doi.org/10.36721/PJPS.2023.36.5.REG.1435-1442.1

Antibacterial activity of phenolic compounds of *Teucrium polium* L.

Mohammed Arafat Mohammed, Younis Saadi Saeed* and Jassim Fatehi Ali
Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq

Abstract: *Teucrium polium* L., a member of the *Lamiaceae* family. The plant's leaf ingredients were extracted with hexane, ethyl acetate, and ethanol, in that order. Column chromatography (CC), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) were used to figure out what phenolic components were in different extracts. Through these methods, three distinct fractions were isolated, originating from ethyl acetate and ethanolic extractions. Further examination of these fractions led to the discovery of 4-Hydroxybenzoic acid, catechol and coumaric acid. The antibacterial activity of isolated components was examined through rigorous testing. To evaluate the efficacy against different types of bacteria, the disc-diffusion assay was employed. This involved testing each fraction against *Staphylococcus aureus*, a Gram-positive bacterium, as well as four Gram-negative bacteria, including *Staphylococcus aureus* and *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. Fraction (F1) was able to stop the growth of *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* well with an inhibition zone of 19–21 mm. Fraction (F2) showed high activity against all types of bacteria used in this study, with an inhibition zone of 19–23 mm. This study's findings suggest that *Teucrium polium* holds potential as a valuable source of natural antibacterial phenolic compounds.

Keywords: *Teucrium polium*, phenolic compounds, antibacterial, HPLC.

INTRODUCTION

Teucrium polium is a naturally occurring perennial herbaceous plant known for its slender, intricately branched stems that can grow up to approximately 45-50cm tall. Leaves lack or have a very short petiole, are oval-elongated and are slightly incised. Leaves have a length of about 3cm. White to light pink flowers are densely arranged towards the top of the branch. distributed widely in Middle Eastern countries, including Iraq, South Western Asia, Europe and North Africa (El Atki *et al*., 2020; Znini *et al*., 2021). *T. polium* (locally called Jaadah) is one of the 300 species of the genus Teucrium. Belongs to the Lamiaceae family. *T. polium* is renowned for its remarkable properties, including its antioxidative, anti-rheumatic, hypolipidemic, anti-inflammatory and hypoglycemic effects. Throughout history, various Teucrium species have been valued in traditional medicine for over two millennia and their applications continue to be prevalent in modern times (Tepe *et al*., 2021; El Atkiet *et al*., 2020).

Several studies have shown that *T. polium* has strong antibacterial properties. Infections caused by bacterial strains are a real problem in our modern health care system because they cause a lot of illness and death (Kumar *et al*. , 2015; Purnavab *et al*., 2015). Most of these infections are caused by bacteria that are resistant to synthetic drugs. Despite its medical importance, it does not show a clear effect on many bacteria. Especially bacteria that have acquired resistance (Khoramian *et al*., 2020; Benali *et al*., 2021). The World Health Organization (WHO) put out a report with a list of the most dangerous multidrug-resistant bacteria that need to be treated with a new antibiotic. But the overuse of antibiotics has become a major cause of the development and spread of strains of microorganisms that are resistant to many drugs (Shakya, 2016; Sevindik *et al*., 2016). For this reason, other sources have been sought to obtain compounds that have the ability to act as natural antibiotics, extracted from plants (Alreshidi *et al*., 2020; Hassan *et al*., 2017).

T. polium has a lot of secondary metabolites, such as polyphenols and flavonoids. These compounds belong to a group of bioactive molecules' called low-molecular-weight secondary metabolites and phenolic compounds are a class of plant secondary metabolites that are widely distributed in plants, contain one or more hydroxy derivatives of benzene rings (Jubair *et al*., 2021; Khameneh *et al*., 2019) and are used for defensive functions against a wide range of pathogens. They have been shown to act as antibacterials when tested, underscoring their potential as pharmaceuticals (Porras *et al*., 2020; Saquib *et al*., 2019).

The objective of this research was to employ chromatographic techniques for the extraction of phenolic compounds from *T. polium*. The investigation aimed to explore the correlation between phenolic compounds and their antibacterial properties, while identifying the optimal solvents for obtaining phenolic-rich extracts from *T. polium*.

**Corresponding author:* e-mail: younissaadisaeed@gmail.com

MATERIALS AND METHODS

Collection, identification and preparation of plant materials

During the flowering season in April and June, the *T. polium* leaves that were not damaged pathogenic and physically were collected from northern Iraq. The leaves were picked up, then put in a sterile plastic bag and taken to the lab. After the plant's identity was confirmed, it was washed with sterile distilled water to get rid of all the dust on the surface. After that, put them in the dark at room temperature (RT, about 25°C) until they were dry, to complete the drying put them into a hot air oven (HAO) and dried at 36°C for about 48 hours. Finally, the dried leaves were ground and turned into a powder and kept at 20C.

Extraction using different solvents by soxhlet

The procedure of Soxhlet extraction based on the sequential solvent system hexane(Hex), ethyl acetate (EtOAc) and ethanol (EtOH), the continuous extraction apparatus was utilized by adding 1000ml of the utilised solvent per 100g of the sample (plant powder). The extraction was carried out for 48-72 hours until the colour of the saxolith-derived solvent vanished. Following that, the extracts were concentrated with an RVE (Rotary Vaccum Evaporator) to produce the crude extract. Finally maintained in the fridge until use and packaged in opaque, sealed glass bottles (Chakraborty *et al*., 2017).

Isolation and purification by column chromatography

The antimicrobial compounds were purified using a technique called silica gel column chromatography (CC). In this process, a column with dimensions of 70cm by 2.0cm (inner diameter) was packed with 250g of silica gel (Sigma Aldrich) with a mesh size of 60-120. The extract from *T. polium* was isolated and fractionated using this column. The sample was loaded onto the column and a solvent was used to elute the compounds at a rate of one drop per minute. Hexane, ethyl acetate and ethanol were employed as mobile phases for rinsing the column. The antibacterial activity of the fractions was assessed and the most active ones were selected for further analysis. The eluent was collected in 5ml fractions and a preliminary identification was performed using thin-layer chromatography (TLC). The fractions containing the *T. polium* extract were then dried using rotary evaporation (Cuellar-Bermudez *et al*., 2015).

Identification by TLC

The crude extract was analyzed using TLC according to a method previously described by Liu *et al*. (2018). In summary, 2µL of each extract were applied as spots on an RP-18 Silica coated TLC plate (Merck, 20 × 20cm), with a 10mm gap between each spot. The plate was then developed in a developing chamber using a mixture of ethyl acetate, isopropanol and water in a ratio of 40:40:30 (v/v) for approximately 60 minutes. After air drying, the TLC plate was examined using a UV-visible spectrophotometer set at 254nm and 365nm to visualize fluorescent compounds (Sunny UV.7804C, Tokyo, Japan). Additionally, vanillin and sulfuric acid were sprayed onto the TLC plate and gentle heating was applied to facilitate the observation of distinct compound colors.

Phenolic compound HPLC analysis

The analytical HPLC system utilized in the study comprised a reversed-phase HPLC with a silica-based C18 column from Agilent Technologies located in Santa Clara, CA, USA. The system was composed of several components, including an SPD-10A UV-VIS detector, an LC-10AT VP pump, an SIL-10AF auto-injector and an SCL-10A VP system controller. To achieve separation, the mobile phase employed a mixture of acetonitrile, water and phosphoric acid in a ratio of 30:70:0.08 (v/v/v). Detection of the separated compounds was performed at a wavelength of 288nm using the built-in SPD-10A UV-Vis detector. The identification of phenolic compounds was accomplished by applying Miklavčič *et al*. (2020) methodology, which involved comparing the retention times of the standards listed in table 1 with those of the peaks observed in the extract.

Microorganism strain origin and selection

The antibacterial properties of extracts derived from *T. polium* were assessed by conducting *in vitro* tests against a selection of highly harmful bacteria. For this study, we opted to screen the effectiveness of these extracts against five human pathogenic bacteria, utilizing strains obtained from our laboratory's stock culture. Our investigation focused on prominent food-borne pathogens, including gram-positive strains such as *Staphylococcus aureus* (ATCC 6538), as well as gram-negative strains like *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 15380) and *Salmonella typhi* (ATCC 19430). Proper storage of these microorganisms was ensured by maintaining them in vials at a temperature of 18°C.

Culture conditions and bacterial strains

The bacterial strains were cultivated on Mueller-Hinton broth-based nutrient agar (NA) at a temperature of 37°C for a duration of 24 hours. Following the incubation period, sub-culturing was performed before conducting any antimicrobial assessments. To prepare the inoculums, the bacteria were suspended in a sterile saline solution consisting of 0.85% NaCl. The optical density (OD) of the suspensions was carefully adjusted or maintained within the range of 0.4 to 0.6 at a wavelength of 405nm, ensuring a cell density of approximately 0.5 McFarland. This OD value corresponds to an expected inoculum containing 106 to 108 colony forming units per mL (CFU/mL) (Amaya-Gómez *et al.,* 2020).

Disc diffusion on agar method

The antibiotic susceptibility test was conducted using the standard disc diffusion method, following a modified protocol based on the work of Uwizeyimana *et al*. (2020). The Mueller-Hinton agar (MHA) plates were prepared and marked with pre-made inoculums. Subsequently, sterile paper discs (6mm, Whatman paper N5) were impregnated with 5µL each of the ethyl acetate (F_1) and ethanol (F_2) fractions, using a solvent composed of 10% v/v dimethyl sulfoxide, 1% v/v tween 80 in deionized water. For the purpose of comparison, positive and negative controls were included, using Amikacin and Gentamycin antibiotics (at a concentration of 5g/mL) and the same solvent as the diluent. The plates were then incubated at 37°C for 24 hours, following an initial period at room temperature. Finally, the antibacterial activity was assessed by measuring the diameter of the inhibition zone surrounding each disc, including the disc itself. Each experiment was repeated three times to ensure reliability.

RESULTS

Composition of phenolic compounds in T. polium ractions

Extraction was processed using Soxhlet. Use the first solvent, hexane, to defat the sample. While the extracts of ethyl acetate and ethanol were used to obtain the fractions by column chromatography, A TLC plate was used to identify phenolic compounds. The initial fraction (F_1) corresponded to the extraction using ethyl acetate, while the subsequent fraction (F_2) was obtained through ethanol extraction. The composition and quantity of phenolic compounds in *T. polium* are presented in table 2, determined by comparing their retention times and peak areas with those of established standards. Within the first fraction (F_1), a prominent peak corresponding to 4-hydroxybenzoic acid was observed, in accordance with its respective standard (fig. 1). As for the second fraction (F_2), two significant peaks were identified as catechol and coumaric acid (fig. 2).

***T. polium* extracts antimicrobial activity**

The effect of phenolic compounds extracted from *T. polium* against the five pathogenic bacterial strains under study, compared with antibiotics using the disc diffusion method, the results showed that there is an inhibitory effect depending on the type of active compounds, the type of bacteria and the concentration gradient. The extracted phenols show antibacterial activity, as shown in table 3.

K. pneumoniae was inhibited at concentrations of 1.25µL/mL for fractions F_1 and F_2. *P. aeruginosa* was inhibited at concentrations of 1.25µL/mL for fractions F_1 and F_2. S. typhi was inhibited at concentrations of 5µL/mL for Fraction F_1. *S. aureus* was inhibited at concentrations of 5µL/mL for fractions F_1 and F_2. *E. coli* was inhibited at concentrations of 5µL/mL for fraction F_1 and 1.25µL/mL for fraction F_2. The extracted phenols can inhibit the growth of *K. pneumoniae* and *P. aeruginosa*, while showing low inhibition effects on *S. typhi*, *S. aureus* and *E. coli*. This corresponds to the fact that the compound in this fraction is 4-hydroxybenzoic acid alone, which can inhibit the growth of *K. pneumoniae* and *P. aeruginosa* efficiently. Fraction F_2 inhibited the growth of *K. pneumoniae*, *P. aeruginosa* and E. coli and showed low inhibition effects on *S. typhi* and *S. aureus*. Due to the occurrence of vanillic acid and quercetin, Fraction F_2 showed a dissimilar antimicrobial effect.

DISCUSSION

T. polium is known for its rich reservoir of natural phenolic compounds, including flavonoids, phenolic acids, anthocyanins and various other phenols. These compounds have gained considerable attention in the medical industry due to their beneficial properties and their non-toxic nature for human health. Consequently, there has been a growing trend in utilizing plants with high phenolic content to develop medical interventions and treatments (Bakari *et al*., 2015; Elmasri *et al*., 2016). Many studies have indicated the phenolic compound content of the extract of Iraqi *T. polium*, which is considered to be very important because it can be considered an antibacterial agent (Khazaei *et al*., 2018; Rahmouni *et al*., 2021). Researchers have found that *T. polium* has a lot of chemical compounds. However, flavonoids are by far the most important chemical marker for this genus and are what give them their medicinal properties. Biosynthetic pathways and methods of extracting phenolic compounds from this genus are also talked about. This is done to learn more and provide enough information for future research (Mihailović *et al*., 2020; Farahmandfar *et al*., 2019; Jarić *et al*., 2020). Methanol and ethyl acetate are highly regarded solvents for extracting phenols and other extractable components from plants. The variance in compound extraction can be attributed to the polarity of the solvent used. The different polarities of the extraction solvents could affect how easily the chemical components of a sample dissolve and how much of the sample can be extracted. Being plant secondary metabolites (Gao *et al.,* 2020; Fattaheian-Dehkordi *et al.,* 2021). The extraction of phenols from plant materials is influenced by their solubility in the solvent used for the extraction process. It has been reported that as the polarity of the solvent went up, the total phenol and total flavonoid content of the extract went up. Most of the time, more polar solvents are better at getting out phenolic compounds. Some phenolic compounds form complexes with carbohydrates and proteins that are easier to get out of methanol and ethyl acetate than from other solvents. This could be a good reason (Etsassala *et al.,* 2021).

Table 1: Standards and retention time for phenolic compounds.

Standards	Retention Time (min)	Conc.(ppm)	Area[1] [mAu*s]
4-Hydroxybenzoic acid	2.901	25	$1.11364*10^4$ (0.01)
Catechol	2.715	25	$7.75188*10^4$ (0.01)
Coumaric acid	3.523	25	116158.325 (0.01)

Mean (n = 5) with coefficient of variation in brackets is Area1.

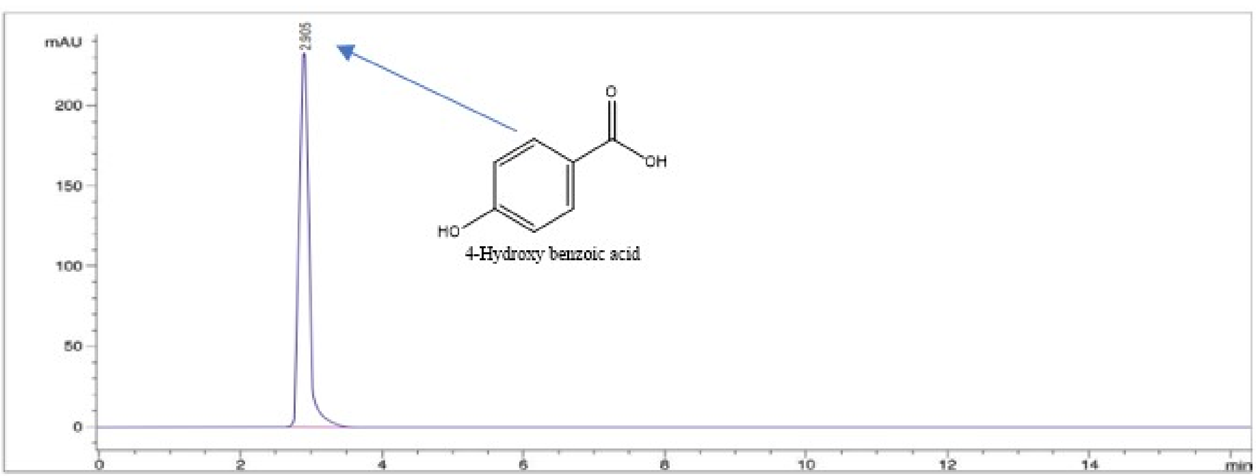
Table 2: Phenolic compounds and retention time in two fractions.

Fractions	No. of Peak	R.t (min)	Conc. (ppm) [c]	Identifed Compounds
F_1	1	2.905	4.5675±0.02	4-Hydroxybenzoic acid
F_2	1	2.717	1.2929±0.02	Catechol
	2	3.512	4.7237 ± 0.03	Coumaric acid

F_1 from ethyl acetate extraction, F_2 from ethanol extraction, c Mean and standard deviation (n = 3).

Table 3: F_1 and F_2 antimicrobial properties.

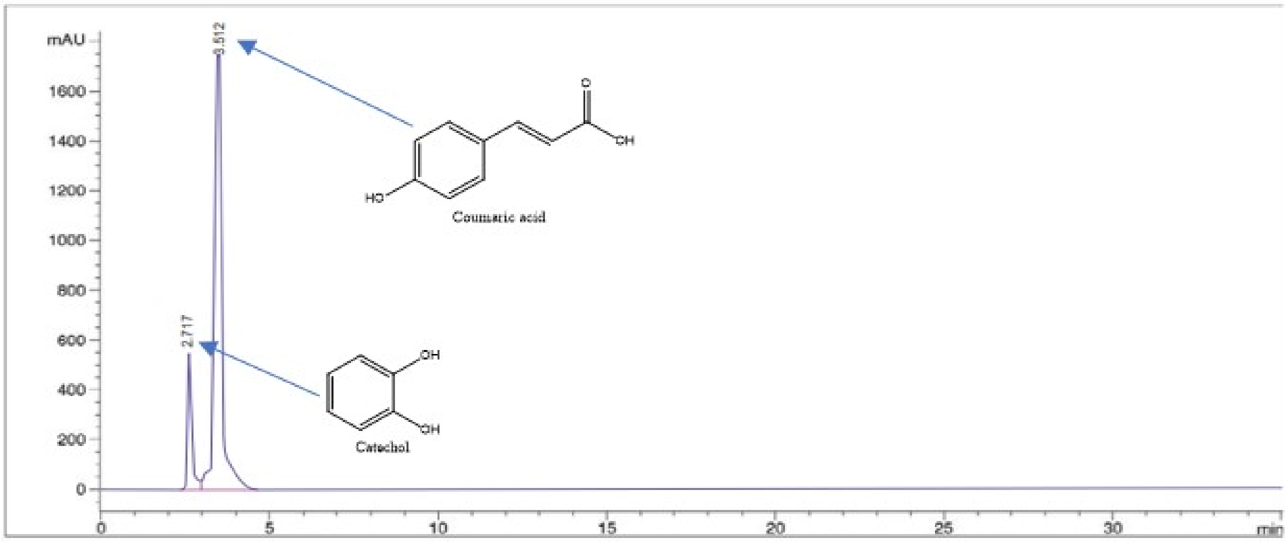
Fractions	Concentration	Zone of Inhibition (mm)				
	µg/mL	*K.pneumoniae*	*P.aeruginosa*	*S.typhi*	*S.aureus*	*E.coli*
F_1	1.25	4	7	0	0	0
	2.5	7	9	0	0	0
	5	9	11	9	8	8
	10	14	17	11	16	13
	20	19	21	16	19	16
F_2	1.25	9	8	0	0	11
	2.5	15	12	0	0	16
	5	17	16	8	6	18
	10	18	19	17	18	19
	20	23	22	19	20	21
Control	Amikacin	22	23	22	21	20
	Gentamycin	20	21	22	24	22




```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   2.905 VV    0.1199 2034.65416  230.99979 100.0000

  Totals :                2034.65416  230.99979
```

Fig. 1: Fraction F_1 HPLC.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.717	BV	0.1389	4009.24834	577.41760	15.4452
2	3.512	VV	0.1008	21948.67122	1698.09765	84.5548
Totals :				25957.91956	2275.51525	

Fig. 2: Fraction F_2 HPLC.

Many studies have shown that extracts from different parts of *T. polium*, like leaves, seeds and flowers, have antimicrobial properties (Abdollahi *et al.,* 2022; Shahraki-Mojahed *et al.,* 2021). The disc agar diffusion technique was used to conduct a preliminary screening of the *T. polium* methanolic extract's antibacterial activity *in vitro* against seven pathogenic bacteria. The tested extract exhibited a significant effect against Gram-negative bacteria. As noted, a moderate effect was observed against Gram-positive bacteria. This is proven by previous studies that Gram-positive bacteria are more affected by plant extracts than Gram-negative bacteria because hydrophobic lipopolysaccharides in the outer membrane provide protection against different agents (Abdollahi *et al.,* 2022; Khoramian *et al.,* 2020; Elsonbaty *et al*., 2020). However, other studies previously conducted on the same plant showed that the ethanolic extract was more effective against Gram-negative bacteria such as *E. coli* and *P. aeruginosa* than Gram-positive as *B. cereus*. It was also recorded in many previous studies that the extracts of *T. polium* have varying effects on both Gram-negative and Gram-positive bacteria. The effect was attributed to several factors, including the plant organ, vegetative cycle stage, climate and soil (Bardsiri *et al*., 2022; Bahramikia *et al.,* 2022; Noumi *et al*., 2020). Other studies indicated that the methanolic extract of *T. polium* was effective in inhibiting the growth of all tested Gram-negative and Gram-positive bacteria; the differences in the effect were due to the differences in the region and climate of the plant. (Toplan *et al*., 2022; zadeh Gharaboghaz *et al*., 2020; Mohammed *et al*., 2022) The ethanolic extract of *T. polium* grown in Iran showed activity against several tested bacteria with different MIC. This extract was effective in inhibiting the growth of *S. typhimurium* and *S. aureus*, with a MIC of 40mg ml-1. While the ethanolic extract of *T. polium* grown in Jordan showed activity against Bacillus anthracis, 10mg ml-1 also represents the minimal bactericidal concentration (MBC). (Masoumipour *et al.,* 2019). Demonstrate that the methanolic extract of *T. polium* is effective against both Gram-positive and Gram-negative bacteria, with the highest inhibitory activity seen against *E. coli* (MIC = 1.2 mg ml-1), while it appeared less affected against S. aureus (MIC = 2.2 mg ml-1) and *P. aeruginosa* (MIC = 2.4 mg ml-1) (Shariatifar *et al.,* 2019). The structure of the cell envelope could explain this difference in susceptibility. Gram-negative bacteria have an outer membrane made of lipopolysaccharides. This membrane separates the periplasmic space from the cytoplasmic membrane, which can stop hydrophobic compounds from moving through the cell. Without this membrane, it would be easier for outside forces to get into gramme-positive cells and change the cytoplasmic membrane (El Atki *et al.,* 2020). Antimicrobial compounds in *T. polium* may stop the growth of bacteria in different ways. So, it may be very useful in treating microbial strains that have become resistant (Kharroubi *et al*., 2021). Bioactive molecules are known as phenols. These compounds cause biological effects because of the way the molecules are built. It contains hydroxyl groups and benzene rings. They can bind to bacterial membrane proteins and form complexes (Zongo *et al.,* 2021). Natural compounds, especially phenolic compounds, may inhibit the growth of bacteria through several mechanisms, thus becoming of high clinical value in the treatment of many resistant bacterial strains and thus considered the mainstay of many applications, including food industries, natural therapies, alternative medicine and pharmaceuticals (Jurić *et al*., 2021).

CONCLUSIONS

The findings of this research revealed the chemical composition and antimicrobial properties of diverse extracts derived from *T. polium*. Three phenolic compounds were successfully isolated and identified. A notable aspect of this study was the utilization of the Soxhlet apparatus for extracting natural compounds from *T. polium* leaves, leading to a significantly higher yield of phenolics such as 4-Hydroxybenzoic acid, Catechol and Coumaric acid compared to previous investigations. The fractions of *T. polium* extracts exhibited varying degrees of antimicrobial activity against the tested bacteria. Fraction F_1 displayed notable inhibition against *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, while Fraction F_2 exhibited the strongest inhibitory effect on both Gram-positive and Gram-negative bacteria employed in this study. The dissimilarity in antimicrobial activities can be attributed to the distinct phenolic compounds identified in each fraction. Nevertheless, further *in vivo* studies and clinical trials are necessary to ascertain the safety and potential applications of these extracts as antimicrobial agents. The outcomes of this study contribute towards the development of a reliable method for extracting phenolic compounds from *T. polium*, which can find applications in various nutraceutical and pharmaceutical contexts.

ACKNOWLEDGMENTS

The researchers would like to express their appreciation to the University of Mosul's College of Education for Pure Sciences for offering valuable resources and support throughout the course of this study. Their assistance significantly contributed to the advancement of this paper.

REFERENCES

Abdollahi S and Raoufi Z (2022). Gelatin/Persian gum/bacterial nanocellulose composite films containing Frankincense essential oil and *Teucrium polium* extract as a nove006C and bactericidal wound dressing. *Journal of Drug Delivery Science and Technology*, **72**(5): 103423.

Alreshidi M, Noumi E, Bouslama L, Ceylan O, Veettil VN, Adnan M and Snoussi M (2020). Phytochemical screening, antibacterial, antifungal, antiviral, cytotoxic and anti-quorum-sensing properties of *Teucrium polium* L. aerial parts methanolic extract. *Plants*, **9**(11): 1418.

Alreshidi M, Noumi E, Bouslama L, Ceylan O, Veettil VN, Adnan M, Danciu C, Elkahoui S, Badraoui R, Al-Motair KA and Patel M (2020). Phytochemical screening, antibacterial, antifungal, antiviral, cytotoxic and anti-quorum-sensing properties of *Teucrium polium* L. aerial parts methanolic extract. *Plants*, **9**(11): 1418.

Amaya-Gómez CV, Porcel M, Mesa-Garriga L and Gómez-Álvarez MI (2020). A framework for the selection of plant growth-promoting rhizobacteria based on bacterial competence mechanisms. *Appl. Environ. Microbiol. . Microbiol.,* **86**(14): e00760-20.

Ashmawy NA, Salem MZ, El Shanhorey N, Al-Huqail A, Ali HM and Behiry SI (2020). Eco-friendly wood-biofungicidal and antibacterial activities of various Coccoloba uvifera L. leaf extracts: HPLC analysis of phenolic and flavonoid compounds. *BioResources*, **15**(2): 4165-4187.

Bahramikia S, Gavyar PHH and Yazdanparast R (2022). *Teucrium polium* L: An updated review of phytochemicals and biological activities. *Avicenna J. Phytomed*., **12**(3): 224.

Bakari S, Ncir M, Felhi S, Hajlaoui H, Saoudi M, Gharsallah N and Kadri A (2015). Chemical composition and *in vitro* evaluation of total phenolic, flavonoid and antioxidant properties of essential oil and solvent extract from the aerial parts of *Teucrium polium* grown in Tunisia. *Food Sci. Biotechnol.*, **24**(6): 1943-1949.

Bardsiri TI, Moayedi S, Meybodi RN, Torbati M and Sorkhani TM (2022). Investigation of antioxidant, antifungal, antibacterial and anti-inflammatory effects of *Teucrium polium* on common pathogens in vaginitis: A review. *Jundishapur J. Nat. Pharm. Prod*., **17**(4): 128060

Benali T, Habbadi K, Bouyahya A, Khabbach A, Marmouzi I, Aanniz T, Chtibi H, Mrabti HN, Achbani EH and Hammani K (2021). Phytochemical analysis and study of antioxidant, anticandidal and antibacterial activities of *Teucrium polium* subsp. *polium* and *Micromeria graeca* (*Lamiaceae*) essential oils from Northern Morocco. *Evid. Based Complement. Alternat. Med.*, **2021** (13): 10.

Chakraborty D, Das J, Das PK, Bhattacharjee SC and Das S (2017). Evaluation of the parameters affecting the extraction of sesame oil from sesame (*Sesamum indicum* L.) seed using soxhlet apparatus. *Int. Food Res. J.,* **24**(2): 691-695.

Cuellar-Bermudez SP, Aguilar-Hernandez I, Cardenas-Chavez DL, Ornelas-Soto N, Romero-Ogawa MA and Parra-Saldivar R (2015). Extraction and purification of high-value metabolites from microalgae: Essential lipids, astaxanthin and phycobiliproteins. *Microb. Biotechnol.,* **8**(2): 190-209.

Darabpour E, Motamedi H and Nejad SMS (2010). Antimicrobial properties of *Teucrium polium* against some clinical pathogens. *Asian Pac. J. Trop. Med.*, **3**(2): 124-127.

El Atki Y, Aouam I, El Kamari F, Taroq A, Lyoussi B, Oumokhtar B and Abdellaoui A (2020). Phytochemistry, antioxidant and antibacterial activities of two Moroccan *Teucrium polium* L. subspecies: preventive approach against nosocomial infections. *Arab. J. Chem.*, **13**(2): 3866-3874.

El Atki Y, Aouam I, El Kamari F, Taroq A, Zejli H, Taleb M, Lyoussi B and Abdellaoui A (2020). Antioxidant activities, total phenol and flavonoid contents of two *Teucrium polium* subspecies extracts. *Mor. J. Chem.*, **8**(2): 8-2.

Elmasri WA, Hegazy MEF, Mechref Y and Paré PW (2016). Structure-antioxidant and anti-tumor activity of *Teucrium polium* phytochemicals. *Phytochem. Lett.*, **15**(2016): 81-87.

Elsonbaty SM, Eltahawy N, Al-dmour SM, Easa S and Qaralleh H (2020). Ionizing Radiation effect on *Teucrium polium*: Phytochemical Contents antioxidant and antibacterial activity. *Arab J. Nucl. Sci. Appl.*. **53**(2): 98-110.

Etsassala NGER, Hussein AA and Nchu F (2021). Potential application of some *lamiaceae* species in the management of diabetes. *Plants*, **10**(2): 279.

F Saoudi M and Rebai T (2021). Therapeutics studies and biological properties of *Teucrium polium* (*Lamiaceae*). *Biofactors*, **47**(6): 952-963.

Farahmandfar R, Asnaashari M and Bakhshandeh T (2019). Influence of ultrasound-assist and classical extractions on total phenolic, tannin, flavonoids, tocopherol and antioxidant characteristics of *Teucrium polium* aerial parts. *J. Food Meas Charact*, **13**(2): 1357-1363.

Fattaheian-Dehkordi S, Hojjatifard R, Saeedi M and Khanavi M (2021). A review on antidiabetic activity of Centaurea spp.: A new approach for developing herbal remedies. *Evid. Based Complement. Alternat. Med.*, **2021**(6): 1-23.

Gao Z, Shen P, Lan Y, Cui L, Ohm JB, Chen B and Rao J (2020). Effect of alkaline extraction pH on structure properties, solubility and beany flavor of yellow pea protein isolate. *Food Res. Int.,* **131**(4): 109045.

Gharaboghaz MNZ, Farahpour MR and Saghaie S (2020). Topical co-administration of *Teucrium polium* hydroethanolic extract and Aloe vera gel triggered wound healing by accelerating cell proliferation in diabetic mouse model. *Biomed. Pharmacother*., **127**(4): 110189.

Hassan SED (2017). Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. *J. Adv. Res*., **8**(6): 687-695.

Jarić S, Mitrović M and Pavlović P (2020). Ethnobotanical features of *Teucrium* species. *In*: S. Jarić, M. Mitrović, and P. Pavlović (Eds.): *Teucrium* Species: Biology and applications, *Springer*, **106**(11): 111-142.

Jubair N, Rajagopal M, Chinnappan S, Abdullah NB and Fatima A (2021). Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria (MDR). *Evid.-Based Compl. Alt. Med*., **2021**(16): 3663315

Jurić T, Mićić N, Potkonjak A, Milanov D, Dodić J, Trivunović Z and Popović BM (2021). The evaluation of phenolic content, *in vitro* antioxidant and antibacterial activity of *Mentha piperita* extracts obtained by natural deep eutectic solvents. *Food Chem*., **362**(15): 130226.

Khameneh B, Iranshahy M, Soheili V and Fazly Bazzaz BS (2019). Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrob. Resist. Infect. Control*, **8**(1): 1-28.

Kharroubi M, Bellali F, Karrat A, Bouchdoug M and Jaouad A (2021). Preparation of *Teucrium polium* extract-loaded chitosan-sodium lauryl sulfate beads and chitosan-alginate films for wound dressing application. *AIMS Public Health*, **8**(4): 754.

Khazaei M, Nematollahi-Mahani SN, Mokhtari T and Sheikhbahaei F (2018). Review on *Teucrium polium* biological activities and medical characteristics against different pathologic situations. *J. Contemp. Med. Sci*., **4**(1): 1-6.

Khoramian Tusi S, Jafari A, Marashi SMA, Faramarzi Niknam S, Farid M and Ansari M (2020). The effect of antimicrobial activity of *Teucrium polium* on Oral Streptococcus Mutans: a randomized cross-over clinical trial study. *BMC Oral Health*, **20**(1): 1-8.

Khoramian Tusi S, Jafari A, Marashi SMA, Faramarzi Niknam S, Farid M and Ansari M (2020). The effect of antimicrobial activity of *Teucrium polium* on Oral Streptococcus mutans: A randomized cross-over clinical trial study. *BMC Oral Health*, **20**(1): 1-8.

Kumar N, Wani ZA and Dhyani S (2015). Ethnobotanical study of the plants used by the local people of Gulmarg and its allied areas, Jammu and Kashmir, India. *Int. J. Curr. Res. Biosci. Plant Biol*., **2**(9): 16-23.

Liu X, Ahlgren S, Korthout HA, Salomé-Abarca LF, Bayona LM, Verpoorte R and Choi YH (2018). Broad range chemical profiling of natural deep eutectic solvent extracts using a high performance thin layer chromatography-based method. *J. Chromatogr. A*, **1532**(12): 198-207.

Masoumipour F, Hassanshahian M, Sasan H and Jafarinasab T (2019). Antimicrobial effect of combined extract of three plants *Camellia sinensis*, *Teucrium polium* and *Piper nigrum* on antibiotic resistant pathogenic bacteria. Iran. *J. Med. Microbiol*., **13**(2): 114-124.

Mihailović V, Katanić Stanković JS and Mihailović N (2020). Phenolic compounds diversity of Teucrium species. *In*: V Mihailović and JS Katanić Stanković

(Eds.): Teucrium species: Biology and applications, Springer, **106**(11): 143-177.

Miklavčič Višnjevec A, Baker P, Charlton A, Preskett D, Peeters K, Tavzes Č and Schwarzkopf M (2020). Developing an olive biorefinery in slovenia: Analysis of phenolic compounds found in olive mill pomace and wastewater. *Molecules*, **26**(1): 7.

Mohammed MA, Ali JF, Saeed YS, Yaseen IH and Ahmad BH (2022). Biological activity of some phenolic compounds extracted from *Agrimonia eupatoria* against several pathogenic bacteria species. *Biodiversitas J. Biol. Divers*., **23**(9): 4912-4917.

Noumi E, Snoussi M, Anouar EH, Alreshidi M, Veettil VN, Elkahoui S and Badraoui R (2020). HR-LCMS-based metabolite profiling, antioxidant and anticancer properties of *Teucrium polium* L. methanolic extract: Computational and *in vitro* study. *Antioxidants*, **9**(11): 1089.

Porras G, Chassagne F, Lyles JT, Marquez L, Dettweiler M, Salam AM, Samarakoon T, Shabih S, Farrokhi DR and Quave CL (2020). Ethnobotany and the role of plant natural products in antibiotic drug discovery. *Chem. Rev*., **121**(6): 3495-3560.

Purnavab S, Ketabchi S and Rowshan V (2015). Chemical composition and antibacterial activity of methanolic extract and essential oil of Iranian *Teucrium polium* against some of phytobacteria. *Nat. Prod. Res*., **29**(14): 1376-1379.

Saquib SA, AlQahtani NA, Ahmad I, Kader MA, Al Shahrani SS and Asiri EA (2019). Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: An *in vitro* microbiological study. *Antibiotics*, **8**(3): 89.

Sevindik E, Abacı ZT, Yamaner C and Ayvaz M (2016). Determination of the chemical composition and antimicrobial activity of the essential oils of *Teucrium polium* and Achillea millefolium grown under North Anatolian ecological conditions. *Biotechnol. Biotechnol. Equip.,* **30**(2): 375-380.

Shahraki-Mojahed L, Behzadmehr R and Beigomi Z (2021). Antimicrobial effects of ethanol, methanol and ethyl acetate *Teucrium polium* and *Citrullus colocynthis* extract on *Pseudomonas aeruginosa*. *Micro Environer*, **1**(01): 26-32.

Shakya AK (2016). Medicinal plants: Future source of new drugs. *Int. J. Herbal Med.*, **4**(4): 59-64.

Shariatifar N, Pirali-Hamedani M, Moazzen M, Ahmadloo M and Yazdani D (2019). Study of the antimicrobial effects of aqueous extract of *Olea europaea, Solanum nigrum, Artemisia sieberi*, *Teucrium polium*, *Glycyrrhiza glabra* on some food-borne pathogenic bacteria. *J. Med. Plants*, **18**(72): 264-273.

Tepe AS, Ozaslan M, KILIC IH and Oguzkan SB (2021). Traditional uses, phytochemistry and toxic potential of *Teucrium polium* L.: A comprehensive review. *Int. J. Plant Based Pharmaceuticals.*, **1**(1): 1-41.

Toplan GG, Göger F, Taşkin T, Genç GE, Civaş A, Işcan G and Başer KHC (2022). Phytochemical composition and pharmacological activities of *Teucrium polium* L. collected from eastern Turkey. *Turk. J. Chem*., **46**(1): 269-282.

Uwizeyimana JD, Kim D, Lee H, Byun JH and Yong D (2020). Determination of colistin resistance by simple disk diffusion test using modified mueller-hinton agar. *Annals of laboratory medicine,* **40**(4): 306-311.

Wiktor A, Mandal R, Singh A and Pratap Singh A (2019). Pulsed light treatment below a critical fluence (3.82 J/cm2) minimizes photo-degradation and browning of a model phenolic (gallic acid) solution. *Foods*, **8**(9): 380.

Znini M, Costa J and Majidi L (2021). Chemical constituents of the essential oil of endemic Teucrium luteum subsp. flavovirens (batt.) Greuter and burdet collected from two localities in Morocco. *J. Essent. Oil Res.*, **33**(2): 197-203.