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Full Length Research Paper

Phytochemical Screening and antimicrobial activity of ethanol and chloroform extract of *Zizyphus nummularis* Wt. & Arm.

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Zizyphus nummularis **Wt. & Arm. (Family: Rhamnaceceae) has ethnomedicinal value used by the local tribe of south Orissa, India for the treatment of typhoid, dysentery and diarrhea. The ethanol and chloroform extracts from various parts of the plant stem, leaves and root were analyzed for the presence of bioactive compounds. In addition, antimicrobial activity of the extracts from various parts where separately assessed against *Salmonella typhimurium*, *Bacillus subtilis* and *Escherichia coli*. Furthermore, minimum inhibitory concentration (MIC) of the extracts was evaluated. Bioactive compounds from all the parts were found to contain tannin, flavonoids, steroids, glycosides and alkaloids in addition to certain other minor compounds. Maximum zone of inhibition was found with the ethanolic root extract against *S. typhimurium* (20.2 mm) as compared to other microbes tested. The MIC of ethanolic root extracts was found to be 7.81 mgml^{-1} against *S. typhimurium* and *E. coli* whereas the same MIC of chloroform extracts was found to be effective against *S. typhimurium*. The study demonstrates that the root of *Z. nummularies* can affectively be used against microbial infection. However, a further study is needed to advocate the safety of the bioactive compounds in therapeutic use.**

Key words: Phytochemicals, antimicrobial activity, zone of inhibition, minimum inhibitory concentration.

INTRODUCTION

India endowed is with different kind of plant species, and is ranked eighth in the world biodiversity (Raman, 2007; Sahoo et al., 2006). Medicinal and aromatic plants constitute a large segment of Indian flora, which provide raw material for pharmaceutical, cosmetics, fragrance and flavor industries (Behera et al*.,* 2008). The uses of plants as well as animals parts as medicine have since been documented in the record of ancient China, India and Egypt (Chopra et al., 1956; Makut et al., 2008). It is also noticed that even today rural people are opted for traditional ways of treatment because of their easy availability and cheaper cost (Mohan et al., 2005).

Zizyphus nummularis Wt. & Arm. belonging to the family Rhamnaceae is frequently used by tribal people of Southeast Orissa for the treatment of typhoid, dysentery and diarrhea. Since no work has been reported earlier regarding the antimicrobial activity of the species, this paper describes the phytochemicals evaluation of different parts of *Zizyphus nummularis* and the efficacy of antimicrobial properties of different plant parts against *Salmonella typhimurium, Bacillus subtilus* and *Escherichia coli.*

MATERIALS AND METHODS

Plant extract preparation

Zizyphus nummularis stem, leaf and root were used for the study. The plant was collected from the rural belt near GIET College, Gunupur. The species was authenticated at the floristic laboratory of Botany Department, Berhampur University, Berhampur and a

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Table 1. Phytochemical screening of the extracts of different parts of *Z. nummularis*.

Compound	Different plant part		
	Stem	Leaf	Root
Tannins	+	+	+
Flavoroids	+	+	+
Steroids	+	+	+
Glycosides	+	+	+
Alkaloids	+	+	+
Saponins	-	-	-

+, Present, -, absent.

Table 2. Zone of inhibition (in mm) of *Z. nummularis* extracts against microorganisms. Values are average of five variables ± SEM.

Microorganism	Ethanolic extract						Chloroform extract					
	Stem		Leaf		Root		Stem		Leaf		Root	
	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%
Salmonella typhimurium	7.4±0.08	13.3±0.12	11.4±0.06	15.6±0.14	15.3 ± 0.09	20.2±0.22	8.6 ±0.02	11.4±0.16	10.8±0.02	14.3 ± 0.14	13.7±0.06	17.4±0.13
Bacillus subtiles	6.4±0.03	10.7±0.14	8.6±0.01	11.6 ±0.19	11.3±0.06	14.4 ±0.11	5.4 ±0.08	8.8±0.18	6.7±0.05	9.6±0.18	8.6±0.04	11.3±0.18
Escherichia coli	5.8 ±0.04	8.9 ± 0.17	7.4± 0.02	9.6± 0.13	9.7 ±0.04	12.8±0.20	5.1±0.03	7.2±0.08	6.6 ±0.03	8.5 ±0.11	7.3 ±0.05	10.4±0.12

voucher specimen was deposited to the department. The uprooted plant was initially washed with tap water to remove dirt present on it. The plant parts were separated and dried in the shade. Dried parts were powdered. Ten gram of each powder was packed in Whatman No. 2 filter paper and Soxhlet in 100 ml of ethanol or chloroform at 80°C for 7 h. The extracts were then filtered by Whatman no.1 filter paper, dried at 37°C and stored at 4°C for future use (Barker and Thomsberry, 1998; Sanches et al., 2005). For antimicrobial assay, the extracts were separately dissolved in 5 ml of 50% dimethyl sulfoxide (DMSO, Hi Media Pvt. Ltd, Mumbai).

Phytochemical analysis

The ethanol extracts of stem, leaf and root was qualitatively analyzed for the presence of bioactive compounds. Tannins and alkaloids were qualitatively determined following the method of Hiremath et al. (1997). Steroids, flavonoids, glycosides and saponins were measured following the methods of Harbone (1973).

Test organisms

The test organisms used for the study were *Salmonella typhimurium* MTCC 98, *Bacillus subtilus* MTCC 441 and *Escherichia coli* MTCC 78. The bacterial strains were obtained from the Institute of Microbial Technology (IMT), Chandigarh, India. The subculture of the microorganisms was maintained in the Laboratory on Luria Bertani (LB) agar slants for future use.

Antimicrobial assays

The antimicrobial activity of the extracts against test microorganisms was studied by agar well diffusion method (Nostro et al., 2000; Anas et al., 2008). One hundred microliter (100 µl) of the each extract was added to the well cut in the Mueller-Hinton agar plate exposed with microorganism by spread plate method. The control well received only 100 µl of 50% DMSO. The inhibition zones were recorded after 72 h of growth at 27°C. The mean of five values was considered as the zone of inhibition.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of each extracts was determined by following the method of Makut et al. (2008). Stock solution was prepared from 0.5 g of each extract dissolved in 4 ml of sterile Muller-Hinton broth. Subsequently, 62.50, 31.25, 15.65, 7.83 and 3.91 mgl^{-1} concentration of solutions were prepared by following two fold serial dilution methods. One millliter (1 ml) of the standardized inoculums (10^6cells/ml) of each test organism was introduced into the extract-nutrient solution separately and then incubated at 37°C for 24 h. The lowest concentration of the extract that inhibited the growth of the microorganisms was recorded.

Result and Discussion

Phytochemical screening of ethanol extracts of

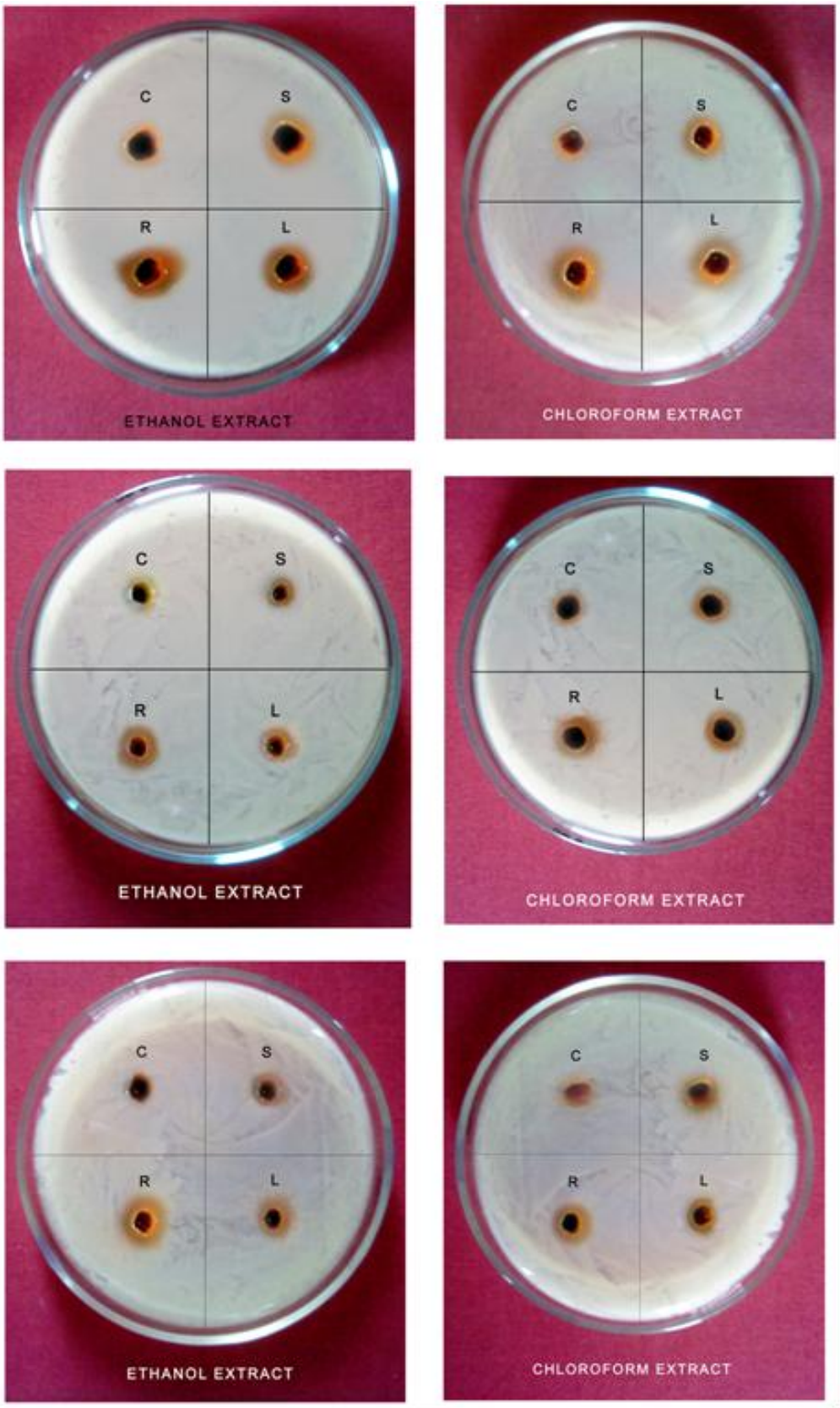

Figure 1. Zone of inhibition by extracts of *Z. nummularris* against microganism :*S. typhimmurium* (a and a1), *B. subtilis* (b and b1) and *E. coli* (c and c1). C : Control, S : Stem extract, L : Leaf extract, R : Root extract.

Table 3. MIC of *Z. nummularis* extracts (mg ml^{-1}) against microorganisms. Data represent average of five replicates.

Microorganism	Ethanolic extracts			Chloroform extracts		
	Stem	Leaf	Root	Stem	Leaf	Root
Salmonella typhimurium	31.25	15.65	7.83	62.50	31.25	7.83
Bacillus subtilis	62.50	31.25	15.65	62.50	31.25	15.65
Escherichia coli	62.50	31.25	7.83	62.50	15.65	15.65

different parts of *Z. nummularis* is given in Table 1. The bioactive compounds such as tannins, flavonoids, steroids, glycosides and alkaloids were found to be present in all plant parts such as stem, leaf and root. However, saponins is absent in all these plant parts.

Table 2 shows the zone of inhibition (in mm) of *Z. nummularis* extracts against three microorganisms such as *S. typhimurium*, *B. subtilus* and *E. coli*. Maximum zone of inhibition was observed with the ethanol root extracts against *S. typhimurium* (20.2 mm) and minimum zone of inhibition was recorded in the chloroform stem extract with *E. coli* (7.2 mm). Other values are intermediate between maximum and minimum values (Figure 1). The extract of root showed greater efficiency as compared to the extract of stem and leaf against all tested microorganisms such as *S. typhimurium*, *B. subtilus* and *E. coli*. Similarly, the growth of *S. typhimurium* was also comparatively highly inhibited than *B. subtilus* and *E. coli* in both ethanol and chloroform root extracts. For the last few decades, many investigations are being made on the antimicrobial properties possessing different kind of plants (Kudi et al., 1990; Sardari et al., 1997; Mohan et al., 2005). It has also been reported that organic extracts of *Zizyphus jujuba* possesses both antioxidant and antilisterial activity (Al-Reza et al., 2009). The antibacterial activity present in the organic extract of *Z. nummularis* can be due to the presence of bioactive compounds. It has been reported earlier that the monoterpene composition of organic compounds are responsible for the antibacterial activity. These compounds destroy the cellular integrity by inhibiting the respiration process in the microbial cell (Helander et al., 1998). Similarly, Leal et al. (2010) established that ceanothane and lupane type triterpenes present in *Zizyphus joazeiro* have bactericidal activity against *Staphylococcus aureus*. However, the antibacterial activity of the crude organic extract may also be correlated to synergistic effect of all the chemical components present in the extract (Dorman and Deans, 2000; Pavithra et al., 2009).

The minimum inhibitory concentrations (MIC) of *Z. nummularis* stem, leaf and root extract against microorganisms are represented in Table 3. It was found that ethanol extract of root had effective MIC with a value of 7.83 $mgml^{-1}$ against *S. typhimurium* and *E. coli.* Similarly, chloroform extract of root had also the same value that is 7.83 $mgml^{-1}$ against *S. typhimurium*. Therefore, the present investigation can be concluded that the bioactive compounds present in the different part of *Z. nummularis* have potent effect to inhibit the growth of pathogenic microorganisms like *S. typhimuium*, *B. subtilus* and *E. coli* and the root extracts have more efficacy than the stem and leaf extracts against the microorganism. However, a further study is needed especially in animal system to advocate the safety of the bioactive compounds in therapeutic use.

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