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In vitro Antimicrobial Activities of *Hygrophila schulli* (Buch.-Ham) Leaf and Root Extracts Against Clinically Important Human Pathogens

R. PRATAP CHANDRAN[1]*, S. MANJU[1], M.V. VYSAKHI[1], P.K. SHAJI[2] and G. ACHUTHAN NAIR[3]

[1]Department of Biotechnology and Research, K. V. M. College of Engineering and Information Technology, K.V.M. College Road, Kokkothamangalam P. O., Cherthala - 688 583, India.
[2]Environmental Resources Research Centre (ERRC), P.B. No. 1230, Peroorkada, P.O. Thiruvananthapuram - 695 005, India.
[3]Chair for Sustainable Development, Indira Gandhi National Open University (IGNOU), Regional Centre, Thiruvananthapuram - 695 002, India.
*Corresponding author E-mail: drpratapchandran@yahoo.co.in

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ABSTRACT

Hygrophila schulli is an important medicinal plant in Indian systems of medicine. The purpose of the present study was to examine the antibacterial and antifungal activities of *H. schulli* collected from Kuttand wetlands, Kerala state, India. Hot and cold extracts of leaf and root in seven solvent each (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) were tested against clinically important bacterial and fungal pathogens. Ciprofloxacin (5 µg/ml) and Amphotericin B (10 µg/ml) was used as a standard drugs for analyzing antibacterial and antifungal activity respectively. The cold methanolic leaf extract showed the highest inhibition zone against *Staphylococcus epidermidis* (16 mm) and *Klebsiella pneumoniae* (15 mm) and the hot ethyl acetate leaf extract showed an inhibition zone of 13 mm against *Pseudomonas aeruginosa.* Different concentrations of cold methanolic extracts were tested against *S. epidermidis* and *K. pneumoniae* and they showed the highest inhibition zones of 27 mm and 26 mm respectively at 400 mg/ml. The results revealed that the leaf extracts of *H. schulli* have promising antibacterial activity than the root extracts. The leaf and root extracts did not show antifungal activity against the tested fungal pathogens.

Key words: Antibacterial, antifungal activity, extracts, *Hygrophila schulli,* inhibition zone, pathogenic microorganism.

INTRODUCTION

The multiple drug resistance of microorganisms is a global concern today in view of the emergence and persistent spread of resistant microbial strains throughout the world owing to their phenotypic plasticity[1-2]. Medicinal plants are a rich source of secondary metabolites and they are exploited from time immemorial as powerful drugs in traditional/alternative healthcare systems. Many pharmaceutical companies are showing great interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is effective, safer and more reliable than synthetic drugs[3]. The demand for plant based medicines and other herbal healthcare products, including pharmaceuticals, food supplements (functional foods), cosmetics (cosmeceuticals), etc. is increasing steadily in both developing and developed countries due to the growing recognition that the natural products are relatively non-toxic, have less side effects and easily available at affordable prices[4].

Botanicals or phyto-pharmaceuticals are very suitable for prophylactic use in order to prevent diseases and also to maintain our normal wellbeing[5].

The extensive use of synthetic compounds led to a decline in the use of plants in modern medicine; however, synthetic drugs often cause considerable side effects, and as a result, people are more favouring the use of natural compounds obtained from plants[6]. During the last two or three decades, rapid increase in the rate of infections, antibiotic resistance in microorganisms, side effects of synthetic antibiotics, together with advances in phytochemistry and identification of new bioactive compounds from plants which are effective against certain diseases, have renewed the popularity of herbal medicines[7-8].

Hygrophila schulli (Buch.-Ham) is a thorny sub-shrub of the family Acanthaceae that grows widely throughout India, Sri Lanka, Myanmar, Indo-China, Tropical Africa and Malaya. The synonyms of *H. schulli* include *H. auriculata* (K. Schum) Heine and *Astercantha longifolia* (L.) Nees. The erect armed sub-shrub with purplish stem generally has eight leaves and six spines at each node. Leaves are whorled, linear-lanceolate, with undulating margins. Flowers form in axillary sessile whorls, with leafy bracts and bracteoles, and pink corolla. Capsules are 1 cm long and seeds orbicular. *H. schulli* (Marsh barbel) is a commonly found associated with and wetlands, which forms large stands by easily colonizing in waterlogged areas.

In ayurvedic literature, it is described as Ikshura, Ikshugandha, and Kokilaksha (having eyes like the Kokila) or Indian Cuckoo. It is classified in the Ayurvedic system of medicine as Seethaveryam, Mathuravipaka and is used for the treatment of a number of conditions, including premeham (diabetes) and athisaram (dysentery)[9-10]. Traditionally, the leaves are used as/in diuretic, jaundice, antibacterial, dropsy, rheumatism, anasaraca, diseases of urinogenital tract, leucor, sweet, sour, bitter, tonic, oleaginous, aphrodisiac, hypnotic, diarrhea, dysentery, urinary calculi, urinary discharge, anti inflammatory, joint pain, biliousness, eye disease, ascites, abdominal troubles, anemia, anuria, gleets, cough, demulcent, stomachic, lumbago, arthritis, gastric disorder and leucorrhoea[11]. *H. schulli* mainly contains lupeol, stigmasterol, isoflavone glycoside, an alkaloid and small quantities of uncharacterized bases [12].

The present investigation was carried out to screen seven extracts (hot and cold) of *Hygrophila schulli* leaf and root against pathogenic bacteria and fungi in order to detect the antibacterial and antifungal properties of samples collected from Kuttanad wetlands, Kerala state, India.

MATERIALS AND METHODS

Chemicals

All the solvents used for the extraction process were of analytical grade and procured from SD Fine Chemicals, Mumbai, India. Ciprofloxacin (10 µg/ml) discs, Amphotericin B (10µg/ml), Mueller Hinton agar and Nutrient agar medium were obtained from Hi-Media Laboratories, Mumbai, India.

Collection of plant material

Fresh and healthy leaf and root samples of *H. schulli* were collected from comparatively undisturbed areas of Kuttanad wetlands, Kerala, India; the taxonomic identity of the plant was confirmed by Dr. T. Shaju, Plant Taxonomist, Division of Plant Systematics and Evolutionary Science, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, (JNTBGRI), Palode, Kerala, and the voucher specimens of the samples were deposited in the Herbarium of Environmental Resources Research Centre (ERRC), Thiruvananthapuram, Kerala. The plant materials was initially cleaned and dried under shade and then pulverized to coarse powder in an electric grinder. The powder was then stored in airtight bottles.

Preparation of extracts

The 30g shade dried powder of *H. schulli* (leaf and roots) was subjected to hot and cold extraction using each of the 250 ml solvents (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) in the increasing order of polarity. The final filtrate of each extract was concentrated using a rotary vacuum evaporator (IKA, RV 10 digital, Germany). The extracts collected were evaporated to dryness and stored in vials for further studies. The percent extractive of each extract from both the plants was calculated by using the formula

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Pathogenic bacterial and fungal strains

Four gram positive bacterial strains, such as *Bacillus subtilis* (MTCC 619), *Bacillus cereus* (MTCC 430), *Staphylococcus simulans* (MTCC 3610), *Staphylococcus epidermidis* (MTCC 3615) and six gram negative strains viz. *Escherichia coli* (MTCC 729), *Pseudomonas aeruginosa* (MTCC 4676), *Salmonella typhi* (MTCC 3216), *Vibrio cholerae* (MTCC 3904), *Klebsiella pneumoniae* (MTCC 432), and *Proteus mirabilis* (MTCC 425) were used in the study. The fungal strains used in the study include *Candida albicans* (MTCC 183), *Trichophyton rubrum* (MTCC 296), *Cryptococcus gastricus* (MTCC 1715), *Aspergillus tubingensis* (MTCC 2425), *Aspergillus flavus* (MTCC 873) and *Aspergillus fumigatus* (MTCC343). All the cultures were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The cultures were maintained at 4ºC and sub cultured in suitable agar slants. All the bacterial and fungal cultures were maintained at 4°C on nutrient agar and potato dextrose agar slants respectively.

Antibacterial assay

The antibacterial sensitivity assay was carried out by disc diffusion method[13] and different solvent extracts of the plant leaves and roots were tested against the selected test bacterial strains. The test bacterial cultures were evenly spread over Mueller Hinton agar plates using a sterile cotton swab. The sterile discs (6 mm in diameter) were impregnated with extract solution and placed in the inoculated agar. The plates were thus incubated at 37°C for 24 to 48 hours. After incubation, the results were observed and the zones of inhibition thus developed were measured with the scale to the nearest in mm. Ciprofloxacin (5 µg/ml) was used as a positive control and respective solvents serves as a negative control. The experiment was done in triplicates and the mean values were presented. The extracts shown high antibacterial activity were further subjected to disc diffusion assay at varying concentrations (5, 10, 25, 50, 100, 200, 300, 400, 500mg/ml) to check for the maximum activity at a definite concentration.

Antifungal assay

Antifungal activity was measured using disc diffusion method[14]. The fungal cultures to be tested were evenly spread over respective agar plates using a sterile cotton swab. Then, sterile paper discs (6 mm diameter) impregnated with suitable extract was placed on agar. The activity was determined after 72 hours of incubation at 27°C. Amphotericin B (10µg/ml) was used as a positive control and respective solvents as negative controls. Inhibition zones were determined after incubation at 27°C for 48 hours. All tests were done in triplicate and the mean values were presented.

RESULTS AND DISCUSSION

The percentage extractive of different solvent extracts of *H. schulli* leaves and roots are given in table 1.The results of showed varying inhibition pattern against bacterial pathogens and the antimicrobial activity was interpreted after 24 hours of incubation. Hot leaf extract of ethyl acetate showed the highest inhibition zone of 13 mm against *P. aeruginosa* and 8 mm against *K. pneumoniae* (Table 2) whereas the control drug ciprofloxacin (5 µg/ml) produced an inhibition zone of 30 mm and 28 mm respectively. Hot extract of acetone and water did not show antibacterial activity against the tested bacterial pathogens. Methanolic hot extract of leaf and root exhibited clear zones of 12 mm against *E. coli* and 12 mm against *P. aeruginosa* respectively and the control drug ciprofloxacin showed an inhibition zone of 32 mm and 30 mm respectively. The results of antibacterial activities of all the other hot extracts showed positive results are given in table 2. Hot extracts (leaf and root) of all the seven extracts (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) did not show inhibition zones against *P. mirabilis*, *S. epidermidis*, *B. cereus*, *S. typhi*, *V. cholerae* and *S. simulans*.

Cold extracts (leaf and root) of hexane, chloroform and water did not show antibacterial activity against all the ten clinically important human pathogens. Highest antibacterial activity was observed in cold methanolic leaf extract against *S. epidermidis* (16 mm), *K. pneumoniae* (15 mm) and 12 mm inhibitory zone against *E. coli.* The methanolic root extract also showed an inhibition zone of 10 mm against *P. aeruginosa.* Nabere *et al*[15] reported that the cold hexane fraction of whole plant extract showed the inhibition zone against *E. coli* (ATCC: 25922) 11 mm. This is contrary to observation made in the present study.

Among the cold leaf extracts, ethyl acetate (15 mm), acetone (11 mm), and methanol (12 mm) showed antibacterial activity against *E. coli,* acetone (12 mm) and methanol (15 mm) was active against *K. pneumoniae* and ethyl acetate (10 mm), and methanol (16mm) against *S. epidermidis. K. pneumoniae* is the most important member of the *Klebsiella* genus of Enterobacteriaceae and it is emerging as an important cause of neonatal nosocomial infection[16]. Nabere *et al*[15] reported that the ethyl acetate fraction showed inhibition zones against *E. coli* (ATCC: 25922) 17.33 mm, *P. mirabilis* (ATCC: 35659) 8.67 mm, *B. cereus* (ATCC: 9144), 11mm, *S. aureus* (ATCC: 6538); 10.67 and *V. cholerae* with 11 mm respectively. In the present investigation, ethyl acetate fraction did not show inhibition zones against *P. mirabilis, B. cereus, S. typhi, V. cholerae* and *S. simulans* (table 3).

The cold acetone (11 mm), dichloromethane (11 mm) and ethyl acetate (12 mm) extract exhibited antibacterial activity against *B. cereus, B. subtilis* and *P. aeruginosa* respectively. In the case of cold extract, it was found that the leaf extract showed better antibacterial property. The inhibition zones exhibited by cold (leaf and root) dichloromenthane, ethylacetate and acetone extracts are given in table 3.

Different concentrations of cold methanolic extracts were tested against *S. epidermidis, K. pneumoniae, E. coli* and *P. aeruginosa.* The results of inhibition zones produced against different concentrations are given in table 4. The antibacterial activity of different concentrations of methanolic extracts against *S. epidermidis* and *K. pneumoniae* are given in figure 1 and 2. The antifungal assay of all the seven extracts of the leaf and root of *H. schulli* did not inhibit the mycelial growth of the five fungal pathogens. Similarly, Esther *et al*[17] reported that the leaf extract showed inhibition against *A. niger* but the extracts did not inhibit *A. flavus* and *A. fumigatus.* This may be due to the absence of antifungal compounds present in *H. schulli.* The control drug amphotericin B (10 µg/ml) showed antifungal activity against all the tested fungal pathogens, *C. albicans* (24 mm), *T. rubrum* (27 mm), *C. gastricus* (23 mm), *A. tubingensis* (28 mm), *A. flavus* (26 mm) and *A. fumigatus* (31 mm) respectively.

Table 1: Percent extractive of *H. schulli* leaves and roots extracts

Plants	Extraction Process	Hexane (%)	Chloroform (%)	Dichloromethane (%)	Ethyl Acetate (%)	Acetone (%) (%)	Ethanol Water (%)	Distilled
Hygrophila schulli (leaves)	Hot	4.425	1.4	0.55	0.475	0.42	1.875	1.75
	Cold	1.1	1.54	0.7	0.56	0.66	3.26	2.1
Hygrophila schulli (root)	Hot	1.1	0.47	0.47	0.73	0.83	9.6	1.4
	Cold	0.76	1.83	1.5	0.83	1.5	3.37	1.58

Table 2: Antibacterial activity of different hot extracts of *H. schulli*

Extraction solvents	Plant part	Diameter of zone of inhibition (mm)									
		E. coli	***K. pneumoniae***	***P. mirabilis***	***S. epidermidis***	***B. cereus***	***P. aeruginosa***	***B. subtilis***	***S. typhi***	***V. cholerae***	***S. simulans***
Hexane	Leaf	11±0.82	-	-	-	-	-	-	-	-	-
	Root	-	10±1.24	-	-	-	-	-	-	-	-
Chloroform	Leaf	-	-	-	-	-	7±0.8	-	-	-	-
	Root	-	-	-	-	-	7±1	-	-	-	-
Dichloro methane	Leaf	8±0.81	-	-	-	-	-	8±1	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	Leaf	-	8±1.63	-	-	-	13±0	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Acetone	Leaf	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Methanol	Leaf	12±0.81	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	12±0	-	-	-	-
Water	Leaf	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin(5 µg/ml)		32±1.15	28±1.52	31±0.57	29±1	27±1	30±0	26±0	30±1.52	31±0	25±0.57

"-" No activity

Table 3: Antibacterial activity of different hot extracts of *H. schulli*

Extraction solvents	Plant part	Diameter of zone of inhibition (mm)									
		E. coli	***K. pneumoniae***	***P. mirabilis***	***S. epidermidis***	***B. cereus***	***P. aeruginosa***	***B. subtilis***	***S. typhi***	***V. cholerae***	***S. simulans***
Hexane	Leaf	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Chloroform	Leaf	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Dichloromethane	Leaf	-	-	-	-	-	-	11±1	-	-	-
	Root	-	-	-	-	-	8±0	-	-	-	-
Ethyl acetate	Leaf	15±0	-	-	10±0.81	-	12±0.81	9±1.63	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Acetone	Leaf	11±0.81	12±1	-	8±1	11±1	7±0	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Methanol	Leaf	12±0	15±0	-	16±0	-	-	-	-	-	-
	Root	-	-	-	-	-	10±1	-	-	-	-
Water	Leaf	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin(5 µg/ml)		32±1.15	28±1.52	31±0.57	29±1	27±1	30±0	26±0	30±1.52	31±0	25±0.57

"-" No activity

Table 4: Antibacterial analysis of different concentration of *H. schulli*

Concentration (mg/ml)	Zone of inhibition (mm)			
	S.epidermidis **against cold methanolic extract**	***K.pneumoniae*** **against cold methanolic extract**	***E. coli*****against cold ethyl acetate extract**	***P.aeruginosa*** **against hot ethyl acetate extract**
5	-	9±0	-	-
10	10±0	10±0	-	-
25	15±1	12±0.8	-	-
50	20±2	14±0.8	-	7±0
100	24±1	23±0	10±0	10±1
200	25±0.8	24±1	13±0.81	11±0
300	26±0	25±0	14±1	13±0.8
400	27±0	26±0	16±2	15±1

"-" No activity

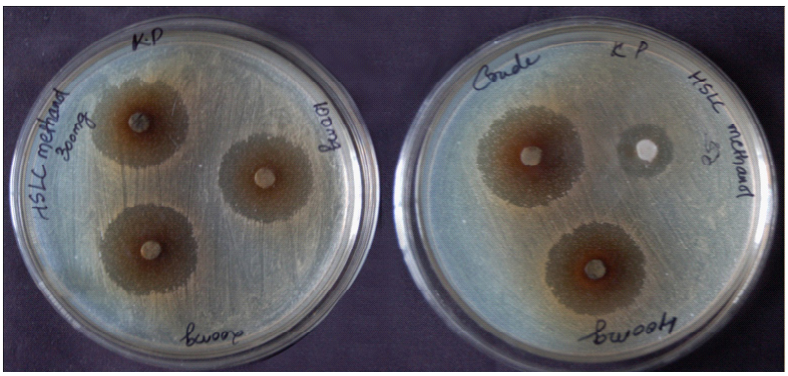

Fig. 1: Antibacterial activity of different concentrations of methanolic extract against *K. pneumoniae*

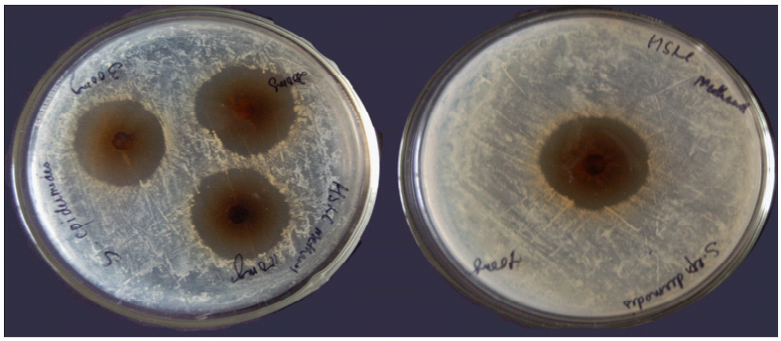

Fig. 2: Antibacterial activity of different concentrations of methanolic extract against *S. epidermidis*

CONCLUSION

The leaf and root extracts of *H. schulli* showed antibacterial activity against the test bacteria. However, leaf extracts were found to be more effective than root extracts. The root extract showed activity only against gram negative bacteria. Out of the various plant extracts examined, the

highest activity was shown by methanolic cold extract of leaf against *S. epidermidis* and *K. pneumoniae.* It was observed that *H. schulli* collected from Kuttanad wetlands, Kerala State, India was also active against *P. aeruginosa.*

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REFERENCES

1. Kunin, C.M. Resistance to antimicrobial drugs: A world wide calamity. *Ann. Intern. Med.*, **118**: 557-561 (1993).
2. Blondeau, J.M. A review of the comparative *in vitro* activities of 12 antimicrobial agents, with a focus on five new respiratory quinolones. *Antimicrob. Agents Chemother.*, **37**: 377-383 (1999).
3. Sujatha, S. Complementary and alternative therapies in palliative care: a transition from modern medicine to traditional medicine in India. *Journal of Cancer pain and Symptom Palliation.*, **1**: 25-9 (2005).
4. Nair, P.K.K. and Shaji, P.K. Phytomedicnes: A Perspective. *Herbal Tech Industry.*, **4**: 15-16 (2008).
5. Md. Sarfaraj Hussain, Sheeba Fareed, Mohd Ali. *Hygrophila auriculata* (K. Schum) Heine: Ethnobotany, phytochemistry and pharmacology. *Asian Journal of Traditional Medicines.*, **5**: 122-131 (2010).
6. Dalal, S. and Sudhir, K.K.K. Phytochemical screening of ethanolic extract and antibacterial activity of *Eclipta prostrate. Asian J. Chem.*, **22**: 7336-7342 (2010).
7. FAO. Medicinal and Aromatic plant in Asia. RAPA Publication (1993).
8. Babu, P.D. and Subhasree, R.S. Antimicrobial activities of *Lawsonia inermis*- a review. *Acad. J. Plant Sci.*, **2**: 231-232 (2009).
9. Nadkarni, A.K. Indian material medica. Vol. 1, Popular Prakashan Private Limited, Bombay, India, pp. 667- 669 (1978).
10. Chopra, R.N., Nayer, S.L. and Chopra, I.C. Glossary of Indian Medicinal plants. National Institute of science and Communication. CSIR Publication (1956).
11. Patra, A., Jha, S., Murthy, P.N. and Satpathy, S. Antibacterial activity of *Hygrophila spinosa* T. Anders leaves - a comparative study. *Int. J. Pharm. Tech. Res.*, **1**: 837-839 (2009).
12. Nikam, D., Mundada, S. and Mishra, D. Kokilaksha: A Potential Ayurvedic Herb. *Int. J. Res. Ayur. Pharm.*, **3**: 780- 782 (2012).
13. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, **45**: 493-496 (1966).
14. Ronald, M.A. Microbiologia. Compania Editorial Continental SA de CV, Mexico, D. F, pp.505 (1990).
15. Nabere, O., Adama, H., Samson, G., Kiessoum, K., Patrice, Z, NagTiero Roland, M., Moussa, C., Martin, K., Millogo, F., Jeanne, Nacoulma, O and Germaine. Antibacterial and phytochemical studies of three *Acanthaceae* species used in Burkina Faso traditional medicine. *J. App. Pharm. Sci.*, **3**: 049-055 (2013).
16. Gupta, P., Murali, P., Murali, M.V., Faridi, M.M.A., Kaul, P.B., Ramachandran, V.C. and Talwar, V. Clinical profile of *Klebsiella* septicaemia in neonates. *Ind. J. Paediatr.***60**: 565-572 (1993).
17. Esther, V.C.J., Saraswathi, R. and Dhanasekar, S. *In vitro* antibacterial and antifungal activities along with x-ray irradiation studies of medicinal plant. *Hygrophila auriculata. Int. J. Pharm. Pharm. Sci.* **4**: 352-358 (2012).