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Antibacterial Properties of Selected Plants used Traditionally by the Rurals of Banda District

Ajeet Kumar Pandey[1]*, Dr. Anuj Bhadauriya[2]

[1] Research Scholar, Shri Krishna University, Chhatarpur M.P.

[2] Professor, Shri Krishna University, Chhatarpur M.P.

Abstract - India is the home to many tribal groups and ranks seventh among the world's biodiversity hotspots. Aboriginal people developed a vast body of knowledge about the plants and their applications through comprehending the surrounding environment to satisfy their requirements. Recent research on conventional medicine has made it possible to identify other plants that produce drugs. Therefore, it is crucial to gather data, provide documentation, and conduct research on ethnomedicine. The aim of is to study the antibacterial properties of selected plants used traditionally by the rurals of Banda district.

Keywords - Antibacterial properties, Traditional Plants, Rurals

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INTRODUCTION

The wealth of indigenous wisdom is completely unknown to individuals in the contemporary world. Tribal communities often reside in diverse geographic and climatic regions with abundant flora and wildlife. They belong to many ethnic groupings. Since the majority of these tribal cultures lack a written language to record information on prescriptions, methods of use, and diagnoses. Many tribal cultures regularly use wild plants as part of their diets, as well as when there is a lack of food or a drought (Narayanan and Anilkumar, 2007). Only 30 species alone account for more than 90% of the world's calories consumed, and 120 species are economically significant on a national scale out of the 3000 edible plant species known to humans (FAO,1993). There are 42 million tribal people in India, and 60% of them live in forests and rely on a variety of edible forest resources (Jain and Chauhan, 1998).

Recent research on conventional medicine has made it possible to identify other plants that produce drugs. Therefore, it is crucial to gather data, provide documentation, and conduct research on ethnomedicine. Indigenous wisdom was losing faster because to the intrusion of western lifestyles, the lack of enthusiasm among younger generations in passing down their traditional knowledge, and the decline in the number of traditional medical practitioners. Therefore, the current research sought to identify plants as the source of novel medications against pathogenic bacteria while also collecting and documenting indigenous knowledge via ethnobotanical survey and antibacterial screening of certain chosen plants.

Study area

This ethnobotanical study was conducted in the Banda region. The district, which is in the Chitrakutdham Division of Uttar Pradesh and has its administrative centre at Banda, is situated between the longitudes of 80o 07' and 81o 34' E and the latitudes of 24o 53' and 25o 55' N. The fieldwork was done in Banda, Naraini, Baberu, and Atarra, the four tahsils that make up the Banda district.

Preparation of media used in antibacterial assay

Three nutritional media are provided for the development of certain bacterial strains. They are Nutrient Broth Medium, Mueller Hinton Agar Medium, and Nutrient Agar Medium.

- **Nutrient Agar Medium** (Hesse,1890)

By routinely subculturing the bacteria in nutrient agar medium, the standard bacterial strains were kept alive. Below are listed the components of nutritional agar media.

- **Ingredients of Nutrient Agar Medium**

Beef extract-	1.5g
Yeast extract-	1.5g
Peotone-	5g

NaCl	-	5g
Agar	-	15g
Water	-	1L
pH	-	7.4

The required amount of distilled water and nutritional agar powder (HiMedia) were mixed to make the nutrient agar slants, which were then gently heated while being swirled until the medium was completely dissolved. Next, 5ml volumes of the medium were put into sterile test tubes, and the lids were tightly fastened. Then, to sanitise them, these test tubes were autoclaved at 15 lbs of pressure for 15 minutes at 121 °C. The test tubes were kept slanted so that they would solidify.

The test organisms developed overnight in an incubator after being inoculated on slants. These tubes were then kept in a refrigerator at 4^{o}C for later use.

- **Nutrient Broth Medium (**Lapage *et al*., 1970**)**

A suspension culture of chosen bacterial strains was created in nutrient broth. The components of the nutritious broth are mentioned below.

- **Ingredients of Nutrient Broth Medium**

Beef extract-	1.5g
Yeast extrac-	1.5g
Peotone-	5g
NaCl-	5g
Water-	1L
pH-	7.4

The nutritional media was obtained from Mumbai's HiMedia Laboratories Private Limited and used in its production. To prepare broth, 13g of pre-made media were cooked in 1 litre of distilled water until completely dissolved. After that, the sterilisation was finished in an autoclave running for 15 minutes at 121 °C under 15 lbs of pressure.

- **Mueller Hinton Agar Medium (**Mueller and Hinton, 1941**)**

The Mueller Hinton Agar Medium was used to assess antibacterial activity utilising disc diffusion sensitivity tests. The dehydrated media were provided by the Mumbai-based HiMedia Laboratories Private Limited. The nutrients in nutritious broth are listed below.

- **Ingredients of Mueller Hinton Agar Medium**

Beef infusion form-	300g
Casein acid hydrol ysate	-
17.50g Starch-	1.5g
Agar-	17g
Water-	1L
pH-	7.4

28gm of dehydrated media were boiled in 1 litre of distilled water until fully dissolved to generate the medium. An autoclave was used to sterilise, as was previously indicated. At least 20 millilitres of sterile medium were put to sterilised petriplates and allowed to set up in an aseptic environment. After cooling, they are either checked for germs or maintained in a refrigerator at 4 degrees Celsius for no more than a couple of weeks.

- **Preparation of Inoculums**

The nutrient inoculums for the test organisms were made by transferring specific bacterial strains from the nutrient slants to nutrient broth medium. Bacterial strains are transferred from the nutrient slant to the nutrient broth via a sterile loop, where they are subsequently cultivated at 37 °C until the broth becomes turbid. If it is found that the turbidity is high, adding sterile broth medium may help to lower it. This murky substance may now be used for immunizations.

- **Antibacterial susceptibility test**

Using the disc diffusion technique, the ability of different plant extracts to halt the development of bacteria was assessed (Lorian, 1996). (Lorian, 1996). To remove any surface moisture, petri plates containing 20 ml of MHA medium were dried in an incubator for 30 minutes at 37–40 oC. Test organisms from the newly created inoculums were distributed throughout the solid medium using a sterile cotton swab. A 6mm sterile disc was used throughout the investigation (HiMedia). A 50 l extract solution comprising around 500 g of plant extract was applied to each disc. Prior to loading, all solvent residues from the discs were completely removed by incubating both the sample-impregnated discs and the control disc with only solvent at 60 °C. These dry discs were incubated at 37 oC for 20 to 24 hours with the medium spread out.

The creation of a distinct inhibitory zone around the disc on the medium allowed for the investigation of the plant extracts' antibacterial properties. Only sensitive cultures developed an identifiable inhibitory zone; resistant strains lacked one. The inhibitory zone's diameter and the disc's total circumference, both stated in millimetres, were measured.

- **Antibacterial activity against Antibiotic**

discs

To compare the antibacterial activity of the plant extracts—which displayed the highest inhibitory activity among the chosen ten plants—with antibiotics, three antibiotic discs—Ampicillin (10 g/disc, HiMedia), Ciprofloxacin (5 g/disc, HiMedia), and Streptomycin (10 g/disc, HiMedia—were used as positive controls. The disc diffusion method was used to examine the ability of antibiotics and plant extracts to stop the development of bacteria (Lorian, 1996).

Statistical analysis

Studies were conducted in three repetitions, and the antibacterial test results were provided as meanstandard deviation with relation to their inhibitory zone.

RESULT AND DISCUSSION

Antibacterial studies

The antibacterial effectiveness of particular plants was examined using the crude extracts. By measuring the inhibition zone around the disc on MHA medium, the activity was evaluated. Results of the disc diffusion test of crude extract in various solvents (Petroleum ether, Acetone, Methanol, and Water) with varying levels of activities are presented in this study.

Methanolic extract from Ageratina adenophora had the strongest antibacterial effect, followed by acetone, while petroleum ether and water extract had no effect. Maximum inhibitory zones were visible against Proteus vulgaris and Salmonella typhi in methanolic extract. Proteus vulgaris and Staphylococcus aureus displayed the maximum inhibitory zone in acetone extract (Table 1).

Table 1: Antibacterial activity of different solvent extracts of Ageratina adenophora

Bacterial strains	50µl			
	PE	AE	ME	WE
Vibrio parahaemolyticus	-	11.17±0.08	17.14±0.05	-
Salmonella typhi	-	16.74±0.34	**36.05±0.07**	-
Bacillus cereus	-	9.26±0.22	15.34±0.47	-
Enterobacter aerogenes	-	14.15±0.58	20.19±0.82	-
Salmonella paratyphi	-	11.3±0.09	16.32±0.24	-
Staphylococcus aureus	-	18.78±0.09	20.27±0.38	-
Escherichia coli	-	10.18±0.26	15.13±0.15	-
Streptococcus haemolyticus	-	12.35±0.21	17.48±0.23	-
Proteus vulgaris	-	**20.41±0.24**	35.40±0.21	-
Klebsiella pneumoniae	-	-	11.33±0.47	-
Pseudomonas aeruginosa	-	-	14.32±0.08	-
Proteus rettgeri	-	15.56±0.34	18.12±0.26	-
Serratia marcesens	-	-	10.23±0.25	-
Vibrio cholerae	-	12.42±0.41	14.19±15	-

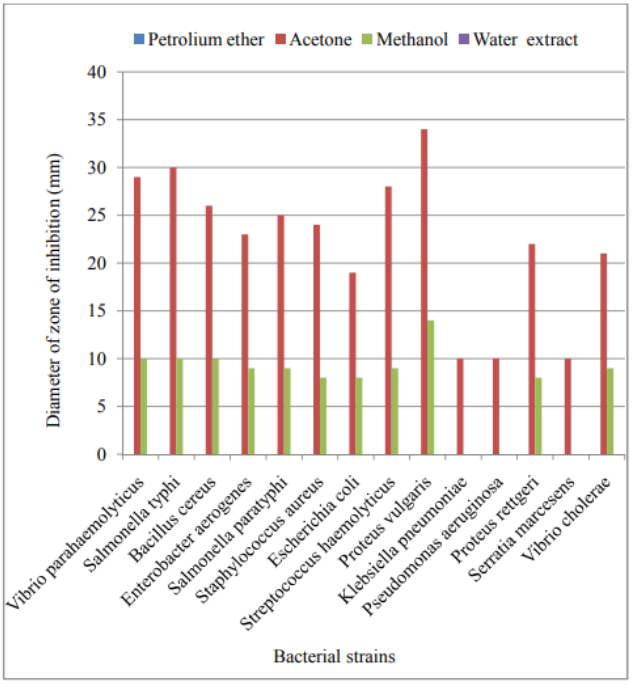
(Values are mean±SD for n= 3 individual observations, PE- Petroleum ether Extract, AE- Acetone Extract, ME- Methanol Extract, WE- Water Extract)

Acetone extract demonstrated maximum activity against all strains in Cissus discolour with the exception of Klebsiella pneumonia. Petroleum ether, extracts of acetone, and extracts of methanol were all susceptible to Streptococcus haemolyticus. All extracts were only resistant to Klebsiella pneumonia (Table 2).

Table 2: Antibacterial activity of different solvent extracts of *Cissus discolor*

Bacterial strains	50µl			
	PE	AE	ME	WE
Vibrio parahaemolyticus	-	12.46±0.32	-	-
Salmonella typhi	-	20.09±0.04	11.05±0.09	-
Bacillus cereus	-	13.51±0.17	-	-
Enterobacter aerogenes	-	14.31±0.24	-	-
Salmonella paratyphi	-	11.36±0.21	-	-
Staphylococcus aureus	-	15.29±0.08	-	-
Escherichia coli	-	13.37±0.09	-	-
Streptococcus haemolyticus	10.12±0.11	12.51±0.59	8.03±0.06	-
Proteus vulgaris	-	**21.26±0.23**	8.14±0.26	-
Klebsiella pneumoniae	-	-	-	-
Pseudomonas aeruginosa	-	20.22±0.24	-	-
Proteus rettgeri	-	7.20±0.26	-	-
Serratia marcesens	-	11.69±0.16	-	-
Vibrio cholerae	-	13.07±0.02	-	-

(Values are mean±SD for n= 3 individual observations, PE- Petroleum ether Extract, AE- Acetone Extract, ME- Methanol Extract, WE- Water Extract)



Graph 1: Antibacterial effects of different solvent extracts of Heracleum candolleanum rhizome against selected bacterial strains. All bacterial strains were sensitive towards acetone extracts, among Proteus vulgaris showed highest activity with inhibition zone 34.15±0.12 . Klebsiella pneumonia, Pseudomonas aeruginosa and Serratia marcesens are insensitive to methanolic extract.

Antibacterial activity against Antibiotic discs

Additionally tested were control discs with just solvents, which had no discernible effects. Three common antibiotics were tested for a comparison study: ampicillin (10 g), ciprofloxacin (5 g), and streptomycin (10 g) (Fig. 3).

Table 3: Shows antibacterial activity of antibiotic discs against selectedbacteria

Bacterial strain	Antimicrobial Disc		
	AMP	CIP	STR
Vibrio parahaemolyticus	20.41±0.28	30.24±0.04	-
Salmonella typhi	10.27±0.22	40.16±0.13	-
Bacillus cereus	21.18±0.20	34.22±0.19	-
Enterobacter aerogenes	18.42±0.53	30.34±0.40	-
Salmonella paratyphi	20.36±0.32	31.17±0.21	-
Staphylococcus aureus	**25.05±0.02**	30.39±0.36	20.26±0.31
Escherichia coli	15.31±0.27	20.58±0.04	-
Streptococcus haemolyticus	23.22±0.25	32.16±0.24	-
Proteus vulgaris	-	35.43±0.38	**26.25±0.36**
Klebsiella pneumoniae	-	25.36±0.42	15.12±0.17
Pseudomonas aeruginosa	-	**40.69±0.04**	25.39±0.34
Proteus rettgeri	17.31±0.13	29.27±0.18	-
Serratia marcesens	-	36.25±0.31	22.29±0.25
Vibrio cholerae	21.44±0.13	28.28±0.24	-

(Values are mean±SD for n= 3 individual observations, AMP- ampicillin, CIP- Ciprofloxacin, STR-Streptomycin)

CONCLUSION

Different plant extracts in aqueous form did not exhibit any measurable effects on the tested bacterial strains. While Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa, and Serratia marcesens are insensitive to ampicillin, all bacterial strains are sensitive to ciprofloxacin. Streptomycin is also not effective against Vibrio parahaemolyticus, Salmonella typhi, Bacillus cereus, Enterobacter aerogenes, Salmonella paratyphi, Escherichia coli, Streptococcus haemolyticus, Proteus rettgeri, and Vibrio cholera.

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Corresponding Author

Ajeet Kumar Pandey*

Research Scholar, Shri Krishna University, Chhatarpur M.P.