

Research Article

Phytochemical analysis and antimicrobial activities of *Kochia indica* (Wight), plant growing in District Karak Khyber Puhktunkhuwa, Pakistan

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Abstract

The medicinal plants play a key role for the treatment of various types of diseases because of bioactive compounds. Study aims, to investigate the antibacterial potency, phytochemical analysis and also perform gas chromatography and mass spectrometry (GC-MS) for isolations of bioactive compounds of *Kochia indica* Wight plant. In antibacterial activity the whole plant extracts of *Kochia indica* Wight, against *S. typhi*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains at different concentrations. According to the results obtained, the ethanol extract of *Kochia indica* was 40%-20.37% (15 mg/ml), 13.11%-19.10% (10 mg/ml) and 11.20%-17.44%, (5mg/ml) concentration over negative control. The best quantitative phytochemical analysis of *Kochia indica* Wight was performed using extracted optical density (OD) and mass chromatography-mass spectrometry (GC-MS) techniques. Results reveal the presence of important antibacterial and phytochemical compounds predominantly 1-Propanol, 2-methyl- (63.27 %), 1,1-Diisobutoxy-isobutane (8.83%), Oleic Acid (7.54%), Cyclohexanone (6.60%), Acetone (3.57%), Hexanoic acid, 2-methylpropyl ester (2.75%), n-Hexadecanoic acid (1.47%), 2-Butenedioic acid (Z)-, dibutyl ester (1.41%), Sebacic acid, ethyl hexyl ester (0.93%), Phytol (1.00%), Octadecanoic acid (0.78%), Cyclopentane carboxylic acid, butyl ester (0.65%), 1-Butanol (0.42%), Propanoic acid, 2-methyl, 2-methylpropyl ester (0.48%), Cyclohexene, 1-(1,1-dimethylethoxy) (0.31%). To detect more effective process and compounds to initiate and develop the most effective approaches and methods to reduce the effectiveness of bacteria on environment as well as in human health.

Keywords: Antibacterial activity; GC-MS analysis; *Kochia indica* Wight; Phytochemical analysis

Introduction

The medicine or drugs obtain from plants are safe and have no side effect as compared to the modern and synthetic medicine and drugs which are not safe having side effect and environment non-friendly. From the primitive times, plants keep the value

medications, aromatic, nutritive as well as flavoring abilities or characters. Now a days, the advancement of modern synthetic drugs and medicine are fully compressed by the drugs and medicine obtained from plants i.e. herbal medicines or drugs [1]. The 25% of drugs and medicine universally approved

provide by the medicinal plants and 121 compounds are in active use and 252 prescriptions thought as elementary and important according to the World Health Organization (WHO). Medicinal plants are a rich source of antibacterial compounds that are effective against natural pathogens. Pakistan has many medicinal plants rich in antibacterial compounds with antibacterial activity. This plant has been used for decades in the production of various medicines [2]. Mainly the drugs which isolated from the medicinal plants worldwide preferred that of synthetic in medicine discipline. The remedial part of a therapeutic plants is used only fresh green leaves and stem, so the quality and quantity of the phytochemical present in that part of the medicinal plant. Such phytochemical have so many chemical constituents such as proteins, gums mucilage, carbohydrates, pectin's, different form of glycoside, phenol compounds, tannins, resins, lipids, fixed and volatile oils, different kinds of alkaloids etc [3].

Therefore, it is necessary and important not only to improve the quality of existing medicines (medicines), but also to find effective new medicines. Numerous studies have shown that herbal medicines have various antifungal and antibiotic properties. Not only are plant extracts cheap and have little impact on the environment, they also minimize synthetic health risks [4].

This study aims to investigate the phytochemical composition and antimicrobial activity of plant extracts. The plant was chosen because there is little scientific evidence for the plant and the need to discover the therapeutic value of herbs. In addition, various compounds found in the *Kochia Indica* plant were measured by gas chromatography and mass spectrometry (GC-MS) analysis.

Materials and Methods

Preparation of plant extracts

Medicinal plants (*Kochia indica* Wight) were collected in district Karak, Khyber pukhtoonkhwa, Pakistan, dried in the shade and then homogenized with fine powder. Using this fine powder, methanol, ethanol, and water extracts were made from these plants according to the method described in [5]. Twenty-five grams of each plant powder was soaked in 100 ml of ethanol for 10 days. The extract was then passed through a muslin cloth, filtered and dried in a 45°C water bath. The resulting past was placed in an amber bottle and stored at 4 °C until use.

Antibacterial activity

The Antibacterial activities was performed by using change of the method is clearly demonstrated and broadly used for activities test [6]. A lapful bacterium was point out from the solution and add o, 1 mili letter saline. After placing the discs, all tests were carried out on the surface of Mueller Hinton agar pre-inoculated with 10 liters of 10 liters of liquid medium MHA containing different crude solvent extracts than 9 mm in diameter. A known solvent without plant extract serves as a control. The standard antibiotics were chloramphenicol 30 mg: disc and streptomycin 30 mg: disc control +ve. The plate was heated at 37° C. for 24 hours and the formation of open areas around the disc was observed.

Phytochemical analysis

The dry powder and extract of *Kochia indica* plant were used for the evaluations of different types of phytoconstituents. The standard method was used for the evaluation of phenols and flavonoids [7].

Gas chromatography and mass spectrometry (GC-MS) analysis of plant extracts

GC-MS analysis of *Kochia indica* Wight ethanol extract shows that the most active and promising antibacterial activity was achieved by Agilent 7890C gas chromatography in

combination with 5975C mass selective detector (MSD) and hypersensitive panchromatic 5 (HP5), mass spectrometry imaging (MSI) separator columns. Here, the length is 30mm, the inside diameter is 0.250mm, and the film thickness is 0.25µm. The deposition, determination and quantity of compounds was carried out by accelerator mass spectrometry (AMDS) using the GCMS library with manual storage index, and the subsequent identification of the compounds was carried out using the NIST17 and Wiley 11 GC (Gas chromatography) spectral libraries.

Statistical analysis

The current analysis of variance was applied using statistical software 16.1. The mean and standard error of the triples were calculated. This solution was compared using the liquid crystal display (LSD) test at a significant level of $\leq 5\%$.

Result and Discussion

Antibacterial activity of plant extracts

Antibacterial potential of *Kochia indica* plants extract were carried out by using different poisoned antibacterial strains (*S. typhi*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The results indicate that the plants have good antibacterial results as compared to the negative control. Among the test plants *Kochia indica* performed best and inhibit the selected bacterial growth as compared to the negative control. Different concentrations of ethanolic extracts of

Kochia indica from 15mg/ml-5mg/ml concentrations significantly reduce the growth of bacteria by 13.40%-20.37% (15mg/ml), 13.11%-19.10% (10mg/ml) and 11.20%-17.44% at concentrations of 5mg/ml lower the negative control shown in (Table 1; Fig.1).

Phenol and Flavonoids quantifications of plant extracts

The quantitative analysis of flavonoids and phenol of the plants extract of *Kochia indica* were investigated by using ethanol extract of different concentrations. The results indicate that all plants have good results for phenols and flavonoids as compared to the negative control. *Kochia indica* have best result for phenol and flavonoids. The Quercetin chemical are used is a standard for flavonoids having $y = 0.0031x + 0.0159$ while the value of $R^2 = 0.9997$. The Catechol chemical was used as a standard for phenol having the values while the value of $y = 0.0012x + 0.0659$ $R^2 = 0.999$ shown in (Fig. 2). In ethanolic extract the maximum quantity of flavonoids substances were estimated by *Kochia indica* (2.80±0.02) at concentrations of 20ug/ml, while the ethanolic extract of the *Kochia indica* plants showed highest quantity of total phenol contents (1.30±0.5) at concentrations of 20ug/ml, and then followed by (1.19±0.05) and 1.09±0.05 at 10ug/ml and 30ug/ml. As compared to the other concentrations the total phenol and flavonoids contents shows more at concentration of 20ug/ml shown in (Table. 2; Fig. 3).

Table1. Antimicrobial activities of ethanolic extract of *Kochia indica* Wight,

Plant/part used	Conc. mg/ml	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
<i>Kochia indica</i> Wight, (Whole plant)	15mg/µl	20.37± 0.23	17.55±0.39	19.84± 0.23	13.40±0.40	19.30±0.38
	10mg/µl	15.33±0.66	13.11±0.43	17.22±0.31	9.12±0.51	19.10±0.40
	5mg/µl	11.13±0.81	9.05±0.51	14.19±0.42	6.07±0.65	17.44±0.66

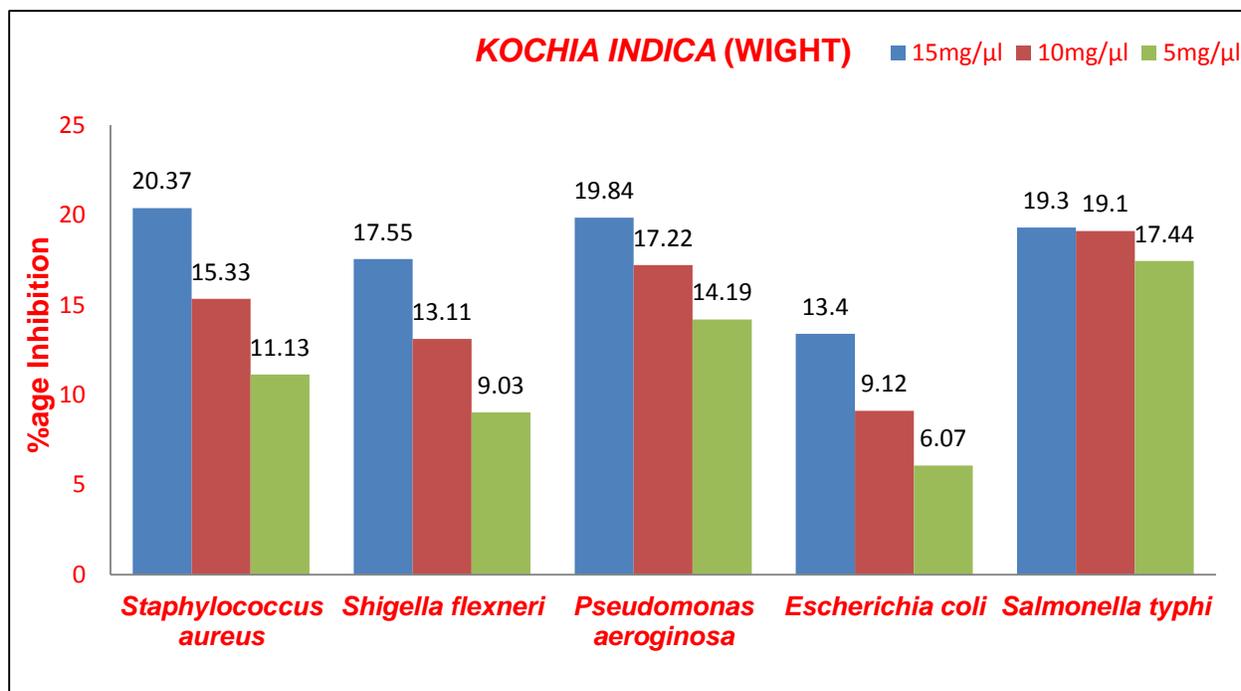


Figure 1. Graph represents the antibacterial activity of plant extracts at various conc.

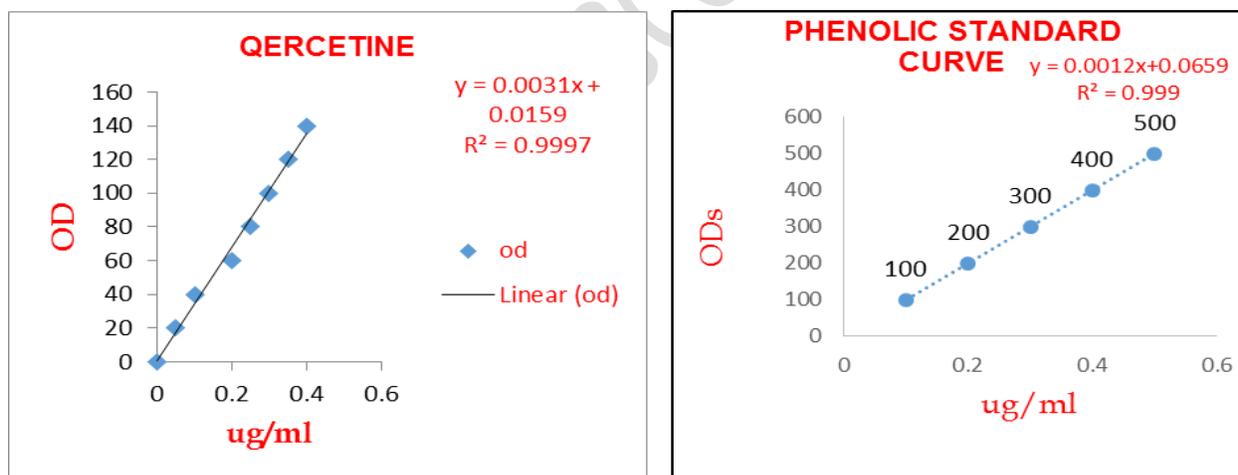


Figure 2. Flavonoids standard curve of Quercetin. Phenol Standard curve of Catechol

Table 2. Total phenol and flavonoids contents of *Kochia indica* Wight, plant

Plant	Parts used	Extracts	Concentrations	Flavonoids μg/ml	Total phenols μg/ml
				mean ± SD	
<i>Kochia indica</i> Wight,	Whole plant	Ethanol	10ug/ml	2.2±0.1	1.19±0.05
			20ug/ml	2.80±0.02	1.30±0.5
			30ug/ml	2.39±0.04	1.09±0.05

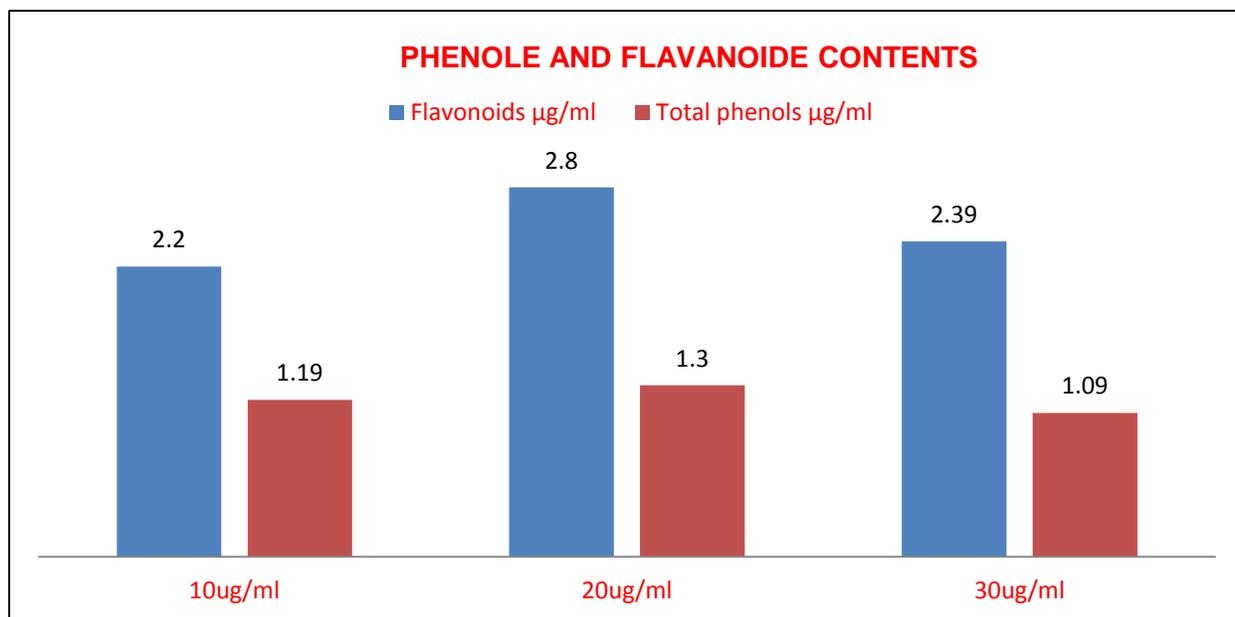


Figure 3. Phenol and Flavanoid contents of *Kochia indica* plants

Gas chromatography and mass spectrometry (GC-MS) analysis

An excellent result-oriented *Kochia indica* extracts was subjected to gas chromatography mass spectrometry (GCMS) analysis. The compound identification of in the extract and molecular weight, molecular formula, retention time and peak area (%). The most prominent and dominant compounds present in ethanolic extracts in *Kochia indica* Wight, were 1-Propanol, 2-methyl- (63.27 %), 1,1-Diisobutoxy-isobutane (8.83%), Oleic Acid (7.54%), Cyclohexanone (6.60%), Acetone (3.57%), Hexanoic acid, 2-methylpropyl ester (2.75%), n-Hexadecanoic acid (1.47%), 2-Butenedioic acid (Z)-, dibutyl ester (1.41%), Sebacic acid, ethyl hexyl ester (0.93%), Phytol (1.00%), Octadecanoic acid (0.78%), Cyclopentane carboxylic acid, butyl ester (0.65%), 1-Butanol (0.42%), Propanoic acid, 2-methyl-, 2-methylpropyl ester (0.48%), Cyclohexene,1-(1,1-dimethylethoxy) (0.31%) are shown in (Table. 3; Fig. 4). Different phenol compounds were detected

from *Kochia indica* which proved their medicative values.

Discussion

The therapeutic medicative plants species are rich source of naturally accruing antimicrobial compounds which play an important role for the development of active, new and effective drugs and medicines. In the literature, the antibacterial activity of these plants has been reported against many plant and human pathogens according to the literature but difficult to study due to choice of solvent, fractionation or extraction method and use of microbes and antibacterial tests [8]. In the recent research, antibacterial potential of medicinal plants extracts using different concentrations of *Kochia indica* were used against *S. typhi*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains. For extractions, dry parts of medicinal plants species were powder and using ethanol as a solvent by using valid and primitive protocol. Mainly the microbial compounds extracted from medicinal plants are may be saturated organic in nature or may be aromatic in

nature, that's why they are easily extracted by using ethanol as a solvent. It is now compatible that different solvents have different solubility for different photochemical [9]. All extracts tested showed antibacterial activity against isolated bacteria. Of these, the plant extract of *Kochia indica* showed the greatest inhibition 13.40%-20.37% (15mg/ml), 13.11%-19.10% (10mg/ml) and 11.20%-17.44% at concentrations of 5mg/ml as compared the negative control respectively. Previous researches investigate the extract of leaves of two plants by using *A. porri* and *Stemphylium* sp. and can observed 62% and 72% of inhibition growth in *A. Porri* and *Stemphylium* sp, respectively [10]. The leaves extract *Eucalyptus* species, five hundred micro liters can form maximum inhibition i.e. 8.6mm by using *A. porri* strain describe by [11]. The investigation shows, that plant extract is directly proportional to

the toxicity and inhibition of bacterial growth i.e. increase plants extract shows more toxicity to bacteria. The plant extract is effective against bacteria and fungus as well as different biological activities due to the presence of various constituents in plants like alkaloid, flavonoids, steroids, triterpenoids, and free hydroxyl groups or phenols compounds. The presence and aggressive release of these secondary compounds is often the cause of biological regulation as they can affect the enzymatic system [12]. GC-MS analysis is a potential tool for accumulating compounds, identifications, and abundances in medicinal plant fractions or extracts. This study identified 15 key compounds from the ethanol extract of *Kochia indica* Wight. The previous investigate 16 phytochemical in ethanolic extract of *Citrus Medica* and quantify 27 compounds i.e. linalyle acetate, α -terpineol, and linalool [13, 14].

Table 3. Compound identified from ethanolic inflorescence extract of *Kochia indica* Wight

S. #	Names of Compounds	Formulas	Molecular weight (g/mol)	Retention time (Min)	Peak Area (%)
1	Acetone	C ₃ H ₆ O	58.08	1.790	3.57
2	1-Propanol, 2-methyl-	C ₄ H ₁₀ O	74.122	2.708	63.27
3	1-Butanol	C ₄ H ₁₀ O	74.121	2.903	0.42
4	Cyclohexanone	C ₆ H ₁₀ O	98.15	7.884	6.60
5	Propanoic acid, 2-methyl-, 2-methylpropyl ester	C ₈ H ₁₆ O ₂	144.21	8.368	0.48
6	1,1-Diisobutoxy-isobutane	C ₁₂ H ₂₆ O ₂	202.334	16.025	8.83
7	Cyclohexene,1-(1,1-dimethylethoxy)	C ₁₀ H ₁₈ O	154.25	16.339	0.31
8	Hexanoic acid, 2-methylpropyl ester	C ₁₀ H ₂₀ O ₂	172.26	17.294	2.75
9	2-Butenedioic acid (Z)-, dibutyl ester	C ₁₂ H ₂₀ O ₄	228.2848	24.775	1.41
10	Cyclopentane carboxylic acid, butyl ester	C ₁₀ H ₁₈ O ₂	170.25	27.213	0.65
11	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.43	43.343	1.47
12	Phytol	C ₂₀ H ₄₀ O	128.1705	46.792	1.00
13	Oleic Acid	C ₁₈ H ₃₄ O ₂	282.47	47.817	7.54
14	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.48	48.255	0.78
15	Sebacic acid, ethyl hexyl ester	C ₁₈ H ₃₄ O ₄	314.4602	50.892	0.93

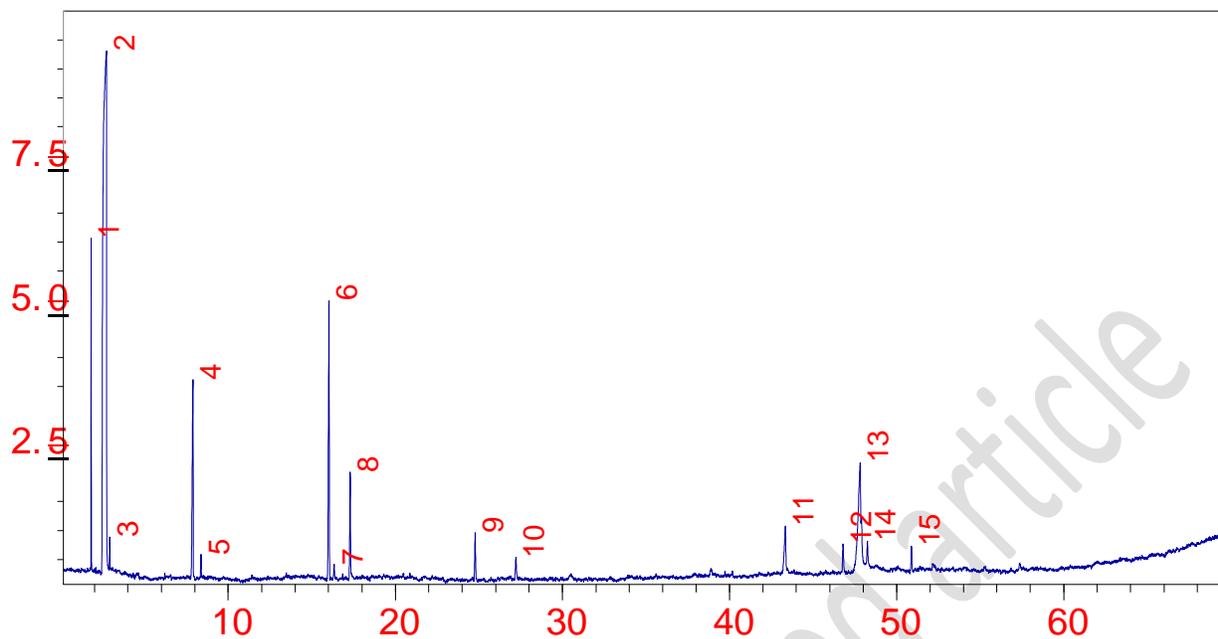


Figure 4. GC-MS Chromatograms of ethanolic extract of *Kochia indica* Wight

Conclusion

This study has shown that traditional use of medicinal plants is effective for antimicrobial activity. The ethanol extract of *Kochia indica* Wight is concluded that it may have antibacterial activity against *S.typhi*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Therefore, further research is needed to identify more compounds to form stable products in order to develop effective management approaches that significantly reduce the effects of bacteria on human health and the environment.

Authors' contributions

Conceived and designed the experiments: H Bibi & S Khan, Performed the experiments: H Bibi & S Khan, Analyzed the data: M Hussain, Contributed materials/ analysis/ tools: G Jan & I Ullah, Wrote the paper: H Bibi, M Hussain & GM Shah.

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