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Phytochemical Analysis and Antibacterial Activities of *Tamarix dioica* Extracts

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Abstract

The objective of this study was to investigate the impact of phytochemical analysis and the antibacterial activity of extracts of stem, flowers and leaves of *Tamarix dioica* Roxb. ex Roth. Four solvents namely, diethyl ether, ethyl acetate, methanol and acetone were selected to obtain extracts from different parts of the plant. The organic solvent extracts were investigated for phytochemical analysis and antibacterial activity against two bacterial strains, namely *Escherichia coli* and *Staphylococcus aureus*. The result of phytochemicals revealed the presence of various constituents, such as phlobatannins, tannins, saponins, alkaloids, phenols, proteins, terpenoids, flavonoids and steroids by using standard procedures. Most of these components were present in methanol and ethyl acetate extract. Therefore, four out of two extracts, such as methanol and ethyl acetate extracts from stems, flowers, and leaves, were used to test their evidence of antibacterial activity. From this, it was observed that the methanol extracts of stem, flowers and leaves of *T. dioica* were highly effective together with *E. coli* and *S. aureus* with a minimum inhibitory concentration (MIC) value of 500 µg/mL. Considering that the ethyl acetate (EA) extracts from the stem, flowers and leaves of *T. dioica* were examined to be ineffective against *E. coli* and *S. aureus* and MIC values were not observed in two strains of bacteria.

Keywords: *Tamarix dioica* extracts, Phytochemical analysis, Antibacterial activities.

Introduction

Plants are important components of the universe and used to cure of several diseases from ancient time. According to the report of the world health organization (WHO), approximately 80% of the world population depends on traditional medicine for its treatment and physical health [1]. These plants have opened the window to the progress of numerous therapeutic agents [2]. In Islam, diseases are treated in two ways, the first is to treat oneself with prayer, and the second is to treat illnesses through medicine [3]. Plants contain a lot of phytochemicals such as amino acids, saponins, tannins, glycosides, alkaloids, fatty acids, sterols, flavonoids and terpenes [4]. These phytochemicals protect plants themselves, but recent research reveals that many of these phytochemicals can protect animals and humans against various diseases [5], including cancer, diabetes and cardiovascular disorders [6]. Phytochemicals are non-nutritive, natural, secondary metabolites and biologically active compounds. However, a large proportion of

phytochemicals has not yet been identified and is still identified by the scientific community [7-9].

T. dioica with the family of Tamaricaceae (common name: Lai) that grows from 1 to 18 m of height includes 60 different species. It is a small tree or evergreen shrub and has vaginate leaves, purple flowers and reddish bark. The *Tamarix* genus feeds on approximately around 250 species of invertebrates and cattle and camels [10]. Although, *Tamarix* hold the height of a small tree and has a deep taproot that can extend 25 to 30 meters below the earth's surface. Mostly, tree is found in Pakistan, Afghanistan, India, Bangladesh, Iran, Bhutan, Nepal, Myanmar and Kashmir. In Pakistan, it is found in Sindh and Khyber Pakhtun Khwa (KPK) provinces [11].

The leaves of *T. dioica* are used as a diuretic (promotes diuresis), carminative and to heal irritation of the liver and spleen. In addition, this plant is used as a constringent for symptoms such as vaginal discharge [12]. *T. dioica* has demonstrated antibacterial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as well as antifungal activity against three microorganisms, *Candida glabrata, Aspergillus fumigates* and *Trichophyton rubrum* [13]. Survey of the literature shows that little work has been found in the phytochemical trace and antibacterial activities of *T. dioica*. Taking into account all these facts, the current study was designed to identify the existence of different phytochemicals in three different parts of *T. dioica* (such as stem, flowers and leaves) and the determination of their antibacterial activity.

Materials and Methods

Chemicals and Reagents

All chemicals (Sigma-Aldrich Chemical Company) were used for biological activity and analysis of phytochemicals. Glacial acetic acid, Chloroform, dimethyl sulfoxide, diethyl ether, ethyl acetate, methanol, acetone, ferric chloride, sulfuric acid, hydrochloric acid, sodium hydroxide, copper(II)sulphate, iodine, potassium iodide, Benedict reagent, Wagner's reagent, Ninhydrin reagent, Fehling solution, Muller Hinton agar (MHA) and distilled water.

Plant Materials (Collection & Identification)

The *T. dioica* collected from Sindh University, New Campus, Jamshoro, Sindh, Pakistan, in the month of September and November 2019 (Longitude: 25.430387 and latitude: 68.280861) and deposited in the herbarium of the Institute of Plant Sciences (IPS), University of Sindh (UoS), Jamshoro, Pakistan. A file and voucher specimen (2671317) of the plant provided by IPS taxonomist, UoS, Jamshoro.

Drying and Crushing of Plant Material

The parts of *T. dioica* were washed thrice with sterilized water to remove dust and contaminated particles, dried in the shade for 15 days, all parts were individually crushed with an electric mixer and the powder it was placed in different containers before analysis.

Preparation of Different Extracts with Various Solvents

Taken 10 g powder of each part of *T. dioica* (e.g. stem, flowers, and leaves) and separately macerated with four different solvents namely; methanol, diethyl ether, acetone and ethyl acetate for seventy two hours. Whatman no. 1, the filter paper was used for filtration processes, and then all the filtrates were examined for phytochemicals and bacterial strains (*E. coli* and *S. aureus*).

The Phytochemical Study of Different Crude Extracts

To identify the phytochemicals in the crude extracts of *T. dioica*, the following procedures were used; the presence of these phytochemicals was detected by color test [4].

Analysis Procedures
Phlobatannins test

1 mL of filtrate was boiled with 1% aqueous HCl (hydrochloric acid). The appearance of a reddish Color confirms the existence of phlobatannins.

Alkaloids test (Wagner's reagent)

(Wagner's reagent composition: 1.27 g iodine + 2 g potassium iodide + distilled water to make final volume 100 mL) 0.5 g of the extract was taken in the test tube and added 2 mL of Wagner's reagent, after few min reddish brown color appeared indicated the presence of alkaloids.

Carbohydrates test (Benedict's test)

(Benedict reagent composition: Solution A: 100 g sodium carbonate +173 g sodium citrate + 800 mL water, dissolve & boil to make solution clear Solution B: 17.3 g of copper sulphate dissolved in 100 mL distilled water)

Benedict reagent (5-8 drops) were added to 2 mL of each extracts, boiled using a water bath for 5 min cooled and reddish brown precipitates were observed.

Cardiac glycosides test (Keller Kelliani's test)

Glacial acetic acid (2 mL) and ferric chloride (1 mL) were added to 5 mL of each extract. The contents were heated, cooled and then the contents were poured into another test tube containing 2 mL conc. sulfuric acid with care. After some time, a purple ring appeared, which may confirmed the presence of glycosides.

Test for flavonoids

20% NaOH (few drops) was added in 2 ml of each extract; the yellow color appeared and turned colorless on adding dilution. HCl confirmed the presence of flavonoids.

Phenols test or Ferric chloride test

Few drops of ferric chloride (5% aqueous solution) were added to each plant extract, the black/blue color appeared in the last one indicates the presence of phenol.

Amino acids and proteins test or 1% ninhydrin solution

5-6 drops of Ninhydrin reagent (10mg ninhydrin + 200 mL acetone) was added to each plant extract (2 mL), then the content was boiled for a few minutes, the purple color appeared confirmed the presence of amino acid.

Saponins test or Foam test

3 mL of distilled water was added in each plant extract, the mixture was shaken vigorously for few min, the foam that appeared at the surface confirmed the presence of saponins.

Tannins test or Braymer's test

Ferric chloride (10%; few drops) was added to each 2 mL plant part extract and observed. The appearance of green/blue color confirmed the presence of tannins.

Terpenoids test or Salkowki's test

Chloroform (1 mL) and conc. Sulfuric acid (few drops) was added in 2 mL of each part of plant extract and then observed. Reddish-brown precipitates indicated the presence of terpenoids.

Proteins test or Biuret test

5-7 drops of 1% $Cu(SO_4)_2$ and 5-7 drops of 5% NaOH were treated with 2 mL of filtrate. The violet color confirmed the presence of proteins.

Determination of antimicrobial activities

The disk diffusion process in Muller Hinton (MHA) medium was used for the antibacterial activity of extracts of stems, flowers and leaves of *T. dioica*. The method to check the antibacterial activity of the three parts was favored with the help of the American Type Culture Collection (ATCC); antibacterial activity against two different microbes was planned; *E. coli* and *S. aureus*. MHA medium was used for the growth of microorganism species [14-15].

Three successive concentrations of 1000, 750 and 500 µg/mL was prepared in dimethyl sulfoxide (DMSO) to test the antibacterial activity of extracts. Therefore, DMSO was used as a negative control. Bacterial suspensions were expanded into solid Petri dishes (90 mm in size) using a sterile cotton swab moistened with bacterial suspensions and adjusted to 10^6 CFU / mL [16].

Then, moistened Whatman No. 1 filter paper (6 mm diameter) with 20 µL of different concentrations was placed on the surface of microbial Petri dishes and placed in an incubator at a temperature of 37 ° C and time period for 24 h. Antibacterial activity of the extracts (stem, flower, and leaf) was recorded against each microbial species by measuring the area of diameter inhibition in millimeters around the discs and the value of MIC was determined. All experiments were carried out three times [17-19].

Results and Discussion

The Analysis of Phytochemicals

Phytochemical examination of plant extracts gave an idea of the presence of what type of class of compounds are present in selected plant. For qualitative analysis of *T. dioca* extract, total of ten phytochemicals were checked such as alkaloids, steroids, glycosides, tannins, phenols, flavonoids, protein, saponins, terpenoids and phlobatannins. The obtained results are tabulated in Table 1.

Table 1. **Phytochemical analysis of various extract of *T. dioica* parts.**

Phytochemicals	Flowers				Stem				Leaves			
	DE	***EA***	***ME***	***AC***	***DE***	***EA***	***ME***	***AC***	***DE***	***EA***	***ME***	***AC***
Phlobatannins	-	-	-	-	-	-	+	+	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	-	+	++	+	-	+	+	+	-	+	++	+
Alkaloids	+	+	++	+	+	+	++	+	+	+	++	+
Phenols	-	+	++	+	-	+	++	+	-	+	++	+
Proteins	-	+	+	+	-	-	-	-	-	+	+	+
Flavonoids	+	+	++	+	+	+	++	+	+	+	++	+
Terpenoids	-	+	+	+	-	+	+	+	-	+	+	+
Steroids	-	+	++	+	+	+	+	+	-	+	++	+

++: highly present, +: moderately present, -: absent
DE: diethyl ether; EA: ethyl acetate; ME: methanol; AC: acetone

Qualitative phytochemical analysis made for the flowers, leaves and stem parts of *T. dioica* revealed the presence of majority of secondary metabolites as shown in Table 1. The majority of these metabolites are present in all parts of *T. dioica* solvent extracts. As we all know that phytochemicals have therapeutic and biological properties, so *dioica* species is expected to have many medicinal uses.

The extraction yield was calculated and the methanol extract was found to have a higher yield than other non-polar solvents, it may be due to the high polarity of the methanol solvents. It can be concluded that the methanol solvent has a higher power to remove the components of plant species than non-polar solvents. It means that the high level of the polarity of the solvent plays an important role in extracting the chemical components of the plants [20]. The absence of steroids in the extract of diethyl ether in flowers and leaves may be due to a low concentration of steroids in these parts that could not be detected. The recovery of the plant's phytochemical can be affected by the chemical properties of the phytochemicals, the dielectric constant, and the chemical composition of the organic solvents. Our previous work reported by Samejo *et al.*, [4], which also showed the analysis of phytochemicals, but in this work, the choice of solvent is different from the present work.

The tabulated results in Table-1 revealed the presence of these phytochemicals such as alkaloids, steroids, glycosides, tannins, phenols, flavonoids, proteins, saponins, terpenoids and phlobatannins, which are well known to possess physiological and medicinal properties [21]. Literature survey revealed that steroids, flavonoids, alkaloids have antibacterial, analgesic and antispasmodic properties. Phenolic compounds have medicinal value such as anti-carcinogen, apoptosis, anti-inflammation, anti-aging, anti-atherosclerosis, cardiovascular protection [21].

Determination of antibacterial activities

The results of the antibacterial activity are shown in Table 2. The general results obtained from the plant parts of the methanol and ethyl acetate extract of *T. dioica* do not represent an interesting antibacterial activity against all the strains tested, including *S. aureus* and *E. coli*. The methanol extract shows some antibacterial activity, but the ethyl acetate extract shows no activity, this may be due to a lower extraction power of antibiotic compounds. Table 2 shows that the MICs of the methanolic extracts of aerial parts of *T. dioica* were found to be higher than the ethyl acetate extract that does not have MIC.

Table 2. **Inhibition zones (mm in diameters) for antibacterial activities of methanol and ethyl acetate extracts of stem, flowers and leaves of *T. dioica.***

Solvents	**Zone of inhibition in mm ± SD**					
Methanol	***E. coli* (MIC 500 µg/mL)**			***S. aureus* (MIC 500 µg/mL)**		
Con. (µg/mL)	Stem	Flowers	Leaves	Stem	Flowers	Leaves
1000	9 ± 0.01	11 ± 0.03	9 ± 0.02	11 ± 0.02	13 ± 0.03	8 ± 0.02
750	7 ± 0.01	8 ± 0.02	6 ± 0.00	6 ± 0.02	8 ± 0.01	5 ± 0.00
500	2 ± 0.00	3 ± 0.00	2 ± 0.00	3 ± 0.001	4 ± 0.01	3 ± 0.00
Control	-	-	-	-	-	-
Ethyl acetate	***E. coli* (MIC 0 µg/mL)**			***S. aureus* (MIC 0 µg/mL)**		
Con. (µg/mL)	Stem	Flowers	Leaves	Stem	Flowers	Leaves
1000	-	-	-	-	-	-
750	-	-	-	-	-	-
500	-	-	-	-	-	-
Control	-	-	-	-	-	-

In recent years, there has been a great demand for research on phytochemicals that possess antimicrobial properties due to their potential use in treating various chronic and infectious diseases.

Reports of the World Health Organization (WHO) showed that approximately 50% of *E. coli* and *S. aureus* were resistant to most antibiotics, such as cephalosporins. The increasing trend in developing antibiotic resistance can be attributed to frequent, unnecessary, and abuse of antibiotics and prolonged hospitalization [22].

Conclusion

The study suggests that the *T. dioica* has the potential for further exploration to identify phytochemicals and antibacterial compounds. The antibacterial activities of methanol extract provide scientific support for its traditional use in folk medicine to treat various diseases.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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