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ASSESSMENT OF ANTIMICROBIAL PROSPECTIVE OF CRUDE EXTRACTS OF LEAVES, BARK AND FRUITS OF *ZIZIPHUSLOTUS* L.

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ABSTRACT

Ziziphus *lotus*, belonging to the Rhamnaceae family, is a deciduous shrub which generally grows in arid and semiarid regions of the globe. The aim of the present study was to assess the antimicrobial activity of solvents with wide range of polarity of various parts of *Z. lotus* against *Klebsiellapneumoniae*, *Staphylococcus aureus*, *Candida albicans*, *Mycobacterium smegamatis*using agar well diffusion method. The collected plant part was processed for themaceration extraction followed by solvent evaporation. The antimicrobial activity was assessed by agar well diffusion method. In the present study, hexane extract of bark was found to have more antibacterial potential against *K. pneumoniae* and water extracts of the same having more antifungal activity against *C. albicans*. The methanolic and chloroform extracts of leaves also exhibited antimicrobial activities. No antimicrobial activity was reported in fruits.

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INTRODUCTION

Ziziphus Lotus (*Z. Lotus*) also known as jujube is a deciduous shrubbelongs to the Rhamnaceae family[1]. This family includes around 135–170 species of Ziziphus. Z. Lotus is widely distributed in India, China, Pakistan, South Korea, Africa and also found in the European continent in Spain, Greece and Sicily[2-4].As this shrub contain various complex phyto-constituent such as polyphenolic compound, flavonoid, alkaloid, saponin, terpenoid. It also has some biological activity. In traditional medicine, both in North Africa region and Middle East region, several parts of Z. lotus are given as anti-urinary troubles agents, anti-diabetes, skin infections, antifever, antidiarrhea, sedative, bronchitis, and hypoglycemic activities[5-7]. This plant bearsa delicious red fruit (jujube) and can be consumed as fresh, dried, and processed food[8]. In the present study, we would focus on the antimicrobial activity of the Z. lotus.

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We have extracted thephyto-constituent from various part of the shrub (bark, leaves, fruit) using various solvents like water, methanol, chloroform, hexane. The four bacterial strain was applied (*S. aureus, K. pneumoniae, M. smegmatis, C. albicans*) following agar well diffusion method.

MATERIALS AND METHODS

Ziziphus lotus (order-Rosales and family-Rhamnaceae) was taken for the present study grown in the local area of North Guwahati, under Tehsil: Bezara of Kamrup district at Latitude: 26.2334 and Longitude: 91.73845. Thefresh leaves, bark and ripen fruit were collected from the *Ziziphus lotus* which were washed with tap water followed byair-dry for 3-4 days in ambient temperatureat 30˚C. The collected plants were identified taxonomically and authenticated. The samples were pulverized using a laboratory grinding machine and collected as powder form. There are four different solvents used (wate**r,** methanol, chloroform, hexane) for the leaves and fruits and six solvents (wate**r,** methanol, chloroform, hexane, 30% ethanol

and 70% ethanol) were applied for barks. In this maceration process, 10gms of dried powder were added in 100ml of each solvent and kept in incubator shaker for overnight using 180 rpm at 37˚C. The solution was then filtered using What man filter paper No.1 and the filtrate was collected on glass petri plates and allowed the solvent to evaporate at room temperature. The dried solvent extract was collected in epitubes. The antimicrobial activity of plant extract was evaluated following agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS)[9] using *Klebsiellapneumoniae, Staphylococcus aureus, Candida albicans, Mycobacterium smegamatis*strains. In the sample well, 1mg of crude sample was used along with positive control in the concentration of 20µl of 100mg/ml ampicillin solution and negative control in the concentration of 20µl of 10% DMSO (DMSO) (Merck (India) Ltd., Mumbai, India).

RESULTS

From the present study, the antimicrobial activity of *Ziziphus lotus* were reported for bark and leaf extracts (Table 1) whereas no activity from fruit extract was reported (data not shown). The hexane extract of bark (S1) showed maximum antimicrobial activity against all the 3 bacterial strains but no antifungal activity was reported against *Candida albicans.* Chloroform extract of bark (S2) showed some antimicrobial activity against *Klebseillapneumoniae* and *Mycobacterium smegmatis* but no activity was reported against *Staphylococcus aureus* and *Candida albicans.* Both the 30% ethanolic extract of bark (S3) and 70% ethanolic extract of bark (S4) exhibited quite similar antimicrobial activity against all the 4 strains, amongst them 70% ethanolic extract exhibited a little more activity than that of 30% ethanolic extract except against *Mycobacterium smegmatis*. Methanol extract of bark (S5) also exhibited antimicrobial activity against all the 4 strains. Water extract of bark (S6) showed a little less antibacterial activity as compared to the other solvent fractions against the 3 bacterial strains but a good antifungal activity against *Candida albicans*. The antimicrobial activity exhibited by different solvent fractions of bark is represented in Figure 2. No antimicrobial activity was reported for hexane (S1) and water extract (S4) of leaf of *Ziziphus lotus*. Methanolic extract (S2) exhibited antimicrobial activity against all the 4 strains. The chloroform extract of leaf (S3) also exhibited little antibacterial activity against the 3 bacterial strains but no antifungal activity was reported. The antimicrobial activity exhibited by different solvent fractions of leaf is represented in Figure 1. Ampicillin was used as positive control against all the strains. A very strong antibacterial activity was reported against *Staphylococcus* aureus and *Klebseillapneumoniae*. Lesser antibacterial activity was reported against *Mycobacterium*

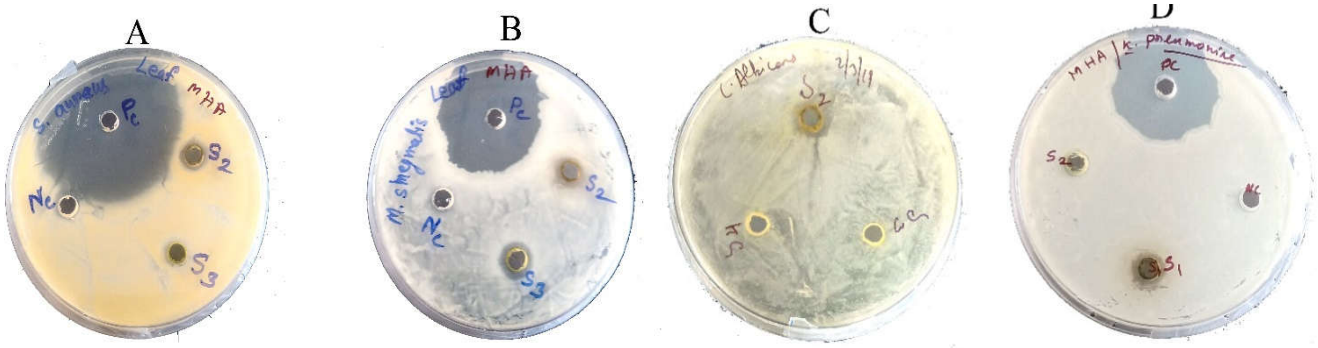

Figure 1. Antimicrobial activity of leaf extracts in methanolic and chloroform solvent.
A. Antimicrobial activity against *S. aureus* B- Antimicrobial activity against *M. smegmatis*
C- Antimicrobial activity against *C. albicans* D- Antimicrobial activity against *K. pneumonia*

Table 1. Zone of Inhibition of Various Solvent Extracts of *Ziziphus lotus* against 3 Bacteria and 1 Fungus Strain

Solvent Fraction		*S. aureus*	*K. pneumoniae*	*M. smegmatis*	*C. albicans*
	Bark	47.333 ± 1.155	42.333 ± 2.517	34.0 ± 1	0
Positive Control	Leaf	49.333 ± 1.155	42.333 ± 2.517	36.333 ± 0.577	0
Negative Control	Bark	0	0	0	0
	Leaf	0	0	0	0
	Bark	14.0 ± 1	24.0 ± 1	14.333 ± 0.577	0
Hexane	Leaf[Ψ]	0	0	0	0
	Bark	0	8.667 ± 1.155	13.0 ± 1	0
Chloroform	Leaf	9.333 ± 0.577	6.667 ± 0.577	11.333 ± 0.577	0
Ethanol 30%	Bark	10.0 ± 1	11.333 ± 0.577	12.667 ± 0.577	11.667 ± 0.577
	Leaf	NA	NA	NA	NA
Ethanol 70%	Bark	10.667 ± 0.577	14.0 ± 1	11.0 ± 0	15.667 ± 1.155
	Leaf	NA	NA	NA	NA
	Bark	10.667 ± 0.577	12.0 ± 0	12.667 ± 1.155	15.333 ± 1.528
Methanol	Leaf	10.667 ± 0.577	11.667 ± 1.155	12.333 ± 0.577	12.667 ± 1.155
	Bark	7.667 ± 0.577	10.667 ± 0.577	9.333 ± 0.577	16.667 ± 0.577
Water	Leaf[Ψ]	0	0	0	0

Zone of Inhibition were measured in millimetre (mm).
All the statistical calculations were done using Graph-Pad Prism 7.0 software.
[Ψ]- Checked twice but have not found any activities.

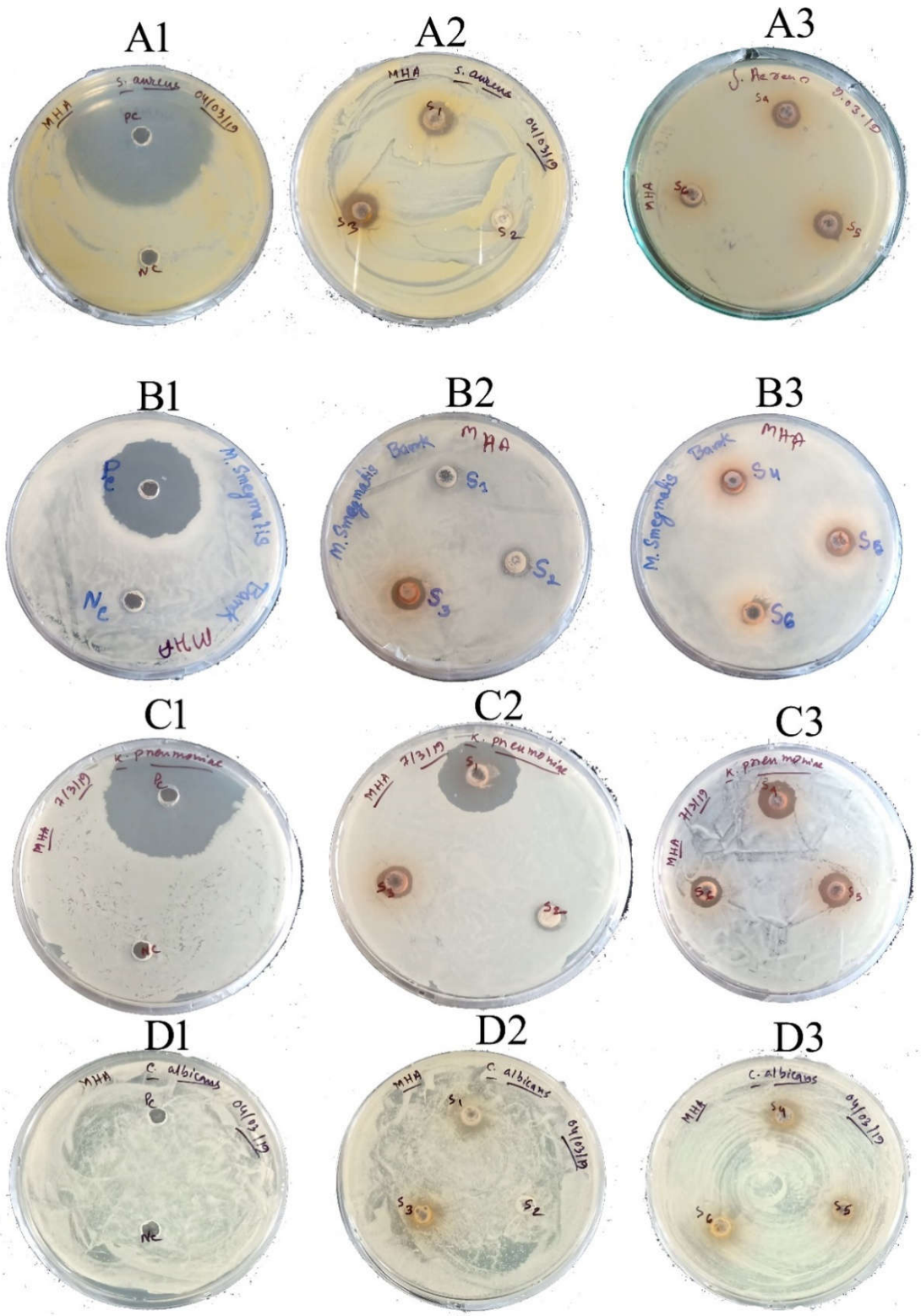

Figure 2. Antimicrobial activity of bark extracts in various solvents (1: positive & negative control, 2: S1, S2 & S3 and 3: S4, S5 & S6)
A1, A2, A3- Antimicrobial activity against *S. aureus*
B1, B2, B3-. Antimicrobial activity against *M. smegmatis*
C1, C2, C3- Antimicrobial activity against *K. pneumoniae*
D1, D2, D3- Antimicrobial activity against *C. albicans*.

*smegmatis.*10% DMSO was used as negative control, although no antimicrobial activity was found for the negative control.

DISCUSSION AND CONCLUSION

From the present assessment, it was observed that crude extract of fruits has not shown any antimicrobial activity whereas crude extracts of leaves and bark showed various antimicrobial activities on various polarized solvents. Hexane extract of bark has more potential antibacterial activity against *K. pneumonia* in comparison to all other solvent fractions and against all bacterial and fungal strains applied. Water extracts of bark exhibited greater antifungal potential against *C. albicans* followed by 70% ethanol and methanol. Whereas, the methanolic crude extracts of leaves showed highest antifungal activity against *C. albicans* and antibacterial activity against *M. smegmatis and K. pneumoniae.* However, the chloroform

fraction, a less polar solvent, exhibited less antibacterial activity against all the three bacterial strains used and no antifungal activity was reported. Plants contain a wide range of compounds like glycosides, tannins, resins, volatile oils, alkaloids, flavonoids, covering a wide range of polarity. The solvents used in this study are having diverse range of polarity and the compounds from the various parts with similar polarity were extracted out in the solvents. Any of these solvents may contain multiple compounds with close range of polarity after extraction and so the activity of the crude extract may not exactly reflect the activity of the main antimicrobial compound(s) present in the plant parts. Separation of the plant extract by column chromatography followed by HPLC after optimizing the mobile phase is required to separate the individual compounds to investigate out the principal antimicrobial compound(s) present in the plant parts.

Conflict of interest: The Authors declare no conflict of interest.

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