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Review Article

Phytochemical Screening, Antimicrobial and Antioxidant Activity of Ziziphus Spina-Christi (L.) (Rhamnaceae) Leaves and Bark extracts

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Abstract

This study aimed to determine the preliminary phytochemical component, the antioxidant and antibacterial activity of the leaves and bark extracts of Ziziphus spina-christi (L.) (Rhamnaceae) against two clinical isolates (Staphylococcus aureus and Escherichia coli species) using the standard method of analysis. The test for the phytochemical component revealed the presence of flavonoids, alkaloids, saponins, tannins, steroids, terpenoids and glycosides. The result of antibacterial activity showed that the bark ethanolic extract exhibit a higher zone of inhibition against all the clinical isolates; Escherichia coli species showed zones of inhibition of 22mm followed by Staphylococcus aureus 15mm. The antioxidant activity of the leaves and bark extracts was evaluated using the standard 2,2 diphenyl-1- picrylhydrazyl (DPPH) 0.5 ml. The antioxidant activity of the leaves water and ethanolic extracts was 30 ± 0.05 and 91 ± 0.02 respectively and the antioxidant activity of the bark water and ethanolic extracts was 44 ± 0.03 and 70 ± 0.02 respectively.

***Keywords**: Ziziphus spina-christi (L.); screening, antioxidant and antimicrobial activities.*

Background

Plants are an essential part of human society since civilization started. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. In the last decades, various plant extracts have been the focus of great interest from researchers because they represent natural resources of new antibacterial agents with possibly novel mechanisms of action. The potential use of these products as an alternative for the treatment of several infectious diseases has been extensively screened. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Therefore, it is of great interest to carry out a screening of these plants to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds [1].

Ziziphus spina-christi (L.), locally known as Sidr, is a multipurpose tree species belonging to the botanical family Rhamnaceae. It is an important cultivated tree and one of the few truly native tree species of Arabia (Saudi Arabia, Jordan, and Egypt) that is still growing along with many newly introduced exotic plants [2]. It is one of the important fruit crops in the dry parts of tropical Asia and Africa. *Ziziphus* spina-christi (L.) fruits are highly nutritious and rich in vitamin *C.*

Ziziphus (Rhamnaceae) species are used in folk medicine to treat blisters, bruises, chest pains, dandruff, fractures, headache, and mouth problems [3]. *Ziziphus spina-christi* (L.) leaves are traditionally used to treat ulcers, wounds, eye diseases, bronchitis, and skin diseases as an anti-inflammatory agent. The seeds are sedative and are used to halt nausea, vomiting and abdominal pain associated with pregnancy [4]. The fresh leaves are applied to the swollen eye at night [5]. The roots are used to cure and prevent skin diseases [6]. Fruits are used to promote the healing of fresh wounds and treat dysentery, bronchitis, coughs and tuberculosis [7]. It is also used to relieve digestive disorders, obesity, urinary troubles and microbial infections [8,9]. Some pharmacological screening studies indicated that the aqueous extract of *Ziziphus spina christi* (L.) root bark has an antinociceptive activity in mice and rats [10] and a central depressant effect in mice [11]. The methanol extract of *Ziziphus spina-christi* (L.) stems bark has antidiarrheal effects in rats [12].

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by [13, 14, 15, 16].

Qualitative analysis

Preparation of reagents: Preparation of Maeyer's reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water. Preparation of Dragendorff's reagent: Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in a 1:1 ratio.

Phytochemical screening for different compounds

Test for Flavonoids

0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: (a) 3ml of the filtrate was mixed with 4 ml of 1% aluminum chloride in methanol in a test tube and the color was observed. The formation of yellow color indicated the presence of flavonols, flavones and chalcones. (b) 3ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the color was observed. A dark yellow color indicated the presence of Flavonoids.(c) 5ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H2SO4. The appearance of the yellow coloration indicated the presence of flavonoids.

Test for alkaloids

0.5 to 0.6 g of various extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Test for Glycosides

Five ml each of the various extract were hydrolyzed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. The formation of a yellow color indicated the presence of glycosides.

Test for steroids

0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The color changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Terpenoids (Salkowski test)

5 ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H2SO4). A layer of reddish-brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for Saponins

0.5 g of various solvent extract was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponin contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in a boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Test for Tannins

0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride (FeCl3) solution was added to the filtrate. The appearance of intense green, purple, blue or black color indicated the presence of tannins in the test samples.

Testing of antimicrobial susceptibility (Disc diffusion method)

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). The bacterial suspension was diluted with a sterile physiological solution to 108cfu/ml (turbidity = Mc far land standard 0.5). one hundred micro decimeters of bacterial suspension were uniformly on the surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No1, 6mm in diameter) were placed on the surface of the MHA and soaked with 20 µdm3 of a solution of each plant extracts. The inoculated plates were incubated at 37C○for 24h in the inverted position. The diameters (mm) of the inhibition zones were measured. The results were expressed in terms of the diameter of the inhibition zone:<9mm, inactive; 9-12mm, partial active; 13-18mm, active; > 18mm, very active.

Result and discussion

Medicinal plants are considered as a repository of numerous types of bioactive compounds possessing varied therapeutic properties. Which includes anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic effects. The results of plant extract under investigation are shown in Table 1. Leaves and bark extracts showed a positive result for the presence of medicinally active constituents. In the Water and ethanolic extract; flavonoids, alkaloids, terpenoids, saponins and tannins were the most common present in the tested plants. While glycosides are absent in leaves water and ethanolic extract and steroids are absent in bark water and ethanolic extract. These findings correlated well with several earlier publications. Plants which rich in a wide variety of secondary metabolites, such as terpenoids, alkaloids, tannins, flavonoids appear biological and pharmacological activities and may have the potential to be used as chemotherapeutic agents or serve as starting material in the development of new antibiotics. Also, the results showed that *Ziziphus spina-christi* (L.) water and ethanolic extracts contain considerable antioxidant activity.

Table 1: Phytochemical screening of Ziziphus *spina-christi* (L.) extracts

S.NO	Active constituents	Leaves		Bark	
		W. Extract	EtOH. Extract	W. Extract	EtOH. Extract
1	Flavonoids	+++	+++	+++	+++
2	Alkaloids	++	+++	++	++
3	Glycosides	-	-	++	+++
4	Steroids	++	++	-	-
5	Terpenoids	++	++	++	++
6	Saponins	+++	+++	+++	+++
7	Tannins	+++	+++	+++	+++

(+++) high, (++) medium, (+) poor, (-) not found, W = Water, EtOH= Ethanol

Table 2: Antimicrobial activity Ziziphus spina-christi (L.) extracts (inhibition zone in mm)

S.NO	Sample Code	*Staphylococcus aureus*	*Escherichia coli*
1	Leaves Water Extract	2	5
2	Leaves EtOH. Extract	7	3
3	Bark water Extract	15	22
4	Bark EtOH. Extract	10	13
Standard	Tetracycline	30	40

Gram negative; Escherichia coli, Gram positive; Staphylococcus aureus EtOH= Ethanol

Activity: <9 inactive, 9-12 partially active, 13-18 active, >18 very active

Table 3: Antioxidant activity of *Ziziphus spina-christi* (L.) extracts

S.NO	Sample Code	%RSA ±SD (DPPH)
1	Leaves water extract	30 ± 0.05
2	Leaves ethanolic extract	91 ± 0.02
3	Bark water extract	44 ± 0.03
4	Bark ethanolic extract	70 ± 0.02
Standard	Propyl gallate	95 ± 0.02

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Conclusion

The results indicated that the water and ethanolic extracts of the *Ziziphus spina-christi* (L.) have considerable antimicrobial and antioxidant activity.

Conflict of Interest

The authors declare that there is no conflict of interest.

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