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Antimicrobial Activity of Methanol Extract of Root Bark of *Hiptage benghalensis* (L) Kurz

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Abstract
The methanol extract of root bark of *Hiptage benghalensis* (L) Kurz. was studied for its antimicrobial activity using disc diffusion method. The activity was assessed by measuring the zone of inhibition and comparing with that of the standard antibiotic, i.e., 10 µg of tetracycline. The tests organisms were *Klebsiela pneumonia* (MTCC – 39), *Escherichia coli* (MTCC – 40) *Micrococcus luteus* (MTCC – 106) and *Pseudomonas aeruginosa* (MTCC – 424). The minimum concentration of the crude extract that inhibits the growth of the test microorganism was determined and recorded as Minimum Inhibitory Concentration (MIC) of the extract on that particular organism.

Keywords: *Hiptage benghalensis*, root bark, methanol extract, disc diffusion, minimum inhibitory concentration.

1. Introduction
A large number of plants used in the traditional medicine have now become a part of the modern world health care system. The World Health Organization (WHO) estimates that 80 % of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of the traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as source of drugs (Fansworth, 1988) [2]. Further evidence of the importance of natural products is provided by the fact that almost half of the world's 25 best-selling pharmaceuticals in 1991 were either natural products or their derivatives (Neill *et. al*., 1993) [6]. Thirty percent of the worldwide sales of drugs are based on natural products (Grabley and Thiericke, 1999) [3].

Mizoram, situated in the north east India, is rich in biodiversity. The people have explored and utilized the potential of the land's rich biodiversity since time immemorial. Many herbal medicines have been developed from traditional knowledge for treating different kinds of diseases some of which have been proven to be really effective many a times. *Hiptage benghalensis* (L) Kurz. belonging to the family Malpighiaceae, known as Raisentur by the Mizo people is also one of the smany medicinal plants which have been used traditionally for treating different ailments. Decoction of the root bark is consumed orally for stomachache, chewed in a raw for diarrhea and the powdered root bark mixed with water for dysentery (Lalnundanga, 2000) [4]. In the present work, the root bark of the plant is studied for its antimicrobial activity as it is used traditionally for treating different microbial infections.

2. Materials and Methods
2.1 Microorganisms: The microorganisms used for the present study are *Escherichia coli* (MTCC-40), *Micrococcus luteus* (MTCC- 106), *Pseudomonas aeruginosa* (MTCC-424) and *Klebsiellla pneumonia* (MTCC- 39), which is obtained from Institute of Microbial Technology (IMTECH), Chandigarh, Punjab, India. The microorganisms are sub-cultured in nutrient broth and incubated at 37˚C for 24 hrs prior to the experiment.

2.2 Preparation and extraction of the plant material: The plant material is obtained from Cherhlun and Ngharchhip, Lunglei District Mizoram, India and authentication is done at Botanical Survey of India, Kolkata. The dried powdered root bark of the plant is extracted with methanol by hot continuous extraction process using soxhlet apparatus for 72 hrs. The crude extract is concentrated and dried using rotary vacuum evaporator.

2.3 Disc diffusion method: The antimicrobial activity of the crude extract is tested on the different test microorganisms mentioned by disc diffusion method (Bauer *et al*., 1966) [1].

2.3.1 Preparation of stock extract solution: The dried methanolic extract of the plant is weighed and dissolved in Dimethyl Sulfoxide (DMSO) made by GR, Merck Specialities Private Limited, Mumbai making 20mg/ml and 10 mg/ml concentrations.

2.3.2 Preparation of test microorganisms: The turbidity of the sub cultured microorganisms is adjusted with sterile distilled water using 0.5 Mc Farland as standard (~1.5 X 10^8 microorganisms/ml).

2.3.3 Preparation of plant extract disc: Paper disc of 5 mm diameter is prepared using Whatman filter no 3. The paper discs are sterilized before applying the extracts. Then, 10 µL of the plant extracts of different concentrations are applied to the sterilized paper discs so that the discs contain 200 µg and 100 µg of the extract respectively, after which the discs are air dried.

2.3.4 Preparation of media: 200 ml. of Nutrient Agar (HiMedia) is prepared by dissolving readymade nutrient agar powder in distilled water. Then the dissolved nutrient agar was sterilized.

2.3.5 Evaluation of antimicrobial activity of plant extract: Antimicrobial activity of plant extract is assessed by disc diffusion method. Agar plates are prepared which are inoculated with the test microorganisms by pour plate method and allowed to dry at room temperature. Then, the paper disc containing two different concentrations of plant extract and antibiotic disc containing 10 µg of tetracycline (HiMedia) were kept carefully on the surface of the agar plate. In addition, paper disc containing DMSO is also kept as negative control to make sure that the solvent used for dissolving the extracts do not have antimicrobial activity. Then, the plates are incubated at 37˚C for 24 hours in inverted position. After incubation is over, the plates are observed for antimicrobial activity. If the extract possesses such activity, the zone of inhibition was measured and compared with the standard antibiotic.

2.4 Minimum Inhibitory Concentration: The minimum inhibitory concentrations of the crude alcoholic extract of *Hiptage benghalensis* (L) Kurz. Is determined on the test organisms where the extract is found to be active by disc diffusion susceptibility test method (Mendoza, 1998) [5].

2.4.1 Preparation of stock extract solution: The dried methanolic extract of the plant is weighed and dissolved in DMSO making 20mg/ml as stock solution. Then, the extract is diluted by two fold dilution making concentrations ranging from 20 mg/ml to 0.3125 mg/ml.

2.4.2 Preparation of test microorganisms: The turbidity of the sub cultured microorganisms is adjusted with sterile distilled water using 0.5 MC Farland as standard (~1.5 X 10^8 microorganisms/ml).

2.4.3 Preparation of plant extract disc: Paper disc of 5 mm diameter is prepared using Whatman filter no 3. The paper discs are sterilized before applying the extracts. Then, 10 µL of the plant extracts of different concentrations are applied to the sterilized paper discs so that the discs contain 200 µg, 100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg and 3.12 µg of the extract respectively, after which the discs are air dried.

2.4.4 Preparation of media: 100 ml. of Mueller Hinton Agar (HiMedia) is prepared by dissolving readymade powder in distilled water. Then the dissolved Mueller Hinton Agar as sterilized.

2.4.5 Evaluation of antimicrobial activity of plant extract: The MIC of plant extract is assessed by disc diffusion method. Agar plates are prepared which are inoculated with the test microorganisms by pour plate method and allowed to dry at room temperature. Then, the paper disc containing seven different concentrations of plant extract and antibiotic disc containing 10 µg of tetracycline (HiMedia)) are kept carefully on the surface of the agar plate. Negative control is also kept which is DMSO. Then, the plates are incubated at 37˚C for 24 hours in inverted position. After incubation is over, the plates are observed for antimicrobial activity and the lowest concentration of the extract inhibiting the growth of microorganism is noted and considered as the MIC for each test microorganism.

3. Results and Discussion

3.1 Disc diffusion method: The antimicrobial activity of the methanol extract of *Hiptage benghalensis* (L.) Kurz is assessed by measuring the zone of inhibition and comparing with that of the standard antibiotic, i.e., 10 µg of tetracycline (Table 1).

Table 1: Antimicrobial activity of the methanol extract of *Hiptage benghalensis* (L.) Kurz on different test microorganisms.

Sl. No.	Test Organisms	Zone of Inhibition (in mm)*		
		200 µg of plant extract	100 µg of plant extract	Standard (10µg Tetracycline)
1.	*Klebsiella pneumoniae* (MTCC No. 39)	9.5	9	16.5
2.	*Escherichia coli* (MTCC No. 40)	8	8.5	17.5
3.	*Micrococcus luteus* (MTCC No. 106)	9	8.5	17
4.	*Pseudomonas aeruginosa* (MTCC No. 424)	9.5	8	21

* The zone of inhibition shown above is the mean of three readings and includes the diameter of the paper disc, i.e., 5 mm.

As shown in Table 1 that the extract is active on all microorganisms *Klebsiela pneumonia* (MTCC – 39), *Escherichia coli* (MTCC–40) *Micrococcus luteus* (MTCC–106) and *Pseudomonas aeruginosa* (MTCC – 424) from the study, it is seen that DMSO, the solvent used for dissolving the extract does not show any antimicrobial activity towards the test organism.

3.2 Minimum Inhibitory Concentration*:* The zone of inhibition exhibited by the different concentrations of the extract on the four test organisms is measured (Table2).

Table 2: Comparison of the antimicrobial activity of the different concentrations of the methanol extract of *Hiptage benghalensis* (L.) Kurz. on selected test microorganisms.

Sl. No.	MTCC No.	Zone of inhibition (in mm)							
		20mg/ml (200 µg/ disc)	10mg/ml (100 µg/ disc)	5mg/ml (50 µg/ disc)	2.5mg/ml (25 µg/disc)	1.25mg/ml (12.5 µg/disc)	0.625mg/ml (6.25 µg/disc)	0.3125mg/ml (3.125 µg/ disc)	Tetracycline (10 µg/disc)
1.	39	9.5	9	8	6.5	7	6.5	-	16.5
2.	40	8	8.5	8.5	7.5	7	6.5	6	17.5
3.	106	9	8.5	7.5	8	7.5	6.5	-	17
4.	424	9.5	8	9	7.5	7.5	7	-	21

The minimum concentration of the crude extract that inhibits the growth of the test microorganism was determined and recorded as MIC of the extract on that particular organism (Table 3).

Table 3: MIC of the methanol extract of *Hiptage benghalensis* (L) Kurz on different test microorganisms.

Sl. No.	Microorganism	MIC
1.	*Klebsiella pneumoniae* (MTCC No. 39)	0.625 mg/ml
2.	*Escherichia coli* (MTCC No. 40)	0.3125 mg/ml
3.	*Micrococcus luteus* (MTCC No. 106)	0.625 mg/ml
4.	*Pseudomonas aeruginosa* (MTCC No. 424)	0.625 mg/ml)

It is seen from the table 3 that the plant extract is quite active at low concentrations, 0.625mg/ml on *Klebsiela pneumonia*, *Micrococcus luteus* and *Pseudomonas aeruginosa* and 0.3125mg/ml on *Escherichia coli.*

4. Conclusion

Since a larger zone of inhibition is seen on the standard antibiotics, it is evident that the extract contains compound which is highly active against the test organisms, isolation and purification of the active constituents may lead to the discovery of new compound better than the standard antibiotic. In conclusion, results of the present study supported the folklore usage of the studied plant and suggested that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggested that they represent an economic and safe alternative to treat infectious diseases. Finally, it is recommended that awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings.

5. References

1. Bauer AW, Kirby WMM. Sherris JC, Turck M**.** Antibiotic susceptibility testing by a standardized single disk method. The American Journal of Clinical Pathology 1966; 45(4):493-496.
2. Fansworth NR. Screening of plants for new medicines. In: Wilson E.O, editor. Biodiversity. National Academy Press Washington. DC, 1998, 83-97.
3. Grabley S, Thierick R. Bioactive agents from natural sources trends in discovery and application. Advance Biochemical Engineering Biotechnology 1999; 64:101-154.
4. Lalnundanga. Study of Medicinal Plants in sub-tropical semi-evergreen forests of Mizoram. Ph.D. Thesis, North Eastern Hill University, Mizoram Campus, Aizawl, Mizoram, 2000.
5. Mendoza MT. What's New in Antimicrobial Susceptibility Testing? Philippine Journal of Microbiology and Infectious Disease 1998; 27(3):113-115.
6. Neill OMJ, Lewis JA. Plant-derived natural products in drug discovery and development. In Kinghorn, A. D., Balandrin, M. F., editors. Human Medicinal Agents from Plants. ACS Symposium Series 1993; 534:48-55