrated significant anti-hemolytic activity and inhibition of albumin eggs denaturation, comparable to a reference anti-inflammatory drug (Nassima *et al*., 2022). As far as we know, the present study is the first to investigate the inhibition of bovine serum albumin denaturation by different extracts of *Bunium incrassatum*.

Plant based antibacterial compounds have enormous therapeutical potential as they can serve the purpose without side effects that are often associated with synthetic antimicrobials (Sukanya *et al*., 2009). The microbial strains selected for this study were chosen due to their clinical relevance as common pathogens associated with human infections. In the present study methanolic and aqueous tubers extracts of *B. incrassatum* showed considerable inhibitory activity against both bacterial and fungal strains. The obtained inhibition varied from 6.00 to 22.56 mm with a highest inhibition zone recorded with *Staphylococcus aureus*. Nevertheless the fungi displayed very low inhibition diameter and varied from 05 to 25.8 mm overall with higher concentration of 25 mg/mL. To sum up, the crude extract containing the above compounds exhibited stronger activity against bacteria than fungi strains. The bioactive molecules thought to be responsible for antimicrobial activity are terpens and sesquiterpens (El kolli *et al.,* 2017) which have been obtained in large amounts in B. incrassatum and B. alpinum EOs (more than 50 % in their chemical composition) and coumarins (bousetta *et al.,* 2015). The antimicrobial activity is also attributed to the presence of fatty acids in the composition (Griffin *et al*., 1999; *Baydar et al., 2004),* and the chemical analysis of *B. incrassatum* EO shows the presence of palmitic acid with 18.39 % (El kolli *et al*., 2017).

The findings of this study demonstrate that the ethanolic extract of Bunium incrassatum exhibits stronger biological activities than the aqueous extract, particularly in antimicrobial, antioxidant, and anti-inflammatory assays. The presence of terpenoids and phenolic compounds in the ethanolic extract is likely responsible for its enhanced biological effects. The differences in

biological activity between the two extracts can be attributed to the solvent's ability to extract different classes of bioactive compounds.

5 CONCLUSION

This comparative study highlights the significance of solvent selection in extracting bioactive compounds from *Bunium incrassatum.* The methanolic extracts exhibited Anti-oxidante, anti-infiammatory and antimicrobial activities compared to the aqueous extract, making it a more promising candidate for pharmacological applications.

However, limitations such as the focus on only two extraction solvents and the lack of in vivo testing need to be addressed. Future research should explore other extraction methods, conduct in vivo studies, and investigate the underlying mechanisms of action for a comprehensive understanding of its therapeutic potential.

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