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Antibacterial Activity of Alkaloids, Phenols Extracts, and Phytochemical Screening of Bioactive Compounds of *Borago officinalis* plant

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Abstract

The overprescription and uses the antibiotics which are produced to rise of drug-resistant bacteria, and this causes a significant global public health threat, this led to discovery phytochemicals compounds such as natural products of medicinal plants which have antimicrobial agent. This study was aimed to evaluate antibacterial activity of alkaloids and phenols extracts of *Borago officinalis* against urinary tract infection UTI in waist province, Gram-positive bacteria such as *Staphylococcus aureus and* Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Phenol extracts were having significant highest antibacterial activity at $p \leq 0.05$ against bacteria than alkaloids. The results showed the *B. officinalis* contains gallic, rutin, alkaloid, glycoside, tannins, and saponins through the estimation of active compounds in plant, The results of High-performance liquid chromatography (HPLC) showed occurrence Gallic acid, Apigenin, Ferulic acid, Qurcetine, Rutin, Sinapic acid compounds, the higher concentration compound was gallic (93.6mg/gm), while, Ferulic acid (44.6 mg/gm) lower concentration in plant. The results showed *S. aureus* was more sensitive for *B. officinalis* extracts, while *P. aeruginosa* was more resistance for each extract.

Keywords: Plant Extracts, *B. officinalis*, Medicinal Plant, HPLC, Urinary Tract Infection UTI.

I. Introduction

The increasing resistance of pathogenic bacteria to conventional antibiotics has become a significant global health concern, limiting the effectiveness of standard therapeutic options. This alarming trend has driven researchers and healthcare practitioners to explore alternative strategies, including the use of medicinal plants, which are known to possess bioactive compounds such as alkaloids, flavonoids, and phenolics with antimicrobial properties. These natural products are gaining attention as potential solutions to combat multidrug-resistant microorganisms due to their diverse mechanisms of action and reduced risk of resistance development (Balouiri *et al*., 2016). Urinary tract infections (UTIs) are indeed more frequent in females compared to males primarily due to anatomical differences, specifically the shorter urethra in females, which allows bacteria to reach the bladder more easily. The female urethra is approximately 1.5 inches long, while the male urethra is about 8 to 9 inches long (Czajkowski *et al.,*2021). Several plant extracts have demonstrated significant anti-arthritic and anti-inflammatory potential. Some of these extracts show promise as complementary or alternative medicines, while others are being investigated for their potential to

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lessen arthritis-related pain and inflammation. (Farooq *et al.,* 2022). Borage *B. officinalis* a species of the Boraginaceae family, is a native herb of the Mediterranean region (Seifzadeh *et al.*, 2020), some studies indicate that Borage is commonly used adjunctively in disorders of the respiratory system, urinary tract, arthritis and skin problems. Biologically active compounds found in borage oil are used as additives in the treatment of atherosclerosis, as well as in the regulation of certain metabolic processes (Pieszak *et al.,* 2012). This plant contains various active constituents that make it useful for medicinal purposes beyond its primary use also includes flavonoids, phenolic acids, and other substances (Ibrahim and AlShammaa, 2023). Is used in the traditional medical practices of various countries and for multiple treatments, including respiratory disorders, colds, influenza, diarrhea, cramps, inflammation, palpitation, hypertension menopause, and post-menopausal symptoms. Its pharmacological properties and biological activities among them antioxidant, antimicrobial, anticancer, anti-inflammatory, insecticidal, antigenotoxic, and anti-obesity activity (Slama *et al.,* 2024). borage is used as a health-improving agent due to its various biological activities. The results of some studies indicate that it can be used as a supportive treatment in respiratory, urinary, and skin disorders, as well as in cardiovascular and inflammatory diseases (Michalak *et al.,* 2023).The aim of this study evaluation antibacterial activity of *R. officinalis* alkaloids, phenols extract against bacteria of urinary tract infection UTI, and defection phytochemicals compounds which have antimicrobial agent of plant natural products through estimation of total active compounds, and HPLC analyses.

II. Material and Methods

Preparation of plant extracts

Crude Alkaloids Extraction

Extraction of crude Alkaloids from *Rosmarinus Officinalis* were carried out according to Harborne (1984) by using 100 g of plant powder was homogenized with 350 ml of 4:1 ethanol: D.W., in electrical blender for 5 minutes, then filtered with muslin cloth and Buchner funnel under reduced pressure by using Whatman No. 1 filter papers. The supernatant was evaporated at 45 °C in a rotary evaporator, drops of 2% sulphuric acid were added until the pH became (1-2), then extracted with chloroform three times in separating funnel The solution was separated into two layers, the lower layer was. chloroform, was neglected. The upper layer was the aqueous layer to be used. Addition of drops of concentrated ammonium hydroxide was added to this layer until pH became (9-10), then extracted was again with chloroform: methanol mixture in ratio of 3:1 twice, and one time with chloroform alone. Two layers appeared, the lower layer was evaporated at 40°C for (1-2) hours. The upper layer, the aqueous layer, was evaporated at 40°C for (1-2) hours, and kept in refrigerator.

Crude Phenols Extraction

Harborne was in charge of extracting crude phenols (1984). 200 g of plant powder was divided into two equal parts, 300 ml of 1% HCL was added to one part, and 300 ml of distal water D.W. was added to the other, then the two parts were placed in an electrical blender for 5 minutes, then boiled water bath for 30-40 minutes, the two parts were cooled and filtered with muslin cloth, and then centrifuged for 10 minutes at 3000 rpm. Both supernatants were combined. NaCl and an equal amount of n-propanol were added to the mixture until the solution was divided into two layers. The bottom layer was separated using an ethyl acetate separating funnel and evaporated using a rotary evaporator at 40°C for (1-2) hours. The top layer was evaporated in a rotary evaporator at 40°C for (1-2) hours, then carried to the oven, where the extract was stored until used.

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Estimation of active compounds

1-Estimation of total phenolic compounds

This method done by according to (salah eddine and ouahrani,2017).

2- **Estimation of total flavonoid content**

This method done by according to (Habibatin *et al*., 2017).

3- **Estimation of total glycosides**

This method done by according to (Tofighi, 2013; Tofighi and Ghazi saeidi, 2016).

4- Estimation of total tannins content

This method done by according to (Abdelkader *et al.,* 2014).

5- Estimation of total alkaloid content

This method done by according to (Ajanal *et al*., 2012).

6- Estimation of total Saponins content

This method done by according to (Ezeabara *et al*., 2014).

Preparation of different concentrations of plant extracts

Alkaloid and phenol extracts were prepared by dissolving a certain weight of each plant extract according to the concentration (25, 50, and 75) mg/ml in ethylene glycol. plant extracts were prepared according to the following equation: the method described by (Shoker *et al*.,2021)

$$\text{Concentration mg/ml} = \frac{\text{Weight}}{\text{Volume}} \times 1000$$

Analysis of chemical composition of the plants extracts

Compounds were analyzed by injecting 100 µL of each sample into a High-Performance Liquid Chromatography (HPLC) system for identification, following the method described by (*Shoker et al.,*2021).

Antibacterial Activity of *B. officinalis* extracts

Agar well diffusion technique was used to appearance the activity of phenolic and alkaloid extracts of *B. officinalis* in vitro and different concentration (25, 50, and 75 mg/ml) of each extracts were placed into the wells with Ethylene glycols as a control. The colony suspension of each bacterial strain was matched to 0.5 McFarland standards, yielding a concentration of 1.510°cfu/ml. Heat sterilized 6 mm cork borers were used to fill agar wells with 25,50,

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and 75 mg/ml of phenols and alkaloid of plant. The plates were incubated for 24 hours at 37°C in triplicate. After incubation, the diameter of the inhibitory zones generated was assessed.

Statistical analysis

Statistical analyses was performed by using SPSS (version 26). Analysis of variance ANOVA was used to compare the significant differences between means. P value of less than (P≤ 0.05) was accepted to indicate statistical significance for each test.

III. Results and Discussion

The yield of alkaloids and phenolic extracts were represented as percentage (%) of the dry weight. The result showed the yield of alkaloids extract of *B. officinalis* was (20.10%) while phenols extract yield was (13.50%).Ibrahim and alshammaa(2023) wich discovered that *B. officinalis* contains various bioactive compounds including alkaloids and phenolic compounds. These phytochemicals contribute to its pharmacological properties as antioxidant, anti-nociceptive, and anti-inflammatory effects enhancing its therapeutic potential in traditional medicine and various health applications . the yield of alkaloids and phenolic extracts were shown in table (1)

Table (1) extracts yield of plants extract of *B. officinalis* expressed as %

Plants Name	Extract Types	Yield (%)
B. officinalis	Alkaloids	20.10
	Phenols	13.50

Estimation of active compounds in plants

The phytochemical analysis of the studied plants revealed clear variation in the levels of bioactive compound in *B. officinalis* involve total phenolic compounds TPC (gallic),total flavonoid compound TFC (rutin,), alkaloid, glycoside ,tannins, and saponins as shown in table (2).The results displayed the higher concentration compound gallic acid (88.8 µg/ml) while, saponins (0.26%) lower concentration in plant .Zemmouri *et al (*2014) discovered in ethanol extract leaves of *B. officinalis* content in Algeria.

Table (2) Estimation of the active compounds in *B. officinalis*

	Active compounds

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Name	Gallic (TPC) (mg/ gm)	Rutin (TFC) (mg/ gm)	Alkaloid %	Glycoside %	Tannins %	Saponins %
B. officinalis	88.8	38.7	1.52	0.41	0.74	0.26

Antibacterial Activity

Analysis of antimicrobial susceptibility revealed substantial resistance among the isolated bacterial strains with resistance patterns differing considerably between Gram-negative and Gram-positive groups which isolated from UTI. Result in table (3) showed *S. aureus* was more sensitive for alkaloid and phenols extracts of .*B officinalis* while *P. aeruginosa* was more resistance for each extract, the inhibition zone of phenols extracts against *S. aureus a* was (25.00 ± 1.00mm, 20.00 ± 2.00 mm, 18.00± 2.0 mm, and 7.33± 1.00 mm) while the inhibition zone of alkaloid extract was (15.67 ± 1.00 mm, 13.00 ± 1.0 mm, 11.33± 1.00 mm, and 6.00 ± 1.57 mm) at (75 mg/ml) concentration, Phenols were having significant highest antibacterial activity at $p \leq 0.05$ against bacteria than alkaloids. *B.officinalis* also showed substantial antimicrobial potential, particularly in the phenol extract. This may be attributed to the presence of flavonoids and other secondary metabolites with known antimicrobial and antioxidant effects. These findings are consistent with Cushnie and Lamb (2005) who showed the antimicrobial mechanisms of flavonoids including inhibition of nucleic acid synthesis and membrane disruption Although *B. officinalis* is more effective against Gram-positive bacteria certain phenolic compounds like gallic acid in its extracts may still contribute to moderate activity against *P. aeruginosa* (Slama *et al*., 2024; Miceli *et al*., 2015).The culture yielded *S. aureus* which showed resistance to oxacillin, gentamicin, moxifloxacin, erythromycin, clindamycin, and vancomycin, while remaining sensitive to trimethoprim-sulfamethoxazole and tigecycline. Resistance to oxacillin confirms the strain as methicillin-resistant *S. aureus* (MRSA), which is commonly associated with multidrug resistance. The additional resistance to vancomycin is notable and may indicate the presence of vancomycin-intermediate or vancomycin-resistant *S. aureus* (VISA/VRSA) a rare but clinically serious finding. Despite this resistance pattern the organism remains sensitive to trimethoprim-sulfamethoxazole and tigecycline, offering limited but viable therapeutic options. Selection of treatment should be based on the infection site severity and patient-specific factors. These findings are consistent with those reported by Hussein *et al.,* (2023) and Raheema *et al.*,(2023), who documented similar resistance trends in clinical *S. aure*us isolates. The culture yielded *P.aeruginosa*, which showed resistance to cefazolin, tigecycline, and gentamicin, while remaining sensitive to piperacillin, cefepime, and imipenem. Resistance to cefazolin and tigecycline is expected, as these agents have limited efficacy against *P. aeruginosa* due to intrinsic mechanisms such as low outer membrane permeability and active efflux pumps. Resistance to gentamicin may be attributed to acquired mechanisms like aminoglycoside-modifying enzymes. The organism's susceptibility to piperacillin, cefepime, and imipenem indicates that these antibiotics remain effective options for managing serious *P. aeruginosa* infections. Antibiotic selection should be tailored based on the infection site patient condition and stewardship guidelines. This resistance profile is consistent with findings reported by Al-Saeedi and Raheema, (2019) and further supported by Raheema *et al*., (2024) who observed similar antimicrobial susceptibility patterns in clinical isolates.

Table (3) Diameters of inhibition zone mm caused by *B. officinalis* extracts against *S. aureus* at diverse concentration.

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Bacterial species	Extract type	Concentration of *Borago officinalis*			LSD
		25%	50%	75%	
S. aureus	Alkaloid	C 9.33 ± 0.57^{b}	CD 14.00 ± 1.00^{a}	C 15.67 ± 1.00^{a}	1.27
	Phenol	A 20.33 ± 1.52^{b}	A 21.67 ± 1.57^{b}	A 25.00 ± 1.00^{a}	1.59
E. coli	Alkaloid	CD 7.00 ± 1.0^{c}	D 11.00 ± 1.0^{b}	CD 13.00 ± 1.0^{a}	1.44
	Phenol	B 17.00 ± 2.0^{a}	AB 18.00 ± 1.0^{a}	B 20.00 ± 2.0^{a}	3.51
K. pneumoniae	Alkaloid	DE 5.67 ± 0.57^{b}	D 12.00 ± 0.57^{a}	D 11.33 ± 1.00^{a}	1.07
	Phenol	B 15.00± 2.00^{a}	BC 17.00 ± 1.00^{a}	B 18.00 ± 2.00^{a}	3.11
P. aeruginosa	Alkaloid	E 4.0 ± 0.57^{b}	E 5.00 ± 1.0^{a}	E 6.00± 1.57^{a}	1.27
	Phenol	DE 5.00 ± 0.57^{b}	E 5.67 ± 0.57^{b}	E 7.33± 1.00^{a}	1.07
LSD		2.71	3.89	2.99	
P value		0.001**	0.001**	0.001**	

Means in the same row with a different small letter are significantly different at (P≤0.05)
Means in the same column with a different capital letter are significantly different at (P≤0.05)

Chemical constituents of the plant extracts

Results of HPLC analysis showed the occurrence of 6 phenolic constituents in B. officinalis including Gallic acid, Apigenin, Ferulic acid, Qurcetine, Rutin, Sinapic acid compounds, the higher concentration compound was gallic (93.6mg/gm), while Ferulic acid (44.6 mg/gm) lower concentration in plant as shown in table (4), Figure (1). All the investigated compounds seemed to have different retention time. while chlorogenic acid, cinnamic acid, and rosemaric acid were absent in plant. (Karimi *et al.,* 2018; Zannou *et al.,* 2024) showed the reverse phase HPLC analyses revealed that the methanolic extract of borage flowers contains pyrogallol, gallic acid, caffeic acid, salicylic acid . Ibrahim *et al.,* (2024) showed by HPLC analysis was Rosemaric acid, Apigenin and caffeic acid.

Table (4) Types and concentration of phenols extract in plant.

Phenolic compounds (ppm)	*B. Officinalis*

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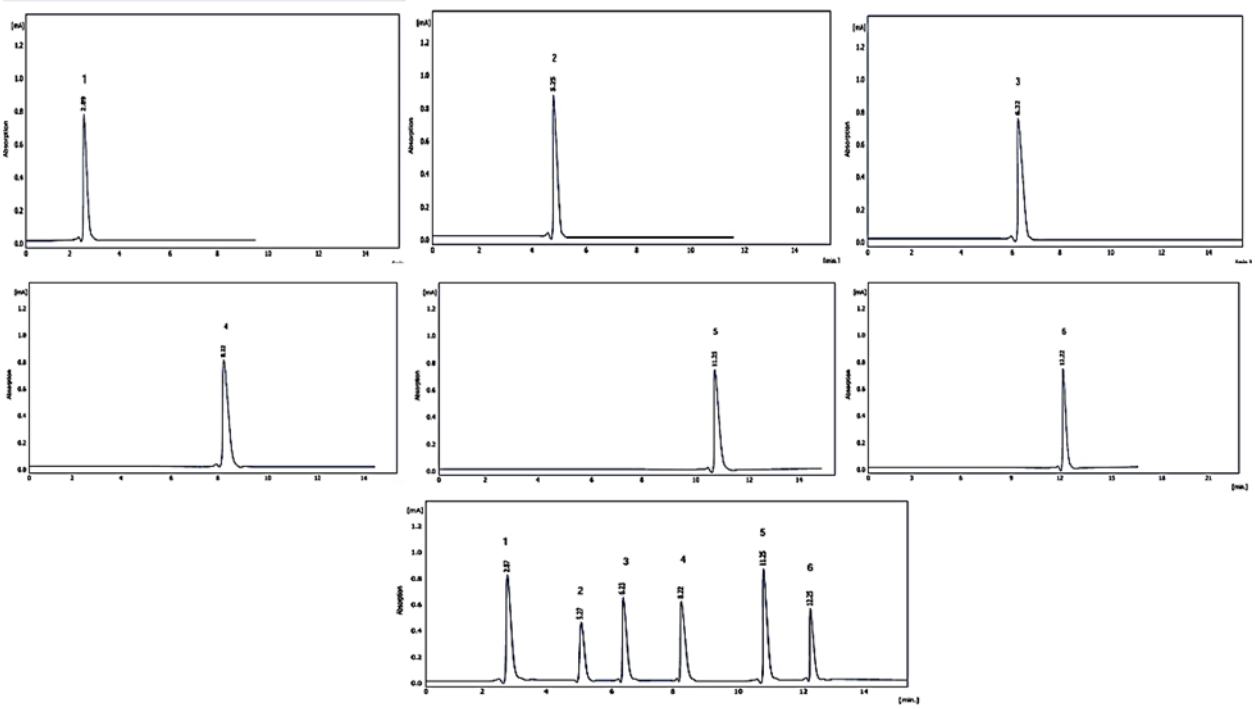
Gallic acid	93.6
Apigenin	68.7
Chlorogenic acid	-
Cinnamic acid	-
Ferulic acid	44.6
Qurcetine	80.7
Rosemaric acid	-
Rutin	77.8
Sinapic acid	62.6
Total concentration (µg/ml)	428



IV. Conclusion

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our study may be concluded that *B. officinalis* plant contains various phytochemical constituents which have antimicrobial agent may assist as a promising natural alternative for developing natural drugs especially, with rise of drug-resistant bacteria.

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