Pak. J. Bot., 57(1): DOI: http://dx.doi.org/10.30848/PJB2025-1(18)

PHENOLIC COMPOSITION, ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ATTRIBUTES OF *EREMOSTACHYS VICARYI* BENTH. EX HOOK. F. FROM SOON VALLEY, SALT RANGE, PAKISTAN

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Abstract

The research work was carried out to study the phenolic composition, antioxidant and antimicrobial activities of *Eremostachys vicaryi* (*E. vicaryi*) by using aqueous and ethanol extracts. The flavonoid content of the extracts varied significantly due to the utilization of two distinct extraction approaches, namely sun-drying and shade-drying. The ethanol extracts obtained from sun-dried samples had a significantly greater flavonoid content of 634.568±2.14 mg/100g CE, whereas the ethanol extracts from shade-dried samples had a lesser content of 253.926±35.30 mg/100g CE. The aqueous extracts obtained from samples that were dried in the sun and in the shade showed flavonoid content values of 148.148±3.70 mg/100g CE and 188.889±3.70 mg/100g CE respectively. The aqueous shade-dried extracts had the highest recorded phenolic content of 1554.00±1.00 mg/100g GAE, while the ethanol sun-dried extracts had the least recorded phenolic content of 401.00±1.00 mg/100g GAE. The antioxidant activity exhibited variation, with the maximum recorded value of 2776.22±2.70 µg/100g AAE reported in aqueous sun-dried extracts and the lowest recorded value of 2093.33±5.63 µg/100g AAE in aqueous shade-dried extracts. Both polar and non-polar extracts were used on four (gram-positive and gram-negative (2 each) bacterial and four fungal strains to determine antimicrobial activity. The maximum antibacterial activity was observed in chloroform extracts, zone of inhibition (ZI) valued at 26±1 mm against *Staphylococcus aureus* and the minimum was observed in aqueous extracts, ZI 8.57 mm against *Psedomonas aeruginosa*. ZI against fungal strains was the maximum for methanol extracts 24±2.51 mm against *Aspergillus niger* and the minimum was for petroleum ether extracts ZI 8±1.52mm against *Aspergillus oryzae*. This study revealed that *E. vicaryi* from Soon Valley of Salt Range, Punjab Pakistan, exhibited a high phenolic profile, significant antioxidant properties and notable antimicrobial activity. These investigations highlighted the plant's potential as a valuable source of natural antioxidants and antimicrobial agents, suggesting its use in pharmaceutical and health supplement applications. Subsequent studies may focus on the identification and extraction of compounds responsible for antimicrobial activity.

Key words: *Eremostachys vicaryi,* Antibacterial, Folin-Ciocalteu reagent, Toxic plants.

Introduction

Due to their astounding number of diverse classes of biochemicals and a wide range of biological functions, plants are more significant to us than animals (Cotton, 1996).

Throughout history, humans have utilized the potential of plants not only for sustenance and protection, but also to improve both mental and physical health (Liu *et al*., 2022). Herbal extracts and traditional recipes have long been essential tools for healers and physicians, and they continue to be the primary form of healthcare for approximately 75-90% of the global rural population (Hamann, 1991). Additionally, plants remain to serve as the primary sources for around twenty-five percent of prescription medications, even in Western medicine (Farnsworth & Morris, 1976). Herbs have been utilized for a diverse array of applications, encompassing medication, nutrition, flavourings, dyeing, beverages, repellents, charms, scents, cosmetics, smoking, and industrial usage. Herbs have been the primary foundation for medicinal treatment since prehistoric times, until the emergence of synthetic medications in the nineteenth century. Currently, herbs are present in forty percent of prescription medications (Alam *et al*., 2010; Phillips, 2023).

Numerous species have been acknowledged for their therapeutic characteristics and positive influence on health, such as antioxidant activity, stimulation of digestion, reduction of inflammation, inhibition of microbial growth, lowering of cholesterol levels, prevention of genetic mutations, and ability to combat cancer (Aaby *et al*., 2004; Neelam & Khan, 2012; Castillo-Lopez *et al*., 2017; Akanbong *et al*., 2021).

Plants have a variety of phytochemical components, including isocatechins, alkaloids, catechins, anthocyanins, flavonoids, isoflavones, lignins, saponins, tannins, phenols and coumarins. As a result of these compounds, plants have potent antibacterial and antioxidant properties (Aqil *et al*., 2006; Li *et al*., 2018; Fusari *et al*., 2019).

Phenolic chemicals present in plants have been confirmed to possess various biological properties, such as antioxidant activity (Kahkonen *et al*., 1999; Niknam & Ebrahimzadeh, 2002; Asif, 2015; Miceli *et al*., 2020). Phenolic compounds possess diverse physiological features including many other vital actions (Puupponen-Pimia *et al*., 2001, Manach *et al*., 2005).

The *E. vicaryi* belongs to the family Lamiaceae (Labiatae) of the genus Eremostachys. Locals in Pakistan refer to it as "Gurganna," "Khalatri," "Rewand-chin,"

"Jangli Tambacco" and "Bishkhaf." It thrives in a variety of environments and frequently grows alongside *Salvia moorcroftiana* Wall. ex Benth. According to observations, "the plant is used for envenoming fish in the Yousafzai tribes nearby Peshawar" (Stewart, 1869).

Most of the unique plants of Soon Valley like *E. vicaryi* are under-explored regarding their phytochemical profile and bioactivities. So, the objective of this study was to investigate the phytochemical composition and biological activities of *E. vicaryi*, with a focus on its antioxidant and antimicrobial properties. The significance of this study lies in its potential to uncover valuable bioactive compounds that can contribute to the development of new therapeutic agents. Given the growing concern over antibiotic resistance and the need for natural antioxidants in the food and pharmaceutical industries. The findings from this research could provide essential insights and alternatives to synthetic drugs and preservatives. Furthermore, understanding the traditional uses of *E. vicaryi* and scientifically validating its medicinal properties can help in preserving indigenous knowledge and promoting sustainable use of local flora.

Material and Methods

Plant material: The plant material *E. vicaryi* was collected from Khabeki Lake, Soon Valley, Salt Range in Punjab, Pakistan with geographical coordinates N 32°36'58.6" and S 072° 12’27.5" located an elevation of 758m above sea level. The specimen was identified according to “Flora of Pakistan” (Nasir & Ali, 1970-2003). The voucher specimen (GS-706) was deposited in the Herbarium of Sargodha University (SARGU) for record and future reference.

Maceration of plant material: The entire plant was dried in both shade and sunlight, and then pulverized into a fine powder. Four types of extracts (solutions) were produced to evaluate the levels of phenols, flavonoids, and antioxidant activity.

Solution (100% Aqueous): Two vessels, one containing shade-dried plant powder and the other carrying sun-dried plant powder, were mixed with 100 mL of water each. The vessels were then gently stirred for period of 8 hours, followed by a day of rest. Finally, the contents were carefully filtered. This meticulous procedure was designed to protect the integrity of the solution for accurate analysis.

Solution (20% ethanol and 80% water): For the second solution, a precise mixture of 20% ethanol and 80% distilled water was prepared in two different containers. One container received sun-dried plant powder, while the other container got shade-dried plant powder. Following an eight-hour period of stirring and subsequent day of resting, both solutions were carefully filtered and transferred to sterile containers to guarantee their accuracy and precision for analytical purposes.

Solution preparation for antimicrobial activity: Dried plant material was macerated by using solvents in accordance to their polarity: water>methanol>chloroform>petroleum ether, these were used for antimicrobial activity.

Total phenolic compounds: The TPC (Total phenolic contents) were calculated by following the Folin-Ciocalteu reagent method, with minor adjustments in accordance with the protocol elucidated by Singleton, 1999. About 500 µL of the sample was placed in test tubes and then mixed with 10% Folin solution. After that, 2000 µL of 20% Na_2CO_3 was added. The mixture was then kept at 30°C for incubation. Absorbance was taken by using a calibrated spectrophotometer at a wavelength of 760 nm.

Total flavonoids: The quantification of FC (Flavonoid Content) was conducted following the methodology described by Jia *et al*., (1999). Two duplicates were produced by mixing a 1 mL sample and distilled H_2O (29mL). 2 mL of the mixture was subjected to treatment with sodium nitrate, aluminum chloride, and 1 M NaOH. The absorbance levels at a wavelength of 510 nm were measured in order to systematically calculate the FC of the samples.

Assessment of total antioxidant potential: The evaluation of antioxidants in the water-soluble extracts of samples followed the methods proposed by Prieto *et al*., (1999). The experiment involved the mixing of 19 mL of distilled water with 1 mL of the extract in test tubes. Following the addition of 4000 µL of an antioxidant solution, the test tubes were incubated at a temperature of 95°C for 90 minutes. The absorbance levels were measured by using spectrophotometer at 695 nm, with a blank sample analyzed prior to the actual samples.

Zone of inhibition assay: Agar well diffusion method was used to investigate the antimicrobial activity (Bauer *et al*., 1966). The analysis of antibacterial activity was done using an autoclaved prepared medium, specifically nutrient-agar following the method described by Cruick-shank *et al*., 1975. For the investigation of antifungal activity, potato dextrose Agar was used by following the method of Johansen, (1940).

Minimum inhibitory concentration (MIC): The method of Murray (1990) was followed to assess the antimicrobial potential of plant samples with lower constancy.

Activity index (AI): The values of MIC were used to determine the Activity index of the extracts by using the formula:

$$\text{Activity index (AI)} = \frac{\text{Zone of inhibition values of sample}}{\text{Zone of inhibition values of standard}}$$

Determination of minimum bactericidal concentration (MBC): A 3 µL sample was mixed with 100 µL of nutrient broth (NB) in each well. This mixture was then diluted in a series to conclude the minimum bactericidal concentration (MBC). Sample was incubated at 20°C for 2 days. The optical density (OD) of each well was determined using a digital colony counter and compared to the standard readings of Ampicillin, which served as the positive control.

Determination of minimum fungicidal concentration (MFC): A 3 µL sample was mixed with 100 µL of nutrient broth (NB) in each well. This mixture underwent a dilution series to determine the minimum fungicidal concentration (MFC) after being incubated at 20°C for 2 days. The optical density (OD) of each well was quantified using a digital colony counter and then compared to the standard readings of Terbinafine, which served as the positive control.

Results and Discussion

The total phenolic content was assessed in comparison with gallic acid. The shade-dried aqueous extracts displayed the highest level of total phenolic content (TPC), measuring at 1554.00±1.00 mg/100g GAE (Gallic acid equivalent). This was followed closely by the sun-dried aqueous extracts, which had a TPC concentration of 1260.67±1.53 mg/100g GAE. Both the sun-dried, and shade-dried extracts indicated significant levels of TPC. The ethanol extracts exhibited the lowest recorded values for total phenolic content, specifically 401.00±1.00 mg/100g GAE and 490.00±1.00 mg/100g GAE, as seen in (Table 1). Bajalan *et al*., (2017) reported similar results for *Eremostachys laciniata* which shows that genus Eremostachys is a rich source of phenolic compounds. The determination of total phenolic content was carried out using the methods used in the study done by Azizuddin *et al*., (2013).

The results obtained from the analysis indicate that the ethanol extract of sun-dried samples exhibited the greatest concentration of flavonoids, measuring at 634.568±2.14 mg/100g CE (Catechin equivalent). While the aqueous extract of sun-dried samples had the least flavonoid content, with a recorded value of 148.148±3.70 mg/100g CE as shown in (Table 2 & Fig. 1). The results of this study emphasize the persistent pattern that ethanol extracts regularly exhibit larger quantities of flavonoids in comparison to aqueous extracts, irrespective of whether the plant matter underwent sun or shade drying procedures.

Table 3 presents the antioxidant content of the aqueous extracts obtained from sun-dried and shade-dried samples. The sun-dried extract demonstrated the maximum antioxidant content, measuring at 2776.22±2.70 µg/100g AAE (Ascorbic acid equivalent). Conversely, the shade-dried extract revealed the smallest antioxidant content, amounting to 2093.3316±5.63 µg/100g AAE (Fig. 2). When comparing the two, it was observed that both the ethanol extracts obtained from specimens that were dried under the sun and the aqueous extracts obtained from samples that were dried in the shade had reduced levels of antioxidants. The findings of this study emphasize the significant antioxidant activity of *E. vicaryi*, suggesting its considerable ability to effectively neutralize free radicals and thus enhance general health and wellness.

Standard disk samples were meticulously created, which were subsequently employed as positive and negative controls in the studies aimed at evaluating the vulnerability of the test microorganisms. In the context of antibacterial research, the measurement of inhibition zones was conducted for bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. It is interesting to note that amikacin demonstrated an inhibition zone of 18mm against *E. coli*, cephalaxin displayed a 13mm zone against *P. aeruginosa*, erythromycin revealed a 22mm zone against *S. aureus* and ampicillin yielded a 19mm zone against *B. subtilis* (Table 4 & Fig. 3). The negative control readings persistently yielded a value of zero, which was used as a baseline to ensure the accuracy of the results (Table 5). It is imperative to point out that the final results of these experiments were contingent upon various factors, such as the pH levels of the media and solvents utilized, as well as the specific temperature conditions upheld during the testing protocols, as elucidated in a study conducted by Tiwari *et al*., (2011).

Table 1. Mean value for phenolic contents.

Solvent	Drying method		Mean
	Sun dry	Shade	
Ethanol	401.00±1.00^{D}	490.00±1.00^{C}	445.50±48.76
Aqueous	1260.67±1.53^{B}	1554.00±1.00^{A}	1407.33±160.67
Mean	830.83±470.86^{B}	1022.00±582.78^{A}	

Units=mg/100g GAE (GAE stands for Gallic acid equivalent). Significantly different mean values do not share a letter

Table 2. Mean value for flavonoid content.

Solvent	Drying method		Mean
	Sun dry	Shade	
Ethanol	634.568±2.14^{A}	253.926±35.30^{B}	444.247±209.68^{A}
Aqueous	148.148±3.70^{C}	188.889±3.70^{C}	168.519±22.56^{B}
Mean	391.358±266.44^{A}	221.407±42.11^{B}	

Units= (mg/100g CE (CE stands for catechin equivalent). Significantly different mean values do not share a letter

Table 3. Mean value for antioxidant.

Solvent	Drying method		Mean
	Sun dry	Shade	
Ethanol	2154.59±2.70^{C}	2424.86±2.70^{B}	2289.79±148.05^{B}
Aqueous	2776.22±2.70^{A}	2093.33±5.63^{D}	2434.77±374.05^{A}
Mean	2465.41±340.48^{A}	2259.10±181.63^{B}	

Units= µg/100g AA (AAE stands for ascorbic acid equivalent). Significantly different mean values do not share a letter

Table 4. Inhibition zone of standard anti-microbial discs (at concentration 25 µg) against selected bacterial strains.

Bacterial strains	Standard disc	Inhibition zone (mm)	Final response
P. aeruginosa	Cephalaxin	13±0.5	R*
E. coli	Amikacin	18±0.5	R*
B. subtilis	Ampicillin	19±0.7	R*
S. aureus	Erythromycin	22±1	R*

R*; Resistant

Table 5. ZI for blank solvents for negative control.

Solvent*	Quantity 10ml	*S. aureus*	*E. coli*	*P. aeruginosa*	*B. subtilis*
a	10	0±0	0±0	0±0	0±0
b	10	0±0	0±0	0±0	0±0
c	10	0±0	0±0	0±0	0±0
d	10	0±0	0±0	0±0	0±0
Final response	N**	N**	N**	N**	N**

*a; Petroleum ether, b; Chloroform, c; Methanol, d; Aqueous; n**; Negligible

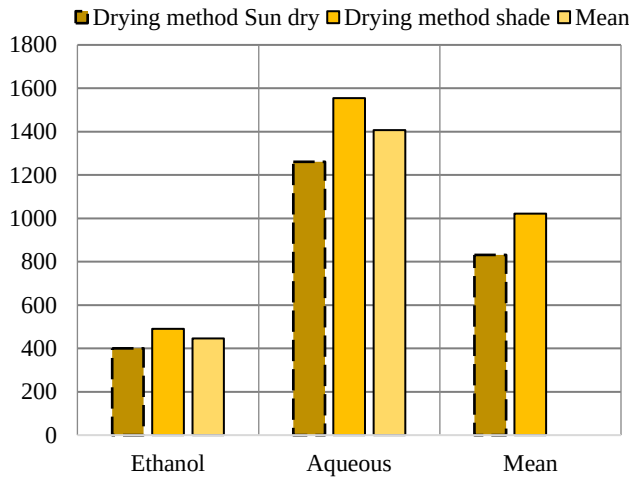

Fig. 1. Mean values for phenolic contents.

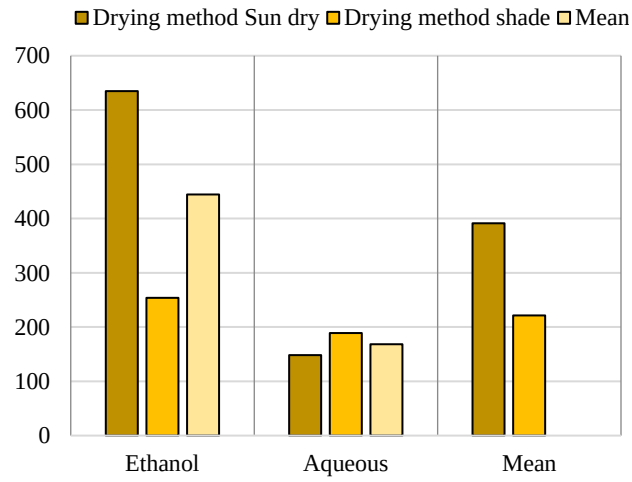

Fig. 2. Mean value of Flavonoid contents.

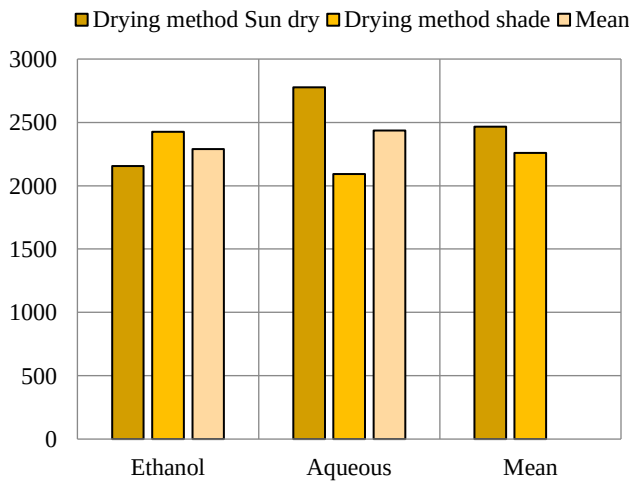

Fig. 3. Mean values for antioxidants.

In this study, four separate types of extracts were used, with each extract being made using different solvents. These extracts were then exposed to testing against *S. aureus*, as shown in (Table 6). Significantly, the chloroform extracts displayed the largest zone of inhibition against *S. aureus*, measuring at 26±1 mm, whereas the methanol extracts revealed the smallest zone of inhibition (13±2 mm) (Table 6). This finding is consistent with the findings reported by Ates & Turgay, (2003), in which they discovered that chloroform extracts derived from *Cinnamomum cassia* had comparable efficacy against *S. aureus*.

In the context of *E. coli*, it was noted that the chloroform extracts demonstrated the highest zone of inhibition (ZI) value, measuring at 22.3±0.57 mm. Conversely, the methanol extracts revealed the lowest ZI value, measured at 13.6±1.15 mm, as indicated in Table 6.

The findings for *B. subtilis* yielded comparable outcomes, with the chloroform extracts showing the maximum ZI value of 19.3±0.57 mm, and the aqueous extracts demonstrated the lowest ZI value of 9.0±1.0 mm, as indicated in Table 6.

In the context of *P. aeruginosa*, it was observed that methanol extracts exhibited the most substantial inhibition zone (ZI) with a mean value of 26.0±1.0 mm, whereas aqueous extracts exhibited the smallest ZI at 8.3±0.57 mm. The results presented in this study align with the findings of a prior investigation conducted by Chah *et al*. (2000), which provided evidence of the antimicrobial properties of methanolic extracts against *P. aeruginosa* (as shown in Table 6 & Fig. 4).

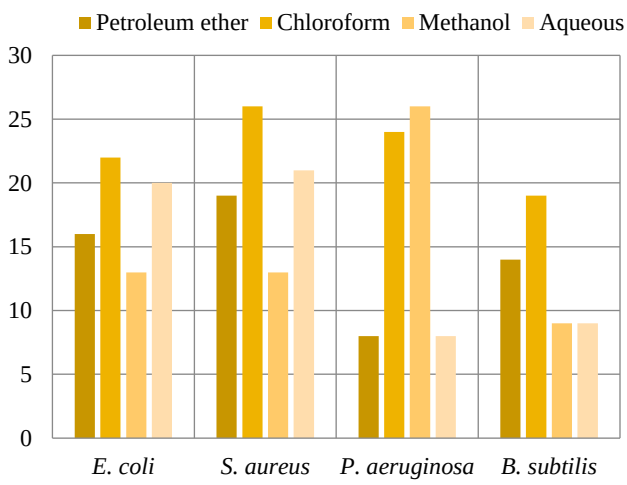

Fig. 4. Zone of inhibition against 4 bacterial strains.

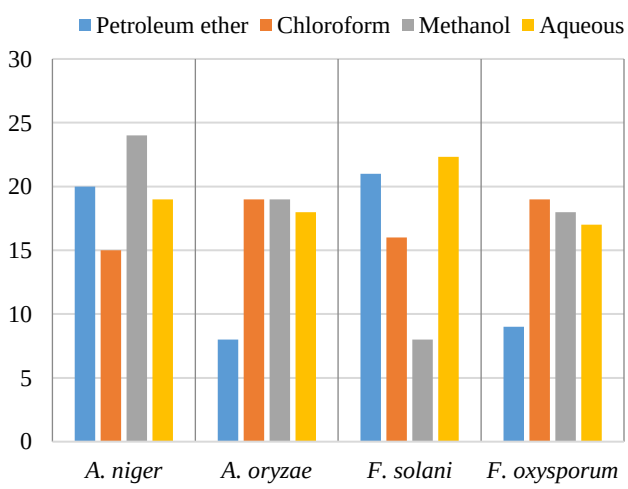

Fig. 5. Zone of inhibition against 4 fungal strains.

Table 6. Zone of Inhibition produced by *E. vicaryi* against bacterial strains.

Extracts *	Inhibition Zone (mm)			
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
a	16.66±1.15	19±1.52	8.66±1.52	14.66±0.57
b	22.33±0.57	26±1	24±1	19.33±1.52
c	13.66±1.15	13±2	26±1	9.33±0.57
d	20.33±1.52	21.66±0.57	8.33±0.57	9±1

*a; Petroleum ether, b; Chloroform, c; Methanol, d; Aqueous

Table 7. Activity index produced by *E. vicaryi* against bacterial strains,

Extracts	Activity index (AI)			
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
a	0.92	0.86	0.66	0.77
b	1.24	1.1	1.84	1.01
c	0.75	0.59	0.79	0.49
d	1.12	0.98	0.64	0.47

*a; Petroleum ether, b; Chloroform, c; Methanol, d; Aqueous

The extracts derived from *E. vicaryi* demonstrated significant antibacterial activity against a range of bacterial species. The chloroform extracts exhibited zone of inhibition (ZI) values ranging from 8±0.57mm to 26±1.1mm, indicating their efficacy against *P. aeruginosa* and *E. coli*. These results are consistent with the results reported by Arora *et al.,* (2015). The aqueous extracts displayed zone of inhibition (ZI) values ranging from 8±0.57mm to 21±0.57mm, whereas the methanol extracts demonstrated ZI values ranging from 9±0.57mm to 26±1mm. The methanol extracts exhibited the most potent antibacterial activity towards *P. aeruginosa*, which aligns with the observations made by Jahan *et al*., (2010). The ZIs of the petroleum ether extracts ranged from 8±1.52mm to 19±1.52mm. A study conducted by Asgharian *et al*., (2017) showed promising antibacterial activity, he used extracts of *Eremostachys macrophylla* and confirmed antimicrobial activity against two bacterial strains these findings along with results obtained from our study suggest that members of this genus might contain possible new antimicrobial compounds.

The determination of the zone of inhibition (ZI) was conducted by employing conventional antifungal discs that included different antibiotics as positive controls. The results showed that these antibiotic discs had moderate blockage against *Aspergillus niger*, *Aspergillus oryzae*, *Fusarium oxysporum* and *Fusarium solani*; more information can be found in (Table 8).

During the evaluation of experimental fungal specimens for the purpose of establishing a negative control, blank solvents were utilized. The resulting zone of inhibition was shown to be insignificant for all four solvents, as clearly shown in (Table 9).

In the examination of the antifungal properties of *E. vicaryi* against *A. niger*, it was noted in (Table 10) that the methanolic extract possessed the most significant zone of inhibition, measured at 24.6±2.5 mm. On the other hand, the chloroform extract showed the smallest zone of inhibition, measuring 15.6±1.51 mm.

In the course of examining the antifungal properties of *E. vicaryi* against *A. oryzae*, it was observed that the methanolic extract displayed the most significant zone of inhibition, measured at 19.3±1.0 mm (Table 10). In contrast, the petroleum ether extract demonstrated the smallest zone of inhibition, measuring 8.6±1.5 mm.

In the study of *E. vicaryi*'s antifungal efficacy against *F. solani* (Table 10 & Fig. 5), it was discovered that the aqueous extract had the greatest zone of inhibition, measuring 22.3±1.52 mm. The methanol extract, on the other hand, had the lowest zone of inhibition, with a value of 8.6±0.57 mm.

During the examination of the antifungal properties of *E. vicaryi* against *F. oxysporum*, it was noted that the chloroform extract had the most significant zone of inhibition, measuring at 19.0±2.0 mm. In contrast, the petroleum ether extract exhibited the smallest zone of inhibition, measuring 9.0±1.0 mm (Tables 10 and 11).

The limited antifungal activity obtained in the aqueous extracts is worth pointing out, as it is consistent with the data reported by Chakraborthy, (2008) about the antifungal activity of *Calendula officinalis* (L.).

Furthermore, it was observed that extracts obtained from *E. vicaryi* had significant efficacy against various fungal strains, with values ranging from 8±1.52mm to 21±2.08mm in petroleum ether. The chloroform extracts presented a diverse array of inhibitory zones, with values ranging from 15±1.52mm to 19±1.52mm. The aqueous extracts displayed inhibition zones that varied between 18±1mm and 22.33±1.52mm. Similarly, the methanol extracts exhibited a broad range of inhibition zones, ranging from 8.6±0.57mm to 24.6±2.51mm. The results reported together underscore the varied antifungal activities of *E. vicaryi* across different solvent extracts. Mimica-Dukic *et al*. (2004) reported considerable antifungal activity from essential oils of *Melissa officinalis* L.

The investigation into the minimum inhibitory concentration (MIC) of *E. vicaryi* was conducted for four bacterial strains (Table 12). The plant extracts of *E. vicaryi* exhibited MIC values of > 0.15±0.07 for *E. coli*, 0.10±0.02 for *S. aureus*, 0.13±0.02 for *P. aeruginosa*, and 0.06±0.01 for *B. subtilis*.

The study focused on determining the minimum bactericidal concentration (MBC) of *E. vicaryi* for four bacterial strains, as presented in (Table 13). Additionally, the investigation also identified the minimum fungicidal concentration for four fungal strains. Regarding bacterial strains, the water extracts derived from *E. vicaryi* had a highest value for minimum bactericidal concentration (MBC) of 100.4 µg against *S. aureus*, whereas the chloroform extracts revealed a minimum MBC of 76.9 µg. In the case of *B. subtilis*, the highest minimum bactericidal concentration (MBC) was seen when using petroleum ether extracts, with a value of 101.2 µg. Conversely, the lowest MBC was recorded when using methanol extracts, with a value of 85.9 µg. In relation to *P. aeruginosa*, the highest minimum bactericidal concentration (MBC) was determined to be 94.2 µg using petroleum ether extracts, while the smallest MBC was discovered to be 70.5 µg using methanol extracts. In the case of *E. coli*, the highest minimum bactericidal concentration (MBC) was determined to be 96.1 µg using chloroform extracts, whereas the lowest MBC was seen using methanol extracts at 80.1µg. Khakshoor & Pazooki, (2014) also engage in a discussion about MBC.

Table 8. Zone of inhibition of standard anti-microbial discs against four strains of fungi.

Fungal strains	Concentration (µg)	Antifungal standard disc	Inhibition zone(mm)	F.R*
A. niger	60	Terbinafine	21 ± 0.5	Intermediate
	60	Griseofulvin	22 ± 1.5	Intermediate
A. oryzae	60	Terbinafine	20 ± 1.5	Intermediate
	60	Griseofulvin	22 ± 2.0	Intermediate
F. oxysporum	60	Terbinafine	24 ± 1.5	Intermediate
	60	Griseofulvin	18 ± 1.0	Intermediate
F. solani	60	Terbinafine	18 ± 2.0	Intermediate
	60	Griseofulvin	18 ± 0.5	Intermediate

F.R*. Final response

Table 10. Zone of Inhibition produced by *E. vicaryi* against fungal strains.

Extracts	Inhibition zone (mm)			
	A. niger	***A. oryzae***	***F. solani***	***F. oxysporum***
Petroleum ether	20 ± 1	8.66 ± 1.52	21.66 ± 2.08	9 ± 1
Chloroform	15.66 ± 1.52	19.66 ± 1.52	16.66 ± 0.57	19 ± 2
Methanol	24.66 ± 2.51	19.33 ± 1.15	8.66 ± 0.57	18 ± 2
Aqueous	19 ± 4.58	18 ± 1	22.33 ± 1.52	17.66 ± 2.08

Table 11. Activity index produced by *E. vicaryi* against fungal strains.

Extracts	Activity index (AI)			
	A. niger	***A. oryzae***	***F. solani***	***F. oxysporum***
Petroleum ether	0.91	0.39	1.2	0.5
Chloroform	0.71	0.89	0.92	1.1
Methanol	1.12	0.87	0.48	1
Aqueous	0.86	0.81	1.24	0.98

Table 12. MIC (Minimum inhibitory concentration) of different strains of bacteria.

E. coli		***S. aureus***		***P. aeruginosa***		***B. subtilis***	
Conc.(mg/mL)	**MIC**	**Conc.(mg/mL)**	**MIC**	**Conc.(mg/mL)**	**MIC**	**Conc.(mg/mL)**	**MIC**
0.9	0.15±0.07	0.4	0.10±0.02	0.7	0.13±0.02	0.2	0.06±0.01

Table 13. MBC (Minimum bactericidal concentration) of four solvents against different strains of bacteria.

Test organism used	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***
Units	**µg**	**µg**	**µg**	**µg**
Petroleum ether	99.4	101.2	94.2	82.8
Methanol	87.2	85.9	70.5	80.1
Water	100.4	93.7	86.5	94.4
Chloroform	76.9	84.3	81.3	96.1

Table 14. MFC (Minimum fungicidal concentration) of four solvents against different strains of fungi.

Test organism used	***F. solani***	***A. oryzae***	***F. oxysporum***	***A. niger***
Units	**µg**	**µg**	**µg**	**µg**
Water	106.1	108.5	100.3	99.3
Methanol	104.5	95.7	96.1	102.3
Petroleum ether	98.4	107.3	101.6	110.4
Chloroform	103.4	112.5	106.9	105.7

The study focused on determining the minimum fungicidal concentration (MFC) of *E. vicaryi* across four different fungal strains, as indicated in (Table 14). Regarding fungal strains, it was observed that the water extracts obtained from *E. vicaryi* demonstrated the highest minimum fungicidal concentration (MFC) of 106.1 µg against *F. solani*, while the petroleum ether extracts revealed the lowest MFC of 98.4 µg. The highest reported maximum fungal growth inhibition concentration (MFC) for *A. oryzae* was found to be 108.5 µg in water extracts, whilst the lowest MFC was recorded at 95.7 µg in methanol extracts. In relation to *F. oxysporum*, the highest maximum microbial fuel cell (MFC) was obtained using chloroform extracts at a concentration of 106.9 µg, while the lowest minimum MFC was recorded with methanol extracts at a concentration of 96.1 µg. In the case of *A. niger*, the highest microbial fuel cell (MFC) value was seen with petroleum ether extracts, measuring 110.4 µg. Conversely, the lowest MFC value was obtained with water extracts, measuring 99.3 µg. The findings presented in this study are corroborated by the research conducted by Nateqi & Mirghazanfari, (2018).

Novelty statement: No phytochemical and pharmacological studies have so far done on this plant except isolation of an isoflavone-vicarin (Imran *et al*., 2012). The results obtained from this study can be considered preliminary data necessary for the elaboration of the study. This intriguing blueprint may impel scientists to thoroughly investigate the possibilities of these natural products for the development of reliable, environmentally-safe, and patent medicines. In short, this plant is a potential candidate for phytomedicine and may be an effective natural antibiotic agent against many prevailing toxic strains of bacteria and fungi that have put a dam in the nose.

Conclusion

No study has been previously reported on assessment of phenolic composition, antioxidant and antimicrobial activities of *Eremostachys vicaryi* so for. This first-ever report provides inaugural comparable baseline data about its total phenolic composition, antioxidant and antimicrobial activities of *E. vicaryi* from the study area (Soon Valley) with unique edaphic and climatic conditions. This plant is unexplored regarding the phytochemical novelty for unique pharmacological activities. Determination and isolation of such compounds may lead to the discovery of new drugs in the field of medicine. In the present research with aqueous extracts from shade-dried plants displayed the highest total TPC while ethanol extracts from sun-dried plants exhibited highest TFC and highest antioxidant activity. Antimicrobial activity was observed against both gram-positive and gram-negative bacteria. Substantial antifungal activity was also observed against different fungal strains. These findings not only contribute to the understanding of *E. vicaryi*'s medicinal properties but also hold implications for drug discovery and the development of novel therapeutic agents. In summary, our study underscores the importance of exploring the pharmacological potential of *E. vicaryi* and highlights its significance as a valuable resource in the field of natural product-based drug discovery.

Acknowledgments

The manuscript is a part of M. Phil dissertation of the second author (Gulfam Shahzad). The authors are thankful to the Higher Education Commission of Pakistan (HEC) (Programme No. 20-4546/ R&D/NRPU/HEC/14/850) for financial support. The authors are also indebted to the laboratory of the Institute of Food Sciences and Nutrition (IFSN) for cooperation and for providing necessary lab. facilities. We are also thankful to the ORIC Department, University of Sargodha, Sargodha who provided us financial support under Project No. UOS/ORIC/ 2016/43 to establish Herbarium and enable us to deposit (preserve) voucher specimen of the plant under voucher number GS-706 for future reference.

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(Received for publication 21 November 2023)