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Research Article

IN VITRO INVESTIGATION OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *HARPAGOPHYTUM PROCUMBENS* SEEDS EXTRACTS

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ABSTRACT

Medicinal plants contain bioactive substances that are highly bioavailable in extracts or pure molecules, making them promising for therapeutic applications and precursors for chemo-pharmaceutical semi-synthesis. *Harpagophytum procumbens* (Devil's Claw) is widely recognized as one of the most potent therapeutic herbs. This study aimed to extract seeds from *H. procumbens* using two types of solvents and to assess both qualitative and quantitative aspects of the extracts. The two extracts were evaluated for antibacterial and anti-biofilm activities using agar well diffusion assays against four bacterial isolates and two yeast isolates. Qualitative analysis identified the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids. The active components detected were: alkaloids (12.69%), flavonoids (3.25%), total phenolic compounds (24.58%), total terpenoids (8.55%), and total steroids (1.25% for methanolic and 4.55% for petroleum ether). Both methanolic and petroleum ether extracts exhibited antioxidant activities of approximately 85.33% and 74.19%, respectively, compared to ascorbic acid, which had an antioxidant effect of 67.99% at a concentration of 200 µg/ml. The extracts demonstrated a broad spectrum of activity against all tested bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*) and fungi (*Candida albicans*, *C. tropicalis*, *C. parapsilosis*). At a concentration of 1000 µg/ml, the seed extracts showed the highest bactericidal activity, with inhibition zones ranging from 10 to 22 mm. Moreover, both extracts exhibited greater anti-biofilm activity at 1000 µg/ml compared to lower concentrations. Our study found that seed extracts of *H. procumbens* possess significant antibacterial and antioxidant activities, particularly at a concentration of 1000 µg/ml.

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INTRODUCTION

Medicinal plants hold significant value as natural sources of bioactive compounds, which can be used to produce pharmaceutical drugs. These natural compounds have the potential to treat a wide range of disorders. Moreover, medicinal plants offer a variety of chemical compounds and elements with potential health benefits. These compounds can be obtained through standardized

extracts or as pure chemicals (Hassan and Ullah, 2019).

Harpagophytum, commonly known as Devil's Claw, is a member of the Pedaliaceae family (Ihlenfeldt, 1988). This plant is a perennial herbaceous species characterized by tubular flowers, spiky hooks, and fruit in the form of capsules (Stewart and Cole, 2005). It is currently facing the threat of extinction in regions such as India, Asia, and Southern Africa. The plant contains a large quantity of active compounds, including alkaloids (Baghdikian et al., 1999), saponins, flavonoids, phenols, terpenoids, volatile compounds, and phytosterols (McGregor et al., 2005; Mariano et al., 2020). Additionally, it is rich in iridoid glucosides (Brendler, 2021).

Devil's Claw has a long history of use in traditional medicine for various ailments, including alleviating constipation (Georgiev et al., 2010), treating arthritis and bacterial infections (Brendler, 2021), managing diabetes and blood disorders (Stewart and Cole, 2005), relieving pain and fever, addressing osteoarthritis and rheumatic diseases, combating malaria and heart disease, mitigating tendonitis and renal dysfunction (Hu et al., 2021), and exhibiting anti-inflammatory and antioxidant properties (Lang et al., 2020).

The bioactive substances in this plant can reduce numerous health issues. These natural compounds can serve as antioxidants and exhibit antibacterial activity through various mechanisms, such as the suppression of DPPH activity. Many chemicals can induce oxidative stress in cells and tissues, leading to the production of reactive oxygen species (ROS). This oxidative stress can cause various types of damage, including the oxidation of DNA, lipids, and proteins. Such damage can contribute to the development of severe conditions, including cancer and vascular diseases (Georgiev et al., 2010).

Antioxidants inhibit oxidative stress in biological molecules by scavenging free radicals, thereby preventing the initiation and progression of oxidative chain reactions (Shah and Modi, 2015). Numerous bacterial strains, both gram-positive and gram-negative, as well as fungi, are capable of causing a broad spectrum of diseases in humans, animals, and plants. This is largely due to their inherent resistance to conventional antibiotics and antifungal medications (Funari and Shen, 2022). Biofilms are aggregations of bacteria bound together by a complex matrix composed of polysaccharides, proteins, and DNA, as explained by Muhammad et al. (2020). These stationary biofilms serve as significant sources of infection on medical devices such as catheters, sutures, and implants (Sharma et al., 2019). This study examined the antibacterial and anti-biofilm activities, antioxidant effects, and phytochemical composition of seed extracts from *Harpagophytum procumbens*. Moreover, it explored the potential biological applications of these extracts as alternative treatments for various infections and diseases.

MATERIALS AND METHODS

Sample collection and extraction process

The fruits of *Harpagophytum procumbens* were collected from Kut City, Iraq. After collection, the fruits were dried, and the seeds were ground. Extraction was then carried out using two different solvents. Petroleum ether, a non-polar solvent, was used for extraction in a Soxhlet apparatus at a solvent-to-sample ratio of 10:1 for 4 h. Methanol, a polar solvent, was employed for extraction over 6 to 8 h at temperatures ranging from 60 to 80°C. The resulting extracts were filtered and dried over 15 h.

Qualitative investigation of bioactive compounds in Devil's Claw

A conventional preliminary phytochemical screening was conducted to identify the bioactive components in *H. procumbens* seed extracts. The principal bioactive components identified by Ali et al. (2022) included tannins, alkaloids, saponins, terpenes, and flavonoids. The petroleum ether and methanolic extracts were analyzed using specific qualitative reagents for each bioactive component:

a. Alkaloid detection

Wagner's and Mayer's reagents were used to determine the presence of alkaloids.

b. Flavonoid detection

Flavonoids were identified using magnesium crystals combined with either 1% hydrochloric acid or concentrated sulfuric acid.

c. Tannin detection

Tannins were detected by adding drops of lead acetate or ferric chloride to each extract.

d. Saponin detection

Saponins were identified by observing foam formation or by using $HgCl_2$ as an indicator. Active chemicals can be identified using a sequential process including the use of chloroform and H_2SO_4, or by employing p-anisaldehyde to detect terpenes.

Quantitative Analysis of Devil's Claw Active Compounds

Total alkaloid estimation

The quantification of alkaloids was performed following

the methodology described by Ajanal et al. (2012). Initially, alkaloids were extracted from the plant material using petroleum ether and methanol. The extracts of *H. procumbens* were dissolved in 2N hydrochloric acid and transferred to a separatory funnel. The solution underwent three rounds of washing with 10 ml of chloroform each time. The pH of the mixture was then adjusted to neutral by adding 0.1N NaOH. Subsequently, 5 ml of Bromocresol Green (BCG) solution and 5 ml of phosphate buffer (pH 7) were added to the mixture.

The quantification of alkaloids was conducted using the Atropine Standard Method. A standard curve was generated by adding varying volumes (0.4, 0.6, 0.8, 1.0, and 1.2 ml) of a 1 mg/10 g atropine solution to the extracts. The samples were then agitated for 1 min with 1, 2, 3, and 4 ml of chloroform, resulting in a final volume of 10 ml. The absorbance of the solution was measured at a wavelength of 470 nm and compared to a blank solution containing the same mixture of extracts without atropine.

Total phenolic estimation

The total phenolic content in the petroleum ether and methanol extracts was determined using the Folin-Ciocalteu reagent. Each 100 ml of extract was mixed with 500 ml of the reagent and 1.5 ml of a 20% Na_2CO_3 solution. After stirring the solution for 2 min, 10 ml of distilled water was added to adjust the volume. The mixture was allowed to react at room temperature for 2 h. The absorbance was measured at a wavelength of 765 nm to determine the phenolic content, which was then compared to a gallic acid calibration curve. The phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry weight. The procedure was conducted three times, as detailed by Laouini et al. (2017).

Total terpenoids estimation

The estimation of total terpenoids was carried out according to the procedure described by Ghorai et al. (2012). A 1.5 g sample of each extract was dissolved in a mixture of 7 ml of methanol and acetonitrile. The solution was shaken for 30 min and then incubated in the dark at room temperature for 24 h. After incubation, the mixture was centrifuged at 6000 RPM for 15 min. From the resulting supernatant, 5 ml was collected, to which 0.5 ml of H_2SO_4 and 1.5 ml of chloroform were added. The mixture was vortexed for 1 min, diluted with methanol, and the volume was adjusted to 10 ml. The absorbance was measured at 538 nm to determine the terpenoid content. Standard samples were prepared using various concentrations of linalool.

Total steroid estimation

The total steroid content was determined using Zak's method (Ziatkis et al., 1953). The sample underwent a reaction with ferric chloride, concentrated sulfuric acid, and a red color indicator. Ferric chloride and the cholesterol reagent were found to produce a reaction, resulting in a colored complex. To prepare the ferric chloride solution, 840 mg of ferric chloride was dissolved in 100 ml of glacial acetic acid. Then, 10 ml of this solution was combined with 100 ml of glacial acetic acid, and 8.5 ml of the resulting solution was further diluted with 100 ml of glacial acetic acid. Next, 10 ml of the cholesterol solution was mixed with 0.85 ml of the ferric chloride stock reagent, and the total volume was adjusted to 100 ml using glacial acetic acid. This final solution, with a concentration of 100 µg/ml, served as the standard working solution.

Approximately 0.1 to 0.2 ml of the *H. procumbens* extract, obtained after triple acidic extraction, was used. A standard solution ranging from 0.5 ml to 2.5 ml was added to a tube. This solution was prepared by mixing 10 ml of cholesterol, 0.85 ml of ferric chloride stock reagent, and 100 ml of glacial acetic acid. The volume was precisely adjusted to 5 ml using the final ferric chloride reagent. A blank solution was prepared by mixing 5 ml of diluted ferric chloride with 4 ml of H_2SO_4. The tubes were then incubated for 30 min to allow the color intensity to develop. Finally, the absorbance was measured at a wavelength of 540 nm to quantify the steroid content.

Determination of antioxidant activity

The antioxidant activity of the two *H. procumbens* seed extracts was determined using the 1,1'-diphenyl-2-picrylhydrazyl (DPPH) assay. A 0.5 ml solution of DPPH in methanol was mixed with either 3.5 ml of petroleum ether extract or methanol extract. The mixture was kept in darkness at room temperature for 20 min. The absorbance of the mixture was then measured at a wavelength of 540 nm. The experiment was repeated three times. The DPPH radical scavenging activity was calculated using the formula provided by Kalabharthi et al. (2021):

$$\% \text{ DPPH radical scavenging activity} = \frac{A0 - A1}{A0} \times 100$$

Where A_0 = absorbance of the blank

A_1 = absorbance of the standard or test

Microbial isolates

The microbes used in this study consisted of four types of bacteria viz. two gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) as well as two species of yeast (*Candida albicans*, *C. parapsilosis* and *C. tropicalis*). Both bacteria and yeast were collected and cultured at the Microbiology Laboratory of the College of Science for Women, University of Baghdad. The identification of these microbes was carried out using various methods (Aslam and Mukhtar, 2023, 2024; Aziz et al., 2024). The bacteria and yeast were cultured in nutrient broth at 37°C for 18 h to achieve a bacterial concentration of 1×10^8 cfu/ml. This preparation was used to study the antibacterial activity of *H. procumbens* seed extracts. The microbial isolates were preserved on slants containing specialized culture media: Nutrient agar for bacteria and Sabouraud dextrose agar for yeast.

Antimicrobial activity of methanolic and petroleum ether extracts of *H. procumbens* seeds

To test the antimicrobial properties of the seed extracts, four different concentrations (1000, 750, 500, and 250 µg/ml) were prepared using methanolic and petroleum ether extracts. The Mueller-Hinton agar (Hi-media) and Sabouraud dextrose agar (Hi-media) were sterilized by autoclaving at 121°C for 15 min at 1.5 bar pressure, according to the manufacturer's instructions. After sterilization, 20 ml of the medium was carefully poured into each Petri plate and allowed to cool and solidify.

The antibacterial activity of the *H. procumbens* seed extracts was assessed using the disc diffusion agar method against the tested bacteria and yeast. Each microbial isolate, at a concentration of 1×10^8 cfu/ml, was spread evenly across the surface of the agar plates using 100 µl of inoculum. Wells with a diameter of 6 mm were then created in the agar using a cork borer. Subsequently, 100 µl of each extract concentration was added to the wells. Sterilized distilled water and dimethyl sulfoxide (DMSO) were used as negative controls. The Petri dishes were incubated at 37°C for 24 h. The diameter of the inhibition zones was measured using a ruler. The experiment was repeated three times to ensure accuracy.

Anti-biofilm activity of methanolic and petroleum ether extracts of *H. procumbens* seeds

The potential effects of *H. procumbens* seed extracts in preventing and inhibiting biofilm formation by pathogenic microorganisms were evaluated by assessing their anti-biofilm activity using 96-well polypropylene microplates. The level of biofilm formation was carefully measured under optimal experimental conditions (Famuyide et al., 2019).

The bacteria and yeasts used in the study were cultured overnight and adjusted to a 0.5 McFarland standard in Lysogeny Broth (LB). The cells were then co-cultured with either methanolic or petroleum ether seed extracts at a concentration of 1000 µg/ml. The negative control consisted of a mixture of DMSO and sterilized distilled water. The co-culture was maintained for 24 h at 37°C with gentle agitation.

A spectrophotometer (Optima Single Beam UV-VIS model) was used to quantify bacterial and yeast growth by measuring absorbance at a wavelength of OD_{560}. Biofilms were stained by adding 100 µl of crystal violet and incubating for 30 min at 25°C. The plates were then emptied and thoroughly dried. The dried crystal violet was dissolved in 95% ethanol (v/v), and the biofilm growth was assessed by measuring absorbance at OD_{570}. The experiments were repeated three times, and results were expressed as mean values with their respective standard deviations.

The ability of the extracts to eliminate preexisting biofilms was assessed using the method described by Famuyide et al. (2019). Biofilm formation was classified into four categories based on the established OD cut-off values (ODc) and biofilm density (Table 1).

Table 1. Classification of biofilm formation according to biofilm density (Davarzani et al., 2021).

Optical Density Values (OD)	Interpretation of biofilm production
$OD \leq ODc$	No biofilm production
$ODc < OD \leq 2 \times ODc$	Weak biofilm production
$2 \times ODc < OD \leq 4 \times ODc$	Moderate biofilm production
$4 \times ODc < OD$	Strong biofilm production

Statistical Analysis

Statistical analysis was conducted to determine the impact of various factors on the research using the SAS (2018) program. Means were compared using one-way ANOVA, followed by the least significant difference (LSD) test to identify statistically significant differences. Data analysis was performed using GraphPad Prism version 6.0, while Microsoft Excel 365 was used for data entry and organization. According to SAS (2018), necessary comparisons were made using Tukey's post hoc test, with a significance level set at $P<0.01$.

RESULTS AND DISCUSSION

Qualitative investigation of *H. procumbens* active compounds

Analysis of *H. procumbens* seed extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in both petroleum ether and methanolic extracts, as shown in Table 2. These findings align with a previous study by McGregor et al. (2005), which identified flavonoids in *H. procumbens*. Additionally, Avato and Argentieri (2019) reported the presence of phenols and flavonoids in alcoholic extracts of the plant. The results are also consistent with research conducted by Mariano et al. (2020). Variability in plant active compounds may be influenced by factors such as light, temperature, growing region, pH, salinity, and soil composition (Cirak and Radusiene, 2019).

Quantitative analysis of *H. procumbens* active compounds

Table 3 presents the quantities of bioactive compounds found in methanol and petroleum ether seed extracts, including steroids, terpenoids, alkaloids, and phenols. The results indicate that the methanolic extract contains the highest quantity of phenolic compounds and the lowest concentration of steroids. Conversely, the petroleum ether extract has the highest concentration of terpenoids and the lowest concentration of steroids. The methanolic extract also shows higher concentrations of alkaloids and phenolics compared to the petroleum ether extract. Notably, the petroleum ether extract contains a higher content of terpenoids and steroids than the methanolic extract.

These findings align with Lang et al. (2022), who reported that phenolic compounds in alcoholic tuber extracts had the highest concentration among the tested chemicals, with alkaloids closely following. The differences in active compound concentrations are likely due to variations in solvent polarity. Polar compounds like phenolics and alkaloids dissolve preferentially in methanol, whereas nonpolar compounds such as terpenoids and steroids are more effectively extracted by nonpolar solvents like petroleum ether (Rustiani et al., 2021).

Table 2: Qualitative analysis of some active compound of *H. procumbens* extracts.

Active compounds	Reagent type	Methanolic extract	Petroleum ether extract
Alkaloids	Wagner	+	+
	Mayer	+	+
Flavonoids	Magnesium crystals and 1% HCl	+	+
	H_2SO_4	+	+
Tannins	Lead acetate	+	+
	$FeCl_3$	+	+
Saponins	Foam	+	+
	$HgCl_2$	+	+
Terpenoids	Chloroform and H_2SO_4	+	+
	p-Anisaldehyde	+	+

+ The compound presence.

Table 3. Qualitative amount of some active compounds of H. procumbens extracts.

Sr. No.	Type of bioactive compounds	Methanolic	Petroleum ether
1	Total alkaloid content %	12.69	3.25
2	Total phenolic content (mg Gallic /100 g)	24.58	8.55
3	Total terpenoid content %	2.58	13.6
4	Total steroid %	1.25	4.55

Antioxidant activity

The DPPH method was employed to assess the antioxidant activity of *H. procumbens* extracts, following the procedure outlined by Kalabharthi et al. (2021). Table 4 presents the significant antioxidant properties of the extracts compared to ascorbic acid. The results indicate that the scavenging activity increased with higher concentrations of *H. procumbens* extract. All five concentrations of the two extracts exhibited significant variations in antioxidant activity. The methanolic extract demonstrated superior antioxidant activity compared to the petroleum ether extract across all concentrations. The antioxidant effect was highest at a concentration of 200 µg/ml, with values of 85.33 for the methanolic extract and 74.19 for the petroleum ether extract. Conversely, at a concentration of 12.5 µg/ml, scavenging activity was lowest, with values of 30.96 for the methanolic extract and 28.67 for the petroleum ether extract. The high antioxidant activity of *H. procumbens* is attributed to its bioactive components, including alkaloids, phenols, flavonoids, steroids, and terpenoids, which are known to neutralize free radicals. These findings are consistent with previous studies that investigated alcoholic extracts of the tuber, as well as research by Kalabharthi et al. (2021), which examined the antioxidant activity of *H. procumbens* leaves.

Table 4. *In vitro* antioxidant activity of *H. procumbens* seed extracts (methanolic and petroleum ether extracts), using DPPH free radical scavenging activity.

Concentration µg/mL	Ascorbic Acid	Methanolic extract	Petroleum ether extract
12.5	25.70 ± 0.62 E	30.96 ± 0.96 D	28.67 ± 1.20 C
25.0	32.72 ± 1.18 D	52.20 ± 1.79 C	34.84 ± 1.52 C
50	46.76 ± 2.55 C	57.91 ± 2.63 C	52.39 ± 2.48 B
100	54.05 ± 1.98 B	65.20 ± 2.05 B	68.29 ± 2.85 A
200	67.99 ± 2.85 A	85.33 ± 3.19 A	74.19 ± 3.02 A
LSD value	6.82 **	8.22 **	7.41 **

Samples against DPPH were calculated as a (mean ± SD) of triplicate tests, with $p < 0.01$ indicating statistical significance.

Means with different capital letters in the same column are significantly different (P≤0.01).

Antimicrobial activity of methanolic and petroleum ether extracts of *H. procumbens* seeds

The antibacterial activity of methanolic and petroleum ether extracts from *H. procumbens* seeds was evaluated using the disc diffusion method against various yeast and bacterial strains. The results, shown in Table 5, reveal significant variations among different extract concentrations (p<0.01). It was observed that the inhibition zone increased with higher extract concentrations, with the most pronounced effect at the 1000 µg/ml concentration. Specifically, Table 5 and Figure 1 illustrate that the inhibition zones around the wells for *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*, *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were 22 mm, 18 mm, 19 mm, 20 mm, 16 mm, 16 mm, and 20 mm, respectively.

Table 5. Diameters of halo zones (mm) formed around discs containing various concentrations of petroleum ether extract from *H. procumbens* seeds.

Microorganisms	Concentrations (µg/mL)				
	1000	750	500	250	Control
E. coli	22 ± 0.51	15 ± 0.9	12 ± 0.41	7 ± 1.55	0
P. aeruginosa	18 ± 0.2	14 ± 0.78	14 ± 1.9	8 ± 2.0	0
S. aureus	19 ± 0.19	15 ± 2.0	12 ± 2.1	8 ± 3.2	0
S. pyogenes	20 ± 1.2	16 ± 1.88	11 ± 0.54	9 ± 0.77	0
C. albicans	16 ± 1.5	13 ± 0.22	13 ± 1.1	7 ± 0.9	0
C. tropicals	16 ± 0.44	12 ± 0.11	10 ± 2.5	6 ± 0.88	0
C. parapsilosis	20 ± 0.23	14 ± 0.31	15 ± 0.27	6 ± 0.66	0

The data are presented as the mean ± SD of triplicate tests, with p < 0.01 indicating statistical significance.

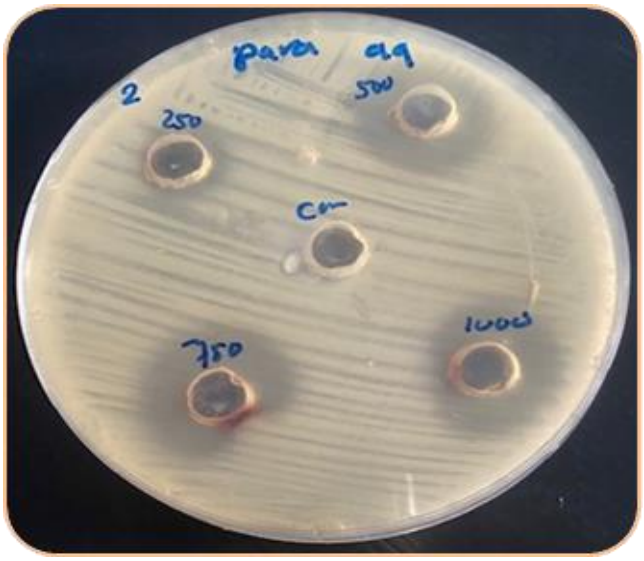

Figure 1. Inhibition zones formed around wells filled with different concentrations of fixed oil extracted from *H. procumbens* seeds against *C. parapsilosis*, compared with a negative control. All plates were incubated at 37°C for 24 hours. Data are presented as mean values ± SD of three replicates.

The antibacterial activity of methanolic extracts from *H. procumbens* seeds was investigated against several pathogens. The results of these examinations are presented in Table 6. The various concentrations exhibited different effects on the studied pathogens. Concentrations of 1000 and 500 µg/ml demonstrated efficacy in inhibiting bacteria and yeast. The inhibition zone diameter increased proportionally with the concentration of the methanolic extracts compared to the control (sterilized distilled water). The largest inhibition zone was observed at a concentration of 1000 µg/ml.

Statistically significant differences were found between the control and the treated samples. The pathogenic strains *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*, *C. albicans*, *C. tropicalis*, and *C. parapsilosis* showed inhibition zones measuring 22, 18, 19, 20, 16, 16, and 20 mm, respectively, as indicated in Table 6.

Anti-biofilm effects of methanolic and petroleum ether extracts on biofilm formation by bacteria and yeast isolates

An anti-biofilm study demonstrated that methanolic and petroleum ether seed extracts of *H. procumbens* inhibited the formation of biofilms by various microbial pathogens. The study employed the crystal violet staining technique, which revealed that methanolic extracts of *H. procumbens* effectively reduced the ability of pathogenic bacterial and yeast isolates to form biofilms - a key virulence factor. The results indicated that biofilm production and the number of attached cells decreased significantly at higher concentrations of 1000 and 750 µg/ml, with inhibition rates reaching 90% and 47%, respectively. In contrast, the lowest concentration tested (250 µg/ml) showed minimal effect on biofilm formation compared to the negative control, as illustrated in Figure 2.

All bars represent the standard deviation. The percentage of biofilm formation was calculated with the negative control set at 100%. The red bars indicate the control, while the green bars represent biofilm inhibition at a concentration of 250 µg/ml, pink bars represent 500 µg/ml, blue bars represent 750 µg/ml, and gray bars represent 1000 µg/ml. One-way ANOVA followed by Tukey's test was used to compare the treatment concentrations against the control.

Table 6: Diameters of the inhibition zones (mm) around discs containing varying concentrations of methanolic extract from *H. procumbens* seeds.

Microorganisms	Concentrations (µg/mL)				
	1000	750	500	250	Control
E. coli	18 ± 0.9	18 ± 0.4	14 ± 0.22	6 ± 1.45	0
P. aeruginosa	20 ± 0.7	14 ± 0.51	16 ± 1.5	8 ± 2.0	0
S. aureus	18 ± 0.16	14 ± 1.8	12 ± 2.1	9 ± 3.2	0
S. pyogenes	20 ± 1.2	16 ± 1.88	17 ± 0.33	8 ± 0.66	0
C. albicans	16 ± 1.4	15 ± 0.10	20 ± 0.1	8 ± 0.9	0
C. tropicals	10 ± 0.40	16 ± 0.9	15 ± 2.5	7 ± 0.66	0
C. parapsilosis	10 ± 0.25	16 ± 0.33	16 ± 0.27	8 ± 0.62	0

The data are presented as mean values ± standard deviation (mean ± SD) from three replicates.

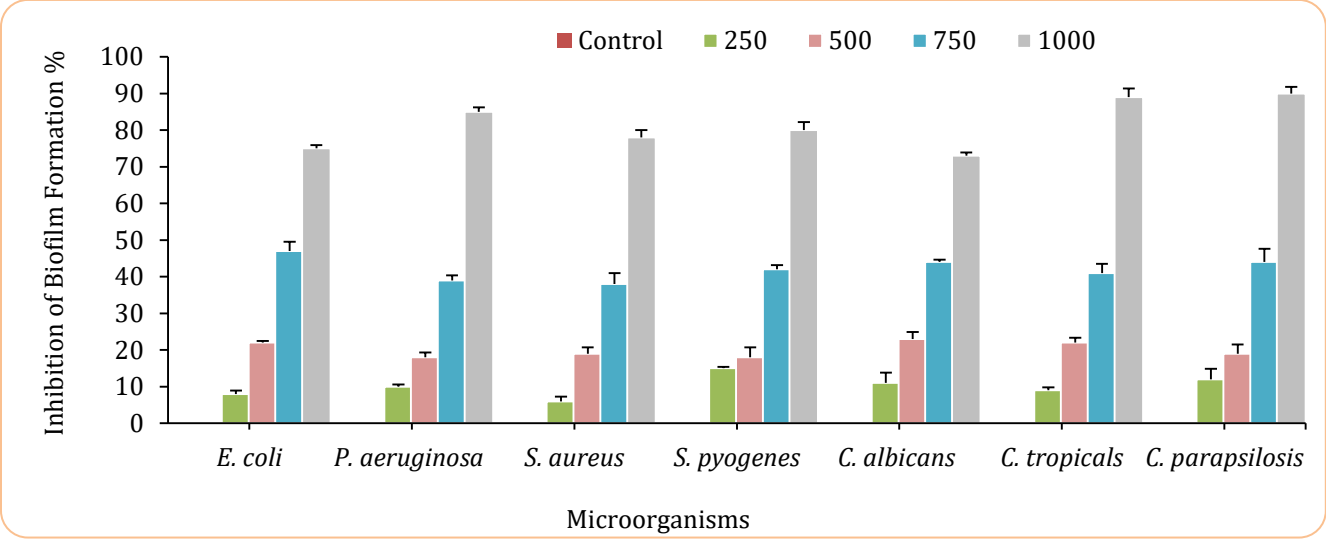

Figure 2. Effects of methanolic extracts from *H. procumbens* seeds at concentrations ranging from 250 to 1000 µg/ml on biofilm growth by pathogenic bacteria and yeast.

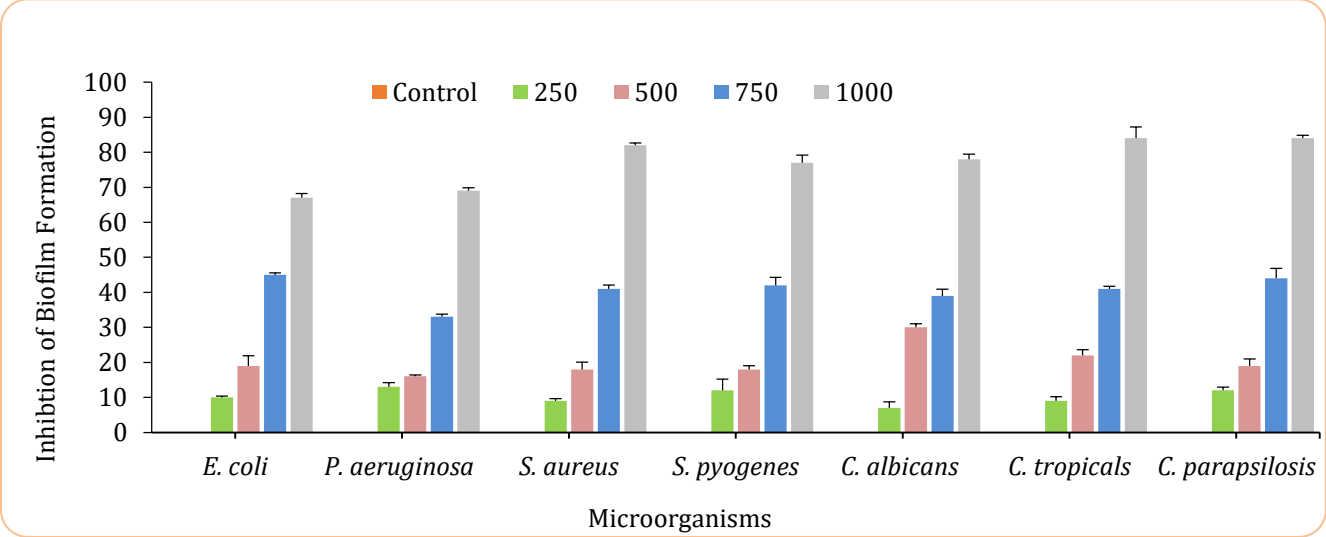

Figure 3. Effects of various concentrations of petroleum ether extracts from *H. procumbens* seeds (250–1000 µg/mL) on biofilm formation by pathogenic bacteria and yeast isolates.

All bars represent standard deviation. The percentage of biofilm formation was calculated with the negative control set at 100%. Red bars indicate the control, while green bars represent inhibition at 250 µg/ml, pink bars represent 500 µg/ml, blue bars represent 750 µg/ml, and gray bars represent 1000 µg/ml. One-way ANOVA followed by Tukey's test was used to compare the effects of all concentrations against the control treatment.

The results indicated significant variation and impact across all three experiments involving the four concentrations of petroleum ether used to extract H. procumbens seeds. These extracts exhibited dose-dependent antimicrobial and anti-biofilm activities. High concentrations (750 and 1000 µg/ml) notably reduced biofilm formation, with inhibition zones of 84% and 45%, respectively. In contrast, the lower concentrations (250-500 µg/ml) had a minimal impact on pathogen formation, as illustrated in Figure 3.

Figure 2 demonstrates that methanolic extracts had more pronounced inhibitory effects against pathogens

compared to petroleum ether extracts. This difference is likely due to the distinct phytochemical classes extracted by solvents of varying polarities. For instance, methanol, a polar solvent, is more effective at dissolving polar compounds such as phenolics and alkaloids, whereas nonpolar solvents like petroleum ether extract nonpolar compounds such as terpenoids and steroids (Rustiani et al., 2021).

These findings align with Lang et al. (2022), which showed that phenolic compounds in alcoholic tuber extracts were present in higher concentrations than other tested chemicals, with alkaloids being the next most prevalent. H. procumbens has recently gained recognition as a potent phytomedicine. However, existing research has not thoroughly explored its effectiveness in inhibiting bacterial and fungal infections (Brendler, 2021), with studies primarily focusing on its anti-inflammatory and antibacterial properties.

This key contributions of the study include evaluating various extracts, isolated chemicals, and extract fractions. The results demonstrated significant antibacterial activity against various bacterial types associated with autoimmune diseases and pathogens in the human gut. Methanolic extracts were particularly rich in tannins, polyphenols, and flavonoids. These bioactive compounds suppress microorganisms by binding to extracellular and soluble proteins in bacterial cell walls (Narayana et al., 2001; Kaur and Arora, 2009). Phenolic substances also inhibit microorganisms through specific enzymatic pathways (Anand et al., 2019). Thus, conducting a phytochemical analysis and purifying bioactive components of Devil's Claw seed extract is crucial for developing effective treatments for drug-resistant bacteria.

CONCLUSION

The studies confirmed that the methanolic and petroleum ether extracts, as well as the fractions of *Harpagophytum procumbens* seeds, contain various bioactive substances, including phenolics, terpenoids, steroids, and alkaloids. Moreover, the research demonstrated that these seed extracts exhibit antioxidant, antibacterial, and anti-biofilm activities against several pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*) and fungi (*Candida albicans*, *C. tropicalis*, *C. parapsilosis*). Future studies should focus on comparing the bioactive substances found in Devil's Claw seeds with commercial antibiotics, or exploring combinations of these bioactive compounds with antibiotics, to identify effective and safe treatments for drug-resistant bacteria.

AUTHORS' CONTRIBUTIONS

HA designed formulated and laid out the study, SAA and GS conducted the experiments and collected data; and SH arranged and analyzed the data; WH provided technical assistance; SAA supervised the work; IA wrote the manuscript; IA proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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