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Bioactivity of *Pancratium Maritimum* Methanolic Extract and its Alkaloid Fractions: Antimicrobial and Anti A-Amylase Properties

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Abstract:
Pancratium maritimum, or sea daffodil, is a species of bulbous plant that belongs to the Amaryllidaceae family, it is among one of the most important alkaloid-containing plant families. Methanolic extract of *Pancartium maritimum* was prepared. The phytochemicals in *P. maritimum* methanolic were identified using UHPLC-MS method. An *in vitro* spectrophotometric assay based on a colorimetric method was used to test the α- amylase inhibitory effect. Antibacterial and antifungal activities were tested against a wide range of relevant microorganisms using the broth dilution method.

This study showed the presence of alkaloids in methanolic extract as hordenine, lycorine and galanthamine, with an inhibitory action similar to acarbose at intermediate concentrations, the methanolic extract demonstrated significant concentration-dependent α-amylase inhibition, reaching 85.2% at 5 mg/mL (IC_{50} = 0.29 mg/mL). Alkaloid fraction displayed the highest antibacterial activity against the ATCC strain of *Pseudomonas aeruginosa* bacteria (MIC=1.125 mg/mL), butanolic fraction exerted a considerable anticandidal activity against *Candida glabrata* and *candida parapsilosis* fungi (MIC=0.031 and MIC=0.125mg/mL respectively).

1. INTRODUCTION

Plants are a source of a vast range of natural products with varied medicinal qualities and are constantly studied to generate innovative medications [1, 2].

These bioactive chemicals such as polyphenols, flavonoids, terpenes and alkaloids provide biological activity against a variety of disease-causing substances [3].

In recent years, research into these phytochemicals has been a major focus in the creation of functional foods, nutraceuticals, and cosmeceuticals, with the discovery that the usage of plant extracts gives bioactive characteristics and positive effects [1].

Diabetes has imposed a significant strain on the health-care system in emerging countries, and it is becoming more prevalent among urban populations. It is anticipated that the majority of individuals have type II diabetes, which is easily controlled with dietary changes, exercise, and medication. Therefore, it is vital to screen medicinal plants scientifically in order to employ them safely and effectively in the traditional medical system [4].

One important digestive enzyme that helps break down dietary starch into smaller carbs is α-amylase. Its inhibition may lessen the pace at which carbohydrates are digested, which may help to mitigate the rise in blood glucose that occurs after a meal. Thus, there has been a lot of interest in natural α-amylase inhibitors as possible substitutes or supplementary agents to synthetic inhibitors, especially those produced from plant secondary metabolites such phenolic compounds and flavonoids [5].

Extracts isolated from medicinal plants have been reported to exhibit various biological activities such as antimicrobial, anti-inflammatory, and antioxidant activities [6].

In this context, *Pancratium maritimum* plants have been widely investigated for their therapeutic potential [7, 8]. The genus *Pancratium* (Amaryllidaceae family) contains approximately 20 species [8]. *P. maritimum* L., often known as marine narcissus, it is a plant species found along sandy shores from the Mediterranean to the Black Sea, as well as parts of the Atlantic coast [9], However extensively dispersed, *P. maritimum* populations have dropped dramatically due to urbanization, tourism development, change and destruction of dune systems, and overharvesting [9].

P. maritimum is utilized in traditional medicine in various Mediterranean nations due to its antimicrobial, antiviral, antimalarial, purgative, analgesic, anticancer, and antioxidant effects [10-15], all these activities are attributed to alkaloids molecules since Amaryllidaceae family is known for producing beneficial alkaloids. In fact, several investigations focused on alkaloids as the principal bioactive ingredients [13].

2. MATERIAL AND METHODS

2.1. Plant Material

Aerial parts (Stems and leaves) of *P. maritimum*, were collected from the island of Djerba in the south of Tunisia during the flowering period in July. The identification of the plant was assessed by Prof. Fethia Skhiri, a botanist at the Hight Institute of Biotechnology in Monastir, Tunisia. A voucher specimen was deposited at our laboratory in the Faculty of Pharmacy, Monastir, Tunisia (MM-H).

2.2. Extract Preparation

50 g of leaves and stems were washed under running water to remove dust, dried at room temperature, and crushed into a fine powder before being macerated three times for four days each time with 500 mL of petroleum ether, ethyl acetate, and methanol. Each extract was filtered and evaporated at low pressure. The extraction yields were calculated [16].

2.3. UHPLC-MS Analysis

The analysis was performed according to our previously published method [17] with some modifications, using the Acquity UHPLC H-Class Waters® System (Guyancourt, France), which was outfitted with two independent pumps, a controller, a strip detector, diodes (DAD), and a QDa electrospray quadrupole mass spectrometer.

An Uptisphere C18-AQ column with dimensions of 2.1 x 100 mm and a particle size of 2.6 micrometers was employed as the stationary phase.

The mobile phase was made up of (A) ultrapure water + 0.1% formic acid (Carlo Erba Reagents®, Val de Reuil, France) and (B) acetonitrile + 0.1% formic acid (Carlo Erba Reagents®, Val de Reuil, France). The column flow rate and temperature were set at 0.3 ml/min and 30°C, respectively. With a precision of 1.2 nm, the wavelength range was chosen between 200 and 790 nm. Ionization was performed in both negative

and positive modes, with masses ranging from 50 to 1250 Da. The values of the cone voltage and capillary voltage were 15 V and 0.8 kV, respectively. The injection volume was set to four microliters. UHPLC-UV-MS analyses were carried out using the following elution gradients: 5% 80% (B) (0-9 min), 100% (B) (9.5-10.5 min), and 5% (B) (11-13 min). All samples were prepared in analytical grade MeOH at 2 mg/mL and centrifuged for 20 minutes. The injections were made with supernatants.

Based on their UHPLC-MS/MS properties, such as chromatographic retention time, precursor ion mass-to-charge ratio (m/z), molecular formula, and fragmentation patterns, alkaloids in the examined fractions were identified. In addition to existing mass spectral databases and literature reports, the obtained mass spectral data were compared with previously published data reported for alkaloids from *Pancratium maritimum* and related species. The agreement between the expected molecular ion and the measured exact mass, as well as the distinctive MS/MS fragment ions reported in the literature, were taken into account when assigning compounds.

2.4. Alkaloid's Extraction

500 mg of methanol extract was suspended in 100 mL water acidified to pH = 2 by HCl 37 %, followed by liquid-liquid partitioning with hexane (4 x 100 mL) and $CHCl_3$ (4 x 100 mL). Next, the water phase was basified to pH = 9-10 by NH_4OH 35 % before partitioning with $CHCl_3$ (4 x 100 mL) which yielded the alkaloid extract, and BuOH (4 x 100 mL). The different masses of the extracts are recorded in the table below [11].

2.5. α- Amylase Inhibition Test of Methanolic Extract

A modified version of the procedures previously reported by [18] was used to conduct the α-amylase inhibition assay in triplicate. The concentrations of the extract used ranged from 0.078125 to 5 mg/mL. Ten microliters of extract and fifty microliters of α-amylase solution (0.5 mg/mL) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) were combined in a 96-well plate and incubated for ten minutes. After the preincubation phase, 100 µL of a 1% starch solution was added to 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride). The mixture was then incubated at 25°C for three minutes.

The reaction was finished with 125 µL of a 3,5-dinitrosalicylic acid reagent. After five minutes in a boiling water bath, it was cooled to room temperature, diluted with 200 µL of deionized water, and its absorbance at 540 nm was measured. In addition to measuring the absorbances of the control samples, which were prepared using a buffer solution instead of the extract and amylase, acarbose was used as the positive control at the same concentration as the extract. Amylase inhibition was computed using the following formula:

α−Amylase inhibition (%) = OD control − OD sample/ OD control ×100

where OD control is the optical density of the control (untreated sample) and OD sample is the optical density of the treated sample, and the IC_{50} was determined from the concentration–response curve as the concentration required to produce 50% inhibition of α-amylase activity [19].

2.6. Evaluation of the Antimicorbial Activity

2.6.1. Microbial Strains

Different reference bacteria strains and fungi have been used in antibacterial and antifungal activity, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27583, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 90030, *Candida tropicalis* ATCC 3123, and *Candida parapsilosis* ATCC 22019. All the above species were

Table 1: Masses of Different Alkaloid's Fractions

Extracts	Masses (mg/mL)
Hexane	22.42
Chloroform (CHCL3l	41.6
Alkaloids	16.68
Butanol (BuOH)	39.5
H2O	47.48

kindly provided by the Laboratory of Microbiology, Hospital of Fattouma Bourguiba, Monastir.

2.6.2. Estimation of Minimum Inhibitory Concentration (MIC) Values

The MIC value of *P.maritimum* extract was determined using the microdilution method in 96-well microtiter plates. The active culture of each bacterial strain was created from an overnight culture in MHB plates, and the suspended active bacterial cell turbidity was held constant at 0.5 Mc Farland standards (108 CFU/mL) or adjusted. The extract was diluted with DMSO (10%).

To determine MBC and MFC (Minimum Fungicidal Concentration), 20 µL of each well with no discernible growth was reinoculated onto Müller-Hinton agar (MH) agar plates and incubated for 24 hours at 37 °C. The smallest amount of extract exhibited no evidence of bacterial or fungal development [20].

2.7. Statistical Analysis

All experiments were performed in three independent experiments, and the results are expressed as mean ± standard deviation (SD). IBM SPSS Statistics 20 was utilized to evaluate the data using one-way ANOVA and the Tukey test. A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed using Graphpad Prisme 7.0 software.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Methanolic Extract by UHPLC-MS

As previously mentioned, [21], the methanolic extract was investigated for its chemical composition *via* UHPLC-MS method. Results are illustrated in Table **2**.

The outcomes revealed the dominance of alkaloids such as lycorine, galanthamine, and hordenine in *P. maritimum* leaf and stem methanolic extract

Phenolic acids in *P. maritimum* extracts were previously identified by several authors [15] such as 5-hydroxy-7-methoxy-2-methylchromone. Previous Studies in Italy demonstrated the presence of lycorine as an alkaloid in the bulb extract of *P. maritimum* [22]. Similarly, galanthamine was identified in the bulbs of Turkish *P. maritimum* [23].

3.2. Determination of α-amylase Inhibitory Effect

Methanolic extract of *P. maritimum* was investigated for its anti α- amylase inhibitory potential and the results showed a concentration dependent inhibition of α-amylase by the methanolic extract with $P<0.05$ (Figure **1**).

At the lowest concentration (0.078125), the extract showed 26.1% inhibition exceeded 50% (51.4%) when the concentration was raised to 0.3125 mg/mL. At 0.625 and 1.25 mg/mL, the inhibition significantly increased to 70.1% and 82.4%, respectively. The activity tended to plateau at higher concentrations, rising only somewhat from 82.4% at 1.25 to 85.2% at 5 mg/mL. It's interesting to note that the extract exhibited inhibition at 0.625 and 1.25 that was either slightly higher or equal to that of acarbose (70.1 vs. 70.2% and 82.4 vs. 80.1%, respectively). At the greatest dosages, however, acarbose continued to be more effective, achieving 99.6% inhibition at 5mg/mL ($p<0.05$).

According to linear interpolation between the values surrounding 50% inhibition, the *P. maritimum* extract's approximate IC_{50} is 0.29 mg/mL, while acarbose's comparable value was estimated to be 0.22 mg/mL.

Table 2: Chemical Composition of *P. Maritimum* Methanolic Extract

	RT	$[M]^+$	Compound
1	0,734	288,15	Zefbetaine isomer
1	0,964	268,12	Zefbetaine
2	1,218	166,07	Hordenine
3	1,431	288,10	Galanthamine
4	2,112	288,16	Lycorine
5	3,764	318,11	Ungiminorine
6	3,907	318,09	Lycorenine
7	4,005	302,13	9-O-demethylhomolycorine
8	4,448	302,12	Haemanthamine
9	4,595	246,17	Haplopine

RT: retention time

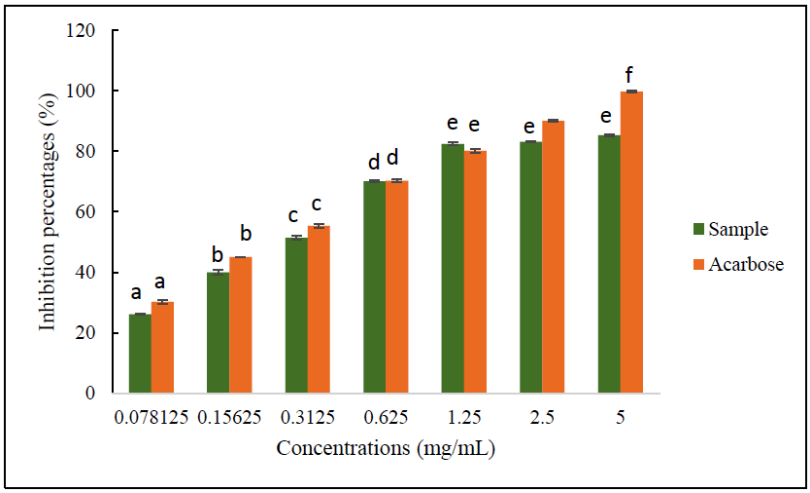

Figure 1: α- Amylase inhibitory effect of *P. maritimum* methanolic extract. According to Tukey's test, means that are followed by the same letter do not differ substantially ($p < 0.05$).

Thus, acarbose reached 50% inhibition at a lower concentration than the tested sample, indicating a higher inhibitory potency under the experimental conditions. Nevertheless, the extract exhibits significant α-amylase inhibitory activity but is generally less powerful than acarbose and showed substantial α-amylase inhibition, reaching more than 80% inhibition at the highest concentration tested. As a result, the extract exhibits significant α-amylase inhibitory activity but is generally less powerful than acarbose.

Pancratium maritimum's methanolic extract significantly inhibited α-amylase in a concentration-dependent manner; the inhibitory activity increased from 26.1% at 0.078125 mg/mL to 85.2% at 5 mg/mL. The inhibition showed a strong inhibitory capacity at comparatively mild dosages, surpassing 70% at 0.625 mg/mL and reaching 82.4% at 1.25 mg/mL. It's interesting to note that at 0.625 and 1.25 mg/mL, the extract's activity was similar to acarbose's, despite acarbose exhibiting more inhibition at the highest concentration examined. The extract's estimated IC_{50} was about 0.29 mg/mL, while acarbose's was about 0.20 mg/mL. This indicates a considerably lower potency but still significant α-amylase inhibitory activity.

The phenolic and flavonoid components of *P. maritimum* may be linked to this action. In fact, earlier phytochemical studies of Tunisian *P. maritimum* have found a number of phenolic acids and flavonol glycosides, with compounds derived from quercetin and isorhamnetin being among the main components [15, 21]. It has been observed that phenolic chemicals and flavonoids interact with α-amylase to inhibit it, which may reduce starch hydrolysis and prolong the digestion of carbohydrates [24-26]. The observed activity thus suggests that *P. maritimum* may be a natural source of α-amylase-inhibitory compounds; however, additional research utilizing isolated constituents and enzyme-inhibition kinetics would be required to identify the compounds and mechanisms responsible for this activity.

The presence of alkaloids and saponins in the methanolic extract of *Pancratium maritimum* may also contribute to the α-amylase inhibitory action. *P. maritimum* is especially rich in Amaryllidaceae alkaloids, such as lycorine, galanthamine, haemanthamine, and related chemicals, according to phytochemical studies. Furthermore, plant secondary metabolites that can block enzymes that hydrolyze carbohydrates, such as α-amylase, include alkaloids and saponins. Therefore, the combined contribution and potential synergistic interactions of the several phytochemical groups found in the extract, such as phenolics, flavonoids, alkaloids, and saponins, may be responsible for the inhibitory impact seen in this investigation [23, 27].

The phytochemical composition of the extract and fractions may be linked to the α-amylase inhibitory activity demonstrated in this investigation. Enzymes that hydrolyze carbohydrates have been shown to be inhibited by a number of kinds of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, and other substances. These substances may interact with α-amylase in a variety of ways, such as by changing its conformation or interacting with the active site of the enzyme. However, rather than isolating particular chemicals, the current investigation

assessed the activity of the extract fractions. Consequently, no particular recognized alkaloid or phytochemical can be linked to the observed α-amylase inhibition. Rather, the activity might be the outcome of multiple components working together, including potential synergistic or additive interactions [28].

To identify the precise chemicals causing the observed activity, more research utilizing isolated compounds and enzyme-inhibition kinetics would be necessary.

3.3. Evaluation of the Antimicrobial Activity of Alkaloids Fractions

The antimicrobial activities of *P. maritimum* alkaloids extracts were assessed, The methanol extract of *P. maritimum* yielded four fractions with variable levels of activity the best was recorded in alkaloids fractions, it was 1 mg/mL against *pseudomonas aeruginosa* bacteria and 4mg/mL toward *E. faecalis,* however, its inhibitory effect was lower than that indicated for the reference antibiotics (Table **3**).

The alkaloid fraction demonstrated the highest antibacterial activity among the studied fractions, while the butanol, chloroform, and hexane fractions demonstrated relatively less activity. This result might be connected to the alkaloid fraction's enrichment of physiologically active alkaloids. Alkaloids may disrupt vital bacterial cellular functions and are known to have antibacterial qualities. Differences in the polarity and chemical composition of the substances divided into these fractions could be the cause of the reduced activity seen for the other solvent fractions. Alkaloids alone cannot, however, be solely responsible for the observed antibacterial activity because the alkaloid fraction may also contain additional co-extracted components that either independently or in concert may contribute to the overall activity [29, 30].

Alkaloids extraction was established by many authors [11, 31, 32], bulbs and flowers of the amaryllidaceous plant *Pancratium maritimum* have been investigated [13] however alkaloids from the mixture of stems and leaves have not yet been studied.

A previous study [10] explored the antimicrobial activity of alkaloids mixture from fruits and flowers that was inactive against *E. faecalis* bacteria .However pure methanolic extract showed low antibacterial activity, which suggests that the alkaloid fraction effectively concentrates the active components responsible for antibacterial activity [33].

Alkaloids mixture of fruits and bulbs was tested for antimicrobial activity by [34]. It was reported that Lycorine alkaloid showed antifungal activity against *Candida albicans* [35]. However higher MIC values were recorded in the hexane fraction (MIC=16 mg/mL against *P. aeruginosa*).

Table 3: Results of MIC and MBC values of Alkaloid Fraction (mg/mL)

Strains	alkaloid fraction			Ciprofloxacin (µg/mL)
	MIC	MBC	MBC/MIC Ratio	MIC
Staphylococcus aureus ATCC 6583	4	8	2	0.031
Pseudomonas aeruginosa ATCC27583	1	2	2	0.125
Enterococcus faecalis ATCC08152	4	8	2	0.125
Escherichia coli ATCC 25922	4	8	2	0.062

MIC: Minimum inhibitory concentration, **MBC:** Minimum bactericidal concentration.

Table 4: Results of MIC and MBC of hexane fraction (mg/mL)

Strains	Hexane fraction			Ciprofloxacin (µg/mL)
	MIC	MBC	MIC	MIC
***Staphylococcus aureus* ATCC 6583**	4	8	0.031	0.031
***Pseudomonas aeruginosa* ATCC27583**	16	32	0.125	0.125
***Enterococcus faecalis* ATCC08152**	2	8	0.125	0.125
***Escherichia coli* ATCC 25922**	8	16	0.062	0.062

MIC: Minimum inhibitory concentration, **MBC:** Minimum bactericidal concentration.

Table 5: Results of MIC and MBC values of chloroform fraction (mg/mL)

Strains	Chloroform fraction			Ciprofloxacin (µg/mL)
	MIC	MBC	MBC/MIC Ratio	MIC
***Staphylococcus aureus* ATCC 6583**	2	4	2	0.031
***Pseudomonas aeruginosa* ATCC27583**	8	16	2	0.125
***Enterococcus faecalis* ATCC08152**	2	4	2	0.125
***Escherichia coli* ATCC 25922**	8	16	2	0.062

MIC: Minimum inhibitory concentration, **MBC:** Minimum bactericidal concentration.

Table 6: Results of MIC and MBC Values of Butanol Fraction (mg/mL)

Strains	Butanol Fraction			Ciprofloxacin (µg/mL)
	MIC	MIC	MBC/MIC Ratio	MIC
***Staphylococcus aureus* ATCC 6583**	2	0.031	2	0.031
***Pseudomonas aeruginosa* ATCC27583**	4	0.125	2	0.125
***Enterococcus faecalis* ATCC08152**	2	0.125	2	0.125
***Escherichia coli* ATCC 25922**	2	0.062	2	0.062

MIC: Minimum inhibitory concentration, **MBC:** Minimum bactericidal concentration.

Table 7: Results of MIC and MFC values of Butanol Fraction (mg/mL)

Strains	Butanol Fraction			Amphotericin B (µg/mL)
	MIC	MFC	MFC/MIC Ratio	MIC
***Candida tropicalis* ATCC 3123**	4	8	2	0.5
***Candida albicans ATCC* 90028**	4	8	2	0.5
***Candida parapsilosis* ATCC 924**	0.125	0.25	2	0.5
***Candida glabrata* ATCC 913**	0.031	0.0625	2	0.5

MFC: minimum fungicidal concentration.

Different pharmaceutical properties of alkaloids were identified from *P. maritimum* including anti-tumor, antiplasmodial, anti-inflammatory, antimicrobial and antioxidant activities, which have been explored previously [36].

Previous studies demonstrated that alkaloids present in the bulbs of *P. maritimum* have therapeutic properties. As an exemple for this molecules Pancratistatin which was reported for its anticancer activity [37]. Recently, alkaloid from *pancartium maritimum* have been explored for its antiviral activity against SARS COV2 virus [38].

All the Alkaloid fractions were also evaluated for their anticandidal activities. Results are summarized in Tables **9**-**10**.

Alkaloid, chloroform, hexane, and butanol fractions demonstrated a considerable antifungal activity with MIC values ranging from 0.031 to 4 mg/mL. The most active extract was butanol fraction of the alkaloids (MIC=0.031 mg/mL against *C. glabrata* and MIC=0.125mg/mL against *C. parapsilosis*) (Table **7**). A reduced MFC value was efficient to exhibit *C. glabrata* yeast (MFC=0.0625 mg/mL).

It's interesting to note that out of all the fractions examined, the n-butanol fraction showed the highest anti-Candida activity. This finding could be explained by the n-butanol fraction's abundance of comparatively polar and moderately polar secondary metabolites, such as flavonoids and phenolic compounds, which have been shown to have antifungal effect against *Candida* species. According to earlier research, flavonoids and phenolic compounds originating from plants can prevent *Candida* from growing and may have an impact on biofilm formation, fungal cell membranes, and other cellular functions [39, 40].

Table 8: Results of MIC and MBC values of alkaloid fraction (mg/mL)

Strains	Alkaloid Fraction			Amphotericin B (µg/mL)
	MIC	MFC	MFC/MIC Ratio	MIC
***Candida tropicalis* ATCC 3123**	4	8	2	0.5
***Candida albicans ATCC* 90028**	4	8	2	0.5
***Candida parapsilosis* ATCC 924**	0.5	1	2	0.5
***Candida glabrata* ATCC 913**	0.25	0.5	2	0.5

MFC: minimum fungicidal concentration

Table 9: Results of MIC and MBC values of hexane fraction (mg/mL)

Strains	Hexane Fraction			Amphotericin B (µg/mL)
	MIC	MFC	MFC/MIC Ratio	MIC
***Candida tropicalis* ATCC 3123**	2	4	2	0.5
***Candida albicans ATCC* 90028**	2	4	2	0.5
***Candida parapsilosis* ATCC 924**	2	4	2	0.5
***Candida glabrata* ATCC 913**	1	2	2	0.5

MFC: minimum fungicidal concentration.

Table 10: Results of MIC and MBC values of chloroform fraction (mg/mL)

Strains	Chloroform Fraction			Amphotericin B (µg/mL)
	MIC	MBC	MBC/MIC Ratio	MIC
***Candida tropicalis* ATCC 3123**	4	8	2	0.5
***Candida albicans ATCC* 90028**	4	8	2	0.5
***Candida parapsilosis* ATCC 924**	1	2	2	0.5
***Candida glabrata* ATCC 913**	1	2	2	0.5

MFC: minimum fungicidal concentration.

Nevertheless, the current study did not specifically look into these mechanisms.

In fact, a recent study reported anti-fungal and antibiofilm activity for an n-butanol fraction of Terminalia catappa, which was rich in tannins and showed activity against different Candida strains [41].

Furthermore, the extremely low minimum inhibitory concentrations (MICs) for the steroidal alkaloids solacongestidine, solafloridine, and verazine come from a previous study that looked at a number of *Solanum* alkaloids. With a minimum inhibitory concentration (MIC) of 0.8 µg/mL, solacongestidine was especially effective against *Candida albicans* [42]. MICs of 8 µg/mL against *Candida albicans* and 4 µg/mL against *Candida tropicalis* were reported in more recent research on the indole alkaloid globospiramine [43].

Depending on the examined microorganism and the fractions under investigation, the antibacterial activity differed significantly. The n-butanol fraction demonstrated the highest activity against the tested strains of *Candida*, while the alkaloid fraction showed the most noticeable antibacterial activity. This discrepancy could be explained by the different chemical profiles of the fractions that arise from solvent partitioning and the phytochemicals' preferential distribution based on their polarity and chemical characteristics [44].

Different cellular structures and susceptibilities to substances derived from plants may also be reflected in the distinct reactions of bacterial and fungal strains. Nevertheless, the current findings do not prove a clear connection between the observed antibacterial action and a particular phytochemical component. Therefore, rather than being proof of a particular mechanism of action, the variations in activity should be seen as

reflecting the overall chemical composition of each fraction and the differential susceptibility of the microbes [45].

4. CONCLUSION

According to our results, stem and leaf methanolic extract of Tunisian *Pancratium maritimum* was rich in alkaloids notably lycorine, galanthamine, and hordenine which led us to investigate more in alkaloid fractions and show the considerable antibacterial and antifungal potentials of these fractions specifically the alkaloid fraction (MIC=1mg/ml against *P. aeruginosa*) and the chloroform fraction (MIC=1mg/ml against *C. parapsilosis* and *C. glabrata*). Moreover, the marked amylase inhibitory effect of methanolic extract of *P. maritimum* further supports the potential of this plant as a valuable source of natural bioactive compounds.

The fractions' varied phytochemical composition, especially their alkaloid and other secondary metabolite concentrations, may be linked to the reported activity. These results, however, should not be immediately applied to therapeutic or nutritional uses; rather, they should be regarded as initial proof of biological activity. To confirm the biological effects and evaluate their potential practical applications, additional research involving the isolation and characterization of individual bioactive compounds, mechanism-based enzyme and antimicrobial studies, thorough toxicity and safety evaluation, and suitable in-vivo studies is required.

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DATA AVAILABILITY STATEMENT

Data is contained within the article.

CONFLICT OF INTEREST

The authors declare no financial or personal interests that could have influenced the work presented in this study.

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