Research Article

Antibacterial Activity of the Ethanol Extract of *Ziziphus xylopyrus* Willd. (Rhamnaceae)

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ABSTRACT

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites, or fungi. Diseases can spread, directly or indirectly, from one person to another. About one-fourth of all the medicines we use, come from rainforest plants. However, scientific studies have been conducted only to a limited extent with few medicinal plants. In the present communication, the successive ethanolic extract of the stem bark of Ziziphus xylopyrus Willd. (Family: Rhamnaceae) was prepared and evaluated for its antimicrobial potential against different gram positive bacteria, namely Staphylococcus aureus ML-59, Salmonella typhimurium NCTC 74, Staphylococcus aureus 29737, Bacillus lycheniformis 10341 and gram negative bacterial strains, namely Escherichia coli K-12 ROW, Shigella sonnei 2, Shigella boydii 8, Vibrio cholera 811, Vibrio cholera 854, Vibrio alginolyteus. The potential antibacterial activity against different bacteria was examined by minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) analysis. MIC values compared with control and zone of inhibition (ZOI) values compared with standard ciprofloxacin. The results revealed that, the ethanolic extract is potent in inhibiting bacterial growth of both gram negative and gram-positive bacteria and comparable with the standard (ciprofloxacin). The 200 µg/ml of ethanolic extract showed the best antibacterial activity as compared to the other concentrations. Hence, this plant can be further subjected to isolation of the therapeutic antibacterials and further pharmacological evaluation.

Keywords: Antibacterial activity, ethanolic extract, MIC, ziziphus xylopyrus willd., ZOI.

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INTRODUCTION

Ziziphus xylopyrus (Family: Rhamnaceae) is a medicinal plant which is commonly found in various parts of Northwestern India [1]. As per the ethnomedicinal information, various parts of this plant possess several medicinal properties. The fruit powder with a pinch of zinger powder thrice in a day is useful for stomachache and indigestion [2]. It also possesses antidepressant, anthelmintic activities as per the available information [3-4]. The reported chemical constituents present in this plant are quercetin and quercitrin in leaves, catechol-type of tannins (8-12 %), oleanolic acid,1-epicatechin,1-leucocyanidin,3,3,4-tri-o-methyl ellagic acid in fruits; tannin (7.2 %), d-7,3',4',-trihydroxyflavan-3,4-diol and oleanolic acid in barks (Anonymous, 2005). The stem wood of the plant is reported to contain triterpinoids [5], alkaloids (xylopyrine-A and B) [6-7] and flavonoids [3]. Traditionally the plant was used in the treatment of diarrhoea, wound healing and boils, which gives as an idea for its antimicrobial activity. The present investigation was done to find out the antimicrobial potential of the ethanol extract of stem bark part against some Gram-positive and Gram-negative bacteria, which gives a scientifically proof for its traditional uses.

MATERIAL AND METHODS

Plant materials

The stem bark of the selected plant was

collected from the forest of Simlipal Biosphere Reserve, Mayurbhanj, Odisha, India in August 2006. The plant material was identified and authenticated taxonomically at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal, India (Ref. no. CNH/I(59) /2006/Tech-II, dated 27-10-2006). A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts

The said plant parts were cleaned, dried under shade and powdered by a mechanical grinder. Hundred grams of the pulverized stem bark was extracted with the solvent, petroleum ether, chloroform, and ethanol in increasing polarity successively in a Soxhlet apparatus. Petroleum ether was used in initial step of extraction for defatting the plant materials followed by chloroform and ethanol. The successive extracts were separately filtered and concentrated at reduced temperature on rotary evaporator.

Phytochemical screening

The extracts of *Z. xylopyrus* Willd. stem bark were subjected to some phytochemical tests to determine the presence of alkaloids (Dragendroff's test), glycosides (Keller-Killiani, Borntrager's, and modified Borntrager's tests), carbohydrates (Fehling's and Molisch's tests), steroids and sterols (Libermann-Burchard test, Salkowski test), tannins (ferric chloride test), proteins and amino acids (Ninhydrin test), tri-terpenoids (tin and thionyl chloride test), saponin (foam test), and flavonoids (NaOH and H_2SO_4 test) [8-9].

Antimicrobial activity

The ethanol extract of *Z. xylopyrus* Willd. stem bark was obtained and tested for the antimicrobial activity against different *Gram-positive (Staphylococcus aureus ML-59 Salmonella typhimurium NCTC 74, Staphylococcus aureus 29737, Bacillus lycheniformis 10341*), and Gram-negative bacterial strains (*Escherichia coli K-12 ROW, Shigella sonnei 2, Shigella boydii 8, Vibrio cholera 811, Vibrio cholera 854, Vibrio alginolyteus*). These bacterial strains were obtained from Jadavpur University, Kolkata-32, India. All subculture microbes used were pure culture preserved as slant agar culture at 4°C. The molten nutrient agar medium containing various concentrations of the extracts (0, 10, 25, 50,100 and 200 µg/ml) were poured and solidified on to sterile 100 mm petridishes to give sterile nutrient agar plates with varying dilutions of the extract. Then these plates were kept in a refrigerator (4°C) for 24 h for uniform diffusion of the extract in the nutrient agar media. The plates were then dried at 37°C for 2 h before spot inoculation [10]. One loop full (diameter 3 mm) of an overnight grown peptone water culture of each test organism was placed in petridish marked by checkerboard technique [11]. The spot inoculated plates were incubated at 37°C for 24 h and the minimum inhibitory concentration values were obtained.

Ciprofloxacin was taken as a standard compound for comparing the results obtained. Two sets of two dilutions (100 and 200 µg/ml) each of *Z. xylopyrus* Willd. stem bark ethanol extract and standard ciprofloxacin (solvent: sterile distilled water) were prepared in sterile McCartney bottles. Sterile nutrient agar plates were prepared and incubated at 37°C for 24 h to check for any sort of contamination. Two sterile filter paper discs (Whatman® filter paper No. 1) of 6 mm diameter were soaked in two different dilutions of the crude extract and placed in appropriate position of the surface of the flooded plate, marked as quadrants at the back of the petridishes. The petridishes were incubated at 37°C for 24 h and the diameter of zones of inhibition were measured in mm. Similar procedure was adopted for the pure ciprofloxacin and the corresponding zone diameters were compared accordingly [12].

RESULTS AND DISCUSSION

Different extracts of of *Z. xylopyrus* Willd. stem bark were obtained. After drying the extracts, colour and physical appearance were observed and presented in (**Table 1**). The percent yield (w/w) of these obtained extracts were also measured (**Table 1**), and it was that the percent yield of ethanolic extract (7.79 %) was maximum in comparison with other extracts (chloroform and petroleum ether).

Table 1: **Yield (%, W/W), Colour and Physical Appearance after Drying of Different Extracts of *Z. Xylopyrus* Willd. Stem Bark**

Extracts	Yield (% w/w)	Colour and physical appearance after drying
Petroleum ether	1.50	Yellowish brown sticky in nature
Chloroform	1.19	Yellowish green amorphous power
Ethanol	7.79	Dark brown crystals

The qualitative phytochemical analysis of *Z. xylopyrus* Willd. bark extracts were performed and the result of this study is presented in (**Table 2**). The petroleum ether extract showed the presence of steroids, only. The chloroform extract showed presence of glycosides, steroids, and carbohydrates. In ethanolic extract, glycosides, carbohydrates, and steroids were present with terpenes and flavonoids.

Table 2: Qualitative Phytochemical Analysis of Different Extracts of *Z. Xylopyrus* Willd. Stem Bark

Phytoconstituents present	Petroleum ether extract	Chloroform extract	Ethanol extract
Alkaloids	-	-	-
Carbohydrates	-	+	+
Glycosides	-	+	+
Proteins and Amino acids	-	-	-
Tannins	-	-	-
Terpenes	-	-	+
Saponins	-	-	-
Flavonoids	-	-	+
Steroids	+	+	+

'+' Present, and '-' = Absent

The ethanol extract was taken for antimicrobial activity due to higher yield value and based on the presence of phytoconstituents. The observations of the MIC study has been tabulated in (**Table 3**) and it was found that the minimum inhibitory concentration of the ethanol extract was found to be varying between 10-200 µg/ml, with respect to most of the test bacteria. The MIC of ethanol extract for bacterial strains like *E. coli* K-12 ROW, *S. sonnei* 2, *S. typhi* 59, *V. cholera* 854 and *S. aureus* ML-59 were found to be 100 µg/ml, for *V. cholera* 811, MIC was 50 µg/ml and for *S. aureus* 2737 and *B. lichniformis* 10341 were at 10 µg/ml. The result of ZOI of the extracts and its comparison with standards antibiotic, ciprofloxacin (100 µg/ml and 200 µg/ml) was recorded in (**Table 4**). The anti-bacterial efficacy of the extract of *Z. xylopyrus* Willd. stem bark was found to decrease in the following order against different tested bacterial strains, *S. typhi* 59, *S. aureus* 2737, *V. alginolyteus*, *S. boydii* 8, *V. cholera* 854, *E. coli* K-12 ROW, *B. licheniformis* 10341. From the results of MIC, ZOI values and their comparison to that of the standard ciprofloxacin, it is evidenced that the ethanol extract was potent against Gram-positive and Gram-negative bacteria.

The compounds responsible for this antibacterial activity had not been investigated. However, preliminary phytochemical analysis of the ethanol extract revealed the presence of tannins, glycosides, flavonoids and steroids. The

antibacterial properties of the plant may be attributed to the individual or combined chemical groups. The findings of the present investigation offer a scientific support to the ethno medicinal use of the plant by the traditional healers.

Table 3: MIC of Ethanol Extract of *Z. Xylopyrus* Willd. Stem Bark against Different Bacteria

Name of bacteria	Growth in nutrient agar containing different concentrations of extract in µg/ml					
	0	10	25	50	100	200
Gram-positive bacteria						
Staphylococcus aureus ML-59	+	-	-	-	-	-
Bacillus licheniformis 10341	+	-	-	-	-	-
Salmonella typhimurium NCTC 74	+	+	+	+	+	-
Staphylococcus aureus 29737	+	+	+	+	+	-
Gram-negative bacteria						
Escherichia coli K-12 ROW	+	+	+	+	+	-
Shigella sonnei 2	+	+	+	+	+	-
Salmonella typhi 59	+	+	+	+	+	-
Vibrio cholera 811	+	+	+	+	-	-
Vibrio cholera 854	+	+	+	+	+	-
Vibrio alginolyteus	+	+	+	+	+	-
Shigella boydii 8	+	+	+	+	+	-

All determinations were done in triplicate '0' Control (without extract); '+' Growth; '-' No growth

Table 4: **Zone of Inhibition Produced By Ethanol Extract and Ciprofloxacin**

Name of bacteria	Zone of inhibition (mm)[a]			
	Ethanol extracts		Ciprofloxacin	
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
Gram-positive bacteria				
Staphylococcus aureus ML-59	9.12 ± 0.50	10.28 ± 0.95	23.50 ± 0.88	28.33 ± 0.75
Bacillus licheniformis 10341	6.24 ± 0.75	7.48 ± 0.67	17.66 ± 0.72	27.28 ± 0.65
Salmonella typhimurium NCTC 74	6.25 ± 1.06	6.84 ± 0.86	23.50 ± 0.86	29.52 ± 0.76
Staphylococcus aurous 29737	6.38 ± 0.68	9.42 ± 0.80	22.26 ± 0.50	25.22 ± 0.80
Gram-negative bacteria				
Escherichia coli K-12 ROW	6.38 ± 0.85	7.48 ± 0.76	10.56 ± 0.52	19.12 ± 0.89
Shigella sonnei 2	6.38 ± 0.55	7.52 ± 0.55	14.33 ± 0.52	18.25 ± 0.78
Salmonella typhi 59	13.00 ± 0.75	14.37 ± 0.75	27.22 ± 0.76	35.22 ± 0.52
Vibrio cholera 811	10.55 ± 0.77	13.58 ±0.65	15.48 ± 0.83	20.12 ± 0.71
Shigella boydii 8	7.48 ± 0.95	9.42 ± 0.66	20.06 ± 0.51	25.14 ± 0.81
Vibrio cholera 854	6.38 ± 0.85	8.50 ± 0.95	22.00 ± 0.75	28.44 ± 0.68
Vibrio alginolyteus	7.57 ± 0.66	10.54 ± 0.60	26.06 ± 0.83	29.03 ± 0.80

[a]Tests are done in triplicate and values were expressed as mean ± standard deviation
Zone of inhibitions was measured as diameters of inhibited zones in mm; ciprofloxacin was used as positive control

CONCLUSION

Based on these results, it is possible to conclude that various extracts of *Z. xylopyrus* Willd. stem bark possess a broad spectrum of activity against a panel of different types of bacteria responsible for the most common bacterial diseases. The ethanol extract of *Z. xylopyrus* Willd. stem bark can potentially be used in the treatment of various infectious diseases caused by various pathogenic bacteria that are showing resistance to currently available antibiotics. These promissory extracts of *Z. xylopyrus* Willd. stem bark open the possibility of finding new clinically effective antibacterial.

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