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Antimicrobial potential of different crude extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* against selected human pathogens

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Abstract

Background: Plants have been a source of medicine to treat various infectious diseases in tribal communities in North East India since time immemorial.

Objective: To evaluate antimicrobial activity of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* leaves against five human pathogenic bacteria.

Method: Ethanolic and Methanolic extracts of the two plants were screened for potential antibacterial activity against 5 bacterial species, namely *Salmonella typhi*, *Escherichia coli*, *Listeria monocytogenes*, *Shigella* and *Serratia* using disc diffusion method. Antibacterial activity of the extracts was performed by agar disc diffusion method. Each experiment was repeated in triplicate sets and the mean values for the inhibition zones from the triplicate results were employed to represent the antibacterial activity of the extracts.

Result: The ethanolic and methanolic extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* showed a broad spectrum of significant antibacterial activity by producing a clear zone of inhibition against all the selected bacterial strains. The clear zones of inhibition in bacterial samples produced by *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* extracts were comparable with the antibiotics.

Conclusion: *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* extracts have demonstrated antibacterial effects particularly on salmonella and *Serratia* as compared to commercially available antibiotics such as ciprofloxacin and chloramphenicol. Thus, *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* could be effective for prevention of bacterial infections and may be considered as an alternative to antibiotic regimens.

Keywords: *Zanthoxylum nitidum*, *drymaria cordata*, crude extracts, antimicrobial activity

1. Introduction

Medicinal plants have been used in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism [1]. Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. *Zanthoxylum nitidum* (Roxb.) DC (*Rutaceae*), called Tez-mui in Assamese, is ethno medicinally prescribed in North-Eastern states India for treatment of various disease conditions like toothache, gingivitis, fever, colic vomiting, diarrhoea and cholera activity [2]. *Zanthoxylum* species has been scientifically studied for several types of biological activities such as larvicidal, anti-inflammatory, analgesic, antinociceptive, antioxidant, antibiotic, hepatoprotective, antiplasmodial, cytotoxic, ant proliferative, anthelmintic, antiviral, anticonvulsant and antifungal. Toothpaste containing *Z. nitidum* extract decreased the incidence of dental plaque and enhanced gingival health [4].

Drymaria cordata is an important plant traditionally used in various parts of the world like Africa, and Asia as folk medicine. It is used for treating snake bite, skin diseases, peptic ulcer, headaches or nephritis, female infertility, sleeping disorders, convulsions, febrile conditions in children and other ailments including cold, headache, coryza, bronchitis, as poultice on sore (to treat aching, inflamed or painful parts), leprosy, tumors, as fumigant for eye troubles [2]. *Escherichia coli*, *Salmonella*, *Shigella* cause enteric infections and have numerous strategies to exploit their host's cellular processes so that they can survive and persist. As the widespread of antibiotic resistance and disease causing agents are surfacing in recent time, it has become great concern to the global health community and development of potential source of novel

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drugs has become of paramount importance. So some commonly used plants with medicinal property of our community could be an excellent source of drugs to fight off this problem.

2. Materials and methods

2.1. Study area

The study was conducted in the Microbiology laboratory, Department of Biotechnology, Gauhati University.

2.2. Procurement of Plant material

The selection of the plants was done on the basis of the information collected from the local community people on their medicinal properties. Through random sampling, healthy and disease free plant materials were procured from the nearby fields and forest in Sibsagar districts with the help of local people. These plant samples were further identified and authenticated by Dr Iswar Chandra Baruah, Principal Scientist, Assam Agricultural University, Jorhat.

2.3. Preparation of crude extract

Plant samples were washed with the help of cleaned and rinsed with distilled water so as to remove the dirt and other disease causing organisms. The samples were then dehydrated at a temperature of 40°C in a hot air oven for 12 hours by spreading evenly on flat trays. After drying, the plants were grinded to make fine powder and stored in airtight containers. For aqueous extraction, 5 g of air-dried powder was taken in a conical flask with 20 ml distilled water and kept in a dark area for two days. After two days the samples were filtered through what man no 1 filter paper. The samples were then evaporated in a water bath to make the volume one fourth of the original volume and stored at 4 °C in airtight bottles. Similarly for alcoholic and methanolic extracts 5 g of each sample was taken in 20 g of organic solvents i.e ethanol and methanol respectively, kept in a dark area for two days and filtered through what man no 1 filter paper. Then the samples were evaporated in a water bath to make the volume one fourth of the original volume and stored at 4 °C in airtight bottles.

2.3. Test organisms

The microbial strains investigated are identified strains and were obtained from National Centre for Microbial research (NCMR), Pune, and India. The studied bacterial strains include *Salmonella typhi*, *Escherichia coli*, *Listeria monocytogenes*, *Shigella* and *Serratia*. Microorganisms were maintained at 4 °C on nutrient agar slants.

2.4. Standard antibiotic

Sterile Muller Hinton Agar plates were prepared and the test organisms were swabbed over the surface of agar plates using sterile cotton swab. The antibiotic discs such as Chloramphenicol against *Salmonella*, Ciprofloxacin against *E. coli* and *Serratia*, Ampicillin against *Shigella* and *Listeria* were placed on the surface of the plates. The plates were incubated at 37°C for 12 hours and after incubation the diameter of the inhibition zones were measured in mm and recorded.

2.5. Inocula preparation

The test organisms were transferred from nutrient agar slants to nutrient agar medium by keeping them at room temperature for overnight to get pure colonies. Then 3-4 colonies were selected to test tube containing nutrient broth. The broth is incubated until the turbidity occurs to the Mc Farland standard.

2.6. Antimicrobial activity Preparation of sterile disc

The antibacterial assay was performed by Agar disc diffusion method [5]. Whitman's No.1 filter paper was punched into 5 mm disc form and they sterilized, each sterile disc was incorporated individually with 10 of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations (10, 100, and 250 µl) along with control (solvent). The plates were incubated for 12 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) and recorded to determine the microbial growth. For each bacterial strain, negative controls were maintained where pure solvents were used instead of the extract.

3. Statistical analysis

Each experiment was repeated in triplicate sets and the mean values for the inhibition zones from the triplicate results were employed to represent the antibacterial activity of the extracts.

4. Result and Discussion

The antibacterial effects of ethanolic and methanolic extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* compared with known antibiotics are described with inhibition zones measured in millimeters.

Table 1: Inhibition zones of Ethanolic extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* at different concentrations:

Extracts	Concentration	E. coli	Salmonella	Listeria	Serratia	Shigella
<i>Zanthoxylum nitidum</i> (Roxb.)	10	10	6	10	17	12
	100	12	14	17	17	15
	250	15	17	13	18	19
<i>Drymaria cordata</i>	10	10	13	0	14	14
	100	12	16	12	15	14
	250	13	23	17	19	17

The findings of the current study reveals that the extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* showed antibacterial activity against most of the tested bacteria at 10mg/ml and 100 mg/ml and 250mg/ml as it is shown in the table 1. The ethanolic extracts of *Zanthoxylum nitidum* (Roxb.) shows feeble to moderate inhibition against all test

bacteria with maximum against *Shigella* (19 mm) and minimum against *Listeria monocytogenes* (13 mm) at highest Concentration (250 mg/ml). The activities decreased with decrease in concentration. Table 1 also describes the antibacterial effect of *Drymaria cordata* against the test organism with maximum against *Salmonella* (22 mm) and

minimum against *E. coli* (13 mm) at 250 mg/ml (highest concentration). Here also activities decreased with

concentration but no inhibition is showed against the bacteria *Listeria* at the lowest concentration i.e 10 mg/ml.

Table 2: shows Inhibition zones of methanolic extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* at different concentrations:

Extracts	Concentration	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>	<i>Serratia</i>	<i>Shigella</i>
<i>Zanthoxylum nitidum</i> (Roxb.)	10	0	0	10	0	12
	100	12	12	10	15	15
	250	17	14	20	19	19
<i>Drymaria cordata</i>	10	0	10	11	5	14
	100	18	13	12	8	14
	250	19	15	14	8	17

The methanolic extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* at different concentrations showed weak activity in comparison with the ethanol extracts. Both the extracts exhibited no inhibitory activity against *E. coli* at lowest concentration. *Zanthoxylum nitidum* shows maximum activity against *Listeria monocytogenes* whereas *Drymaria cordata* showed maximum activity against *E. coli* at highest concentration (250mg/ml). The table 1 and 2 shows that ethanolic and methanolic extracts of *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* were effective against most Gram-positive and Gram-negative bacteria tested, thereby indicating a broad spectrum of activity. When compared with the commercially available antibiotics, Ethanolic extract of *Zanthoxylum nitidum* produced zone of inhibition (23mm) similar to that of inhibition zone produced by antibiotic chloramphenicol (23mm) particularly for *Salmonella typhimurium* bacteria. Similarly, it showed comparable inhibition zone produced by antibiotic ciprofloxacin (19 mm) against *Serratia*. The methanolic extract of *Zanthoxylum nitidum* showed zone of inhibition (19 mm) similar to that of inhibition zone produced by antibiotic ciprofloxacin (19 mm) at the highest concentration i.e 250mg/ml. Related reports shows the *in vitro* antibacterial activity of aqueous and ethanol extracts from the stem bark and root of *Zanthoxylum nitidum* (Roxb.) against *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *Sarcina lutea*, *Bacillus subtilis* and two Gram-negative bacteria, *Klebsiella pneumoniae*, and *Escherichia coli*. All extracts at higher concentrations showed varying degrees of inhibitory activity against all bacteria [3]. The aerial parts of different extracts of *Drymaria cordata* Willd were tested for antibacterial efficacy against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus pumilis* and *Pseudomonas aeruginosa*. The effects produced by all the extracts were found to have significant activities against all the tested organisms [8].

5. Conclusion

From the findings of the study, it has reiterated and confirmed the evidence found in scientific literature, that *Zanthoxylum nitidum* (Roxb.) and *Drymaria cordata* do contain immense antimicrobial properties. This would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin to fight off various infectious diseases as using these natural source can be regarded as nutritionally safer option. The antimicrobial activities may be due to presence of some important phytochemicals like phenols, alkaloids, saponins, tannins, steroids, and flavonoids. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

6. Reference

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