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Research Article

PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF *HUGONIA MYSTAX* L. (LINACEAE)

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ABSTRACT

In this paper, an ethnomedicinal plant, *Hugonia mystax* L., was evaluated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of root extracts showed significant activity against various human pathogens. The antimicrobial activity revealed the medicinal potential of *H. mystax* to develop a drug against various human ailments.

Keywords: Ethnomedicine, *Hugonia mystax*, Constituents, Antimicrobial activity, Drug development.

INTRODUCTION

The genus *Hugonia* L., (family: Linaceae) represents about 40 species in the world, of which two species namely *Hugonia mystax* L., and *H. ferruginea* Wight & Arn., were reported from India[1,2]. The plant, *Hugonia mystax* L., locally known in Tamil language as *Modirakanni*, is commonly distributed in the thorny scrubs and tropical dry evergreen forests of Tamil Nadu. Ethnobotanically roots were used for dysentery[3] and snake bite[4], and also used for anthelmintic, astringent, fever, inflammation, Rheumatism[5-9]. Biological activities such as analgesic, anti-inflammatory and ulcerogenic were reported[10].

After the scrutiny of literatures, only scanty work has been reported on root portion. Hence in the present study, preliminary phytochemical screening and antimicrobial activity of various extracts of roots in *Hugonia mystax* were reported.

MATERIALS AND METHODS

Collection of Plant material

The roots materials were collected from the Marakanam forest vicinity of Villupuram district, Tamil Nadu. The collected plant materials were botanically identified by compared the herbarium specimen submitted at French Institute, Pondicherry. The herbarium specimens were prepared and deposited at the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Lawspet, Puducherry, for future reference.

Preparation of the Extracts

The collected root materials were chopped into small pieces separately, shade-dried and coarsely powdered using a pulverizor. The coarse powders were subjected to successive extraction with organic solvents of increasing polarity such as petroleum ether, chloroform and ethanol by Soxhlet method. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed in *vacuo* and stored at 4°C. The resulted extracts were used for preliminary phytochemical screening and antimicrobial studies.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical tests followed by the Standard methods [11,12].

In vitro antimicrobial activity

All the three extracts (petroleum ether, chloroform and ethanol) extracts were used to prepare various concentrations such as 100, 50 and 25 mg/ml respectively. These were used for antimicrobial activity. For aqueous extract, roots were collected and 30 g each of plant materials were weighed, chopped and divided into 3 portions. Each portion was crushed by grinding in a mortar, transferred to a suitable glass bottle and added 50 ml each of distilled water. First glass bottle was autoclaved at 10 lbs for 20 min. The second was boiled (100ºC) for 20 min. The third was mechanically shaken (200 rpm) under cold conditions for 2 h. The extracts were filtered off using cheesecloth and 0.45 µ filter papers, transferred into sterile closed containers and considered as 100% extract. By adding sterile distilled water, 50% of the extracts were prepared[13].

Test microorganisms

The following bacterial strains and fungal strains were used for the study of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH, Chandigarh includes Gram-negative bacteria such as *Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhi* (MTCC 733), *Vibrio parahaemolyticus* (MTCC 451) and *V. vulnificus* (MTCC 1145) and Gram-positive bacteria such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and *Streptococcus pneumoniae* (MTCC 655) and four fungi viz., *Aspergillus flavus*, *A. fumigatus*, *A. niger* (MTCC 1344) and *Candida albicans* (MTCC 227) respectively.

Determination of antimicrobial activity

Agar well-diffusion method[14] was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for fungal pathogens. Respective proper controls of solvent plant extracts were also maintained. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded.

RESULTS

Preliminary Phytochemical Screening

The results of preliminary phytochemical screening were given in the Table 1. It revealed the presence of carbohydrates, flavonoids, phenolic groups, saponins, tannins and terpenoids in all the extracts. Steroids were present in both the petroleum ether and ethanol extracts while alkaloids were present only in ethanol extract. Moreover, amino acids, anthraquinones, catechins, coumarins, gum, oil & resins, proteins and quinones were absent in all the extracts.

Antimicrobial activity

The results of antimicrobial activity of roots of *H. mystax* were given in the Table 2. All the tested extracts showed concentration-dependent activity against the tested microorganisms.

For petroleum ether extract, the zone of inhibition recorded was ranged between 21 and 25mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 25mm each against *S.aureus* and *S. pneumoniae* at 100mg/ml, 24mm against *S.aureus* at 50mg/ml, 22mm each against *B.subtilis* at 50 and 25mg/ml, *S.aureus* at 25mg/ml, *S.pneumoniae* at 50mg/ml concentration respectively.

Table 1: shows preliminary phytochemical screening of various extracts on the roots

Constituents	Petroleum Ether	Chloroform	Ethanol
Alkaloids	-	-	+
Aminoacids	-	-	-
Anthraquinones	-	-	-
Carbohydrates	+	+	+
Catechins	-	-	-
Coumarins	-	-	-
Flavonoids	+	+	+
Gum, oil & resins	-	-	-
Phenolic groups	+	+	+
Proteins	-	-	-
Quinones	-	-	-
Saponins	+	+	+
Steroids	+	-	+
Tannins	+	+	+
Terpenoids	+	+	+

+=present, **-=**absent

Table 2: shows antimicrobial activity of various extracts of roots.

Tested Microorganisms	Solvent extracts									Aqueous extracts									Standard drug (µg/ml)
	Petroleum ether (mg/ml)			Chloroform (mg/ml)			Ethanol (mg/ml)			Autoclaved (mg/ml)			Boiled (mg/ml)			Cold (mg/ml)			
	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	10
Gram-positive bacteria																			
B. subtilis	23	22	22	-	-	-	28	26	25	24	22	20	21	20	19	23	22	21	33 (A)
S. aureus	25	24	22	-	-	-	27	26	25	25	23	22	23	22	20	20	18	16	35 (A)
S. pneumoniae	25	22	21	-	-	-	23	22	21	21	20	20	19	18	17	15	14	13	32 (Cl)
Gram-negative bacteria																			
E. coli	27	26	25	-	-	-	31	30	29	22	21	19	22	21	20	17	16	15	33 (A)
P. aeruginosa	23	21	21	-	-	-	25	24	23	27	26	25	21	20	19	19	17	16	34 (A)
P. vulgaris	20	16	14	24	23	22	32	31	30	19	17	16	24	23	21	24	23	22	35 (Cl)
S. typhi	23	22	22	21	19	15	26	24	23	20	18	17	-	-	-	-	-	-	35 (Cf)
V. parahaemolyticus	24	23	22	-	-	-	33	32	31	24	20	18	22	21	16	-	-	-	34 (K)
V. vulnificus	20	17	14	22	20	18	28	26	25	19	17	16	24	23	21	24	23	22	35 (K)
Fungi																			
A. flavus	23	22	21	16	15	14	27	26	25	20	17	16	26	25	24	19	18	17	30 (P)
A. fumigatus	22	19	18	-	-	-	22	21	20	17	15	14	19	18	18	18	15	14	32 (P)
A. niger	29	27	26	-	-	-	32	31	29	-	-	-	-	-	-	-	-	-	31 (P)
C. albicans	21	19	18	20	19	16	28	25	24	26	25	24	25	24	20	20	19	18	31 (P)

(Measurements in mm indicates the zone of inhibition); A-Ampicillin, C-Clotrimaxazole, Cf-Ciproflaxacin, K-Kanamycin, Penicillin

In gram-negative bacteria, the zone of inhibition recorded was ranged between 14 and 27mm. Maximum zone of inhibition was recorded as 27, 26 and 25mm each against *E.coli* at 100, 50 and 25mg/ml, 24mm against *V. parahaemlyticus* at 100mg/ml, 23mm each against *P.aeruginosa* and *S.typhi* at 100mg/ml and against *V. parahaemolyticus* at 50mg/ml, 22mm each against *S.typhi* at 50 and 25mg/ml and against *V. parahaemolyticus* at 25mg/ml concentration respectively. In fungi, zone of inhibition recorded was ranged between 18 and 29mm. Maximum zone of inhibition was recorded as 29, 27 and 26mm against *A.niger* at 100, 50 and 25mg/ml, 23, 22 and 21mm against *A.flavus* at 100, 50 and 25mg/ml and 21mm against *C.albicans* at 100mg/ml concentration respectively.

For chloroform extract, the zone of inhibition recorded was ranged between 15 and 25mm against gram-negative bacteria. Maximum zone of inhibition was recorded as 24, 23 and 22mm against *P. vulgaris* at 100, 50 and 25mg/ml, 22mm against *V. vulnificus* at 100mg/ml concentration. Moreover, it did not show any activity against gram-positive bacteria. In fungi, zone of inhibition recorded was ranged between 21 and 25mm. Maximum zone of inhibition was recorded as 20mm against *C.albicans* at 100mg/ml concentration while it did not show any activity against *A.fumigatus* and *A.niger*.

For ethanol extract, the zone of inhibition recorded was ranged between 21 and 28mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 28mm against *B. subtilis* at 100mg/ml, 27mm against *S.aureus* at 100mg/ml, 26mm each against *B. subtilis* and *S. aureus* at 50mg/ml, 25mm each against *B. subtilis* and *S. aureus* at 25mg/ml, 23, 22 and 21mm against *S. pneumoniae* at100, 50 and 25mg/ml concentrations respectively. In gram-negative bacteria, the zone of inhibition recorded was ranged between 23 and 33mm. Maximum zone of inhibition was recorded as 33, 32 and 31mm each against *V. parahaemolyticus* at 100, 50 and 25mg/ml, 32mm against *P. vulgaris* at 100 mg/ml, 31mm each against *E. coli* and *P. vulgaris* at 100, 50mg/ml respectively. 30mm each against *E. coli* at 50mg/ml and *P. vulgaris* at 25mg/ml, 29mm against *E. coli* at 25mg/ml, 28mm against *V. vulnificus* at 100mg/ml, 26mm each against *S. typhi* at 100mg/ml, *V. vulnificus* at 50mg/ml, 25mm each against *P. aeruginosa* at 100mg/ml and *V. vulnificus* at 25mg/ml, 24, 23mm each against *P. aeruginosa* and *S. typhi* at 50, 25mg/ml respectively. In fungi, zone of inhibition recorded was ranged between 20 and 32mm. Maximum zone of inhibition was recorded as 32, 31 and 29mm against. *A. niger* at 100, 50 and 25mg/ml, 28mm against *C. albicans* at 100mg/ml, 27, 26mm against *A. flavus* at 100 and 50mg/ml, 25mm each against *C. albicans* at

50mg/ml and *A. flavus* at 25mg/ml, 24mm against *C. albicans* at 25mg/ml, 22, 21 and 20mm against *A. fumigatus* at 100, 50 and 25mg/ml respectively.

For cold extract, the zone of inhibition recorded was ranged between 13 and 23mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 23, 22 and 21mm against *B. subtilis* at 100, 50 and 25mg/ml, 20mm against *S. aureus* at 100mg/ml respectively. In gram-negative bacteria, the zone of inhibition recorded was ranged between 15 and 24mm. Maximum zone of inhibition was recorded as 24, 23 and 22mm each against *P. vulgaris* and *V. vulnificus* at 100, 50 and 25mg/ml concentration while it did not show any activity against *V. parahaemolyticus* and *S. typhi*. In fungi, zone of inhibition recorded was ranged between 14 and 20mm. Maximum zone of inhibition was recorded as 20mm against *C. albicans* at 100mg/ml concentration and it did not show any activity against *A. niger*.

For boiled extract, the zone of inhibition recorded was ranged between 17 and 23mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 23 and 22mm against *S. aureus* at 100 and 50mg/ml, 21mm against *B. subtilis* at 100mg/ml, 20mm each against *B. subtilis* at 50mg/ml and *S. aureus* at 25mg/ml respectively. In gram-negative bacteria, the zone of inhibition recorded was ranged between 16 and 24mm. Maximum zone of inhibition was recorded as 24, 23mm each against *P. vulgaris* and *V. vulnificus* at 100, 50mg/ml, 22mm each against *E. coli and V. parahaemolyticus* at 100mg/ml, 21mm each against *P. aeruginosa* at 100mg/ml, *E. coli* and *V. parahaemolyticus* at 50mg/ml, *P. vulgaris* and *V. vulnificus* at 25mg/ml, 20mm each against *P. aeruginosa* at 50mg/ml and *E. coli* at 25mg/ml respectively. In fungi, zone of inhibition recorded was ranged between 18 and 26mm. Maximum zone of inhibition was recorded as 26, 25 and 24mm against *A. flavus* at 100, 50 and 25mg/ml, 25, 24 and 20mm against. *C. albicans* at 100, 50 and 25mg/ml concentration while it did not shows any activity against *A. niger*.

For autoclaved extract, the zone of inhibition recorded was ranged between 20 and 25mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 25mm against *S. aureus* at 100mg/ml, 24mm against *B. subtilis* at 100mg/ml, 23mm against *S. aureus* at 50mg/ml, 22mm each against *B. subtilis* at 50mg/ml *and S. aureus* at 25mg/ml, 21,20mm against *S. pneumoniae* at 100, 50 and 25mg/ml respectively. In gram-negative bacteria, the zone of inhibition recorded was ranged between 16 and 27mm. Maximum zone of inhibition was recorded as 27, 26 and 25mm each against *P. aeruginosa* at 100, 50 and 25mg/ml, 24mm against *V. parahaemolyticus* at 100 mg/ml, 22 and 21mm against *E. coli* at 100, 50mg/ml, 20mm each against *S. typhi* at 100mg/ml and *V. parahaemolyticus* at 50mg/ml respectively. In fungi, zone of inhibition recorded was ranged between 14 and 26mm. Maximum zone of inhibition was recorded as 26, 25 and 24mm against. *C. albicans* at 100, 50 and 25mg/ml, 20mm against *A. flavus* at 100mg/ml concentration while it did not shows any activity against *A. niger*.

DISCUSSION

Medicinal plants remain an important source of new drugs, new drug leads and New Chemical Entities (NCEs)[15]. This indicates the chemical potential of the extracts to facilitate the process of chemical isolation[15, 16]. It has been reported that many medicinal plants are rich in varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids[17,18]. These secondary plant metabolites exert a wide range of biological activities on physiological systems[19]. In the present study, preliminary phytochemical screening showed the presence of flavonoids, phenolic groups, saponins, tannins and terpenoids in all the extracts. Flavonoids are reported to possess anti-oxidant, anti-proliferative, antitumor, anti-inflammatory, pro-apoptotic activities with molecular targets[20-27]. The health-promoting effects of flavonoids may relate to interactions with key enzymes, signaling cascades involving cytokines and transcription factors, or antioxidant systems[28]. Due to the presence of these many compounds the extracts possess the medicinal potential to develop novel therapeutic agents.

Regarding antimicrobial activity, petroleum ether, ethanol and aqueous extracts (cold, boiled and autoclaved) extracts showed maximum zone of inhibition against both gram-positive and gram-negative bacteria while chloroform extract showed maximum zone of inhibition against only gram-negative bacteria but no activity against gram-positive bacteria. This studies revealed that the medicinal and chemical potential of the extracts to develop new broad spectrum antibiotics against various diseases. McCutcheon [29] reported that most of the plant extracts showed activity against gram-positive than gram-negative bacteria. But in our present study, chloroform extract possess inhibitory activity against gram-negative than gram-positive bacteria. In support to his view, Garvey [30] reported that most of the plant extracts possess activity against gram-negative bacteria. This revealed the medicinal potential against gram-negative bacteria. In fungi, all the extracts showed inhibitory activity which revealed the antibiotic potential of these extracts to develop new leads. Thus all the extracts except chloroform extract possess antibiotic potential to develop broad spectrum antibiotic leads for the treatment of various diseases. Thus the significant inhibitory against the tested microorganisms may be due to the presence of various classes of phytochemicals such as flavonoids, phenolic groups, steroids saponins tannins and terpenoids as suggested by previous reports[31-34]. The significant activity of the results against the fungi, *A. flavus* and *Candida albicans* provides additional confirmation to the phenolic compounds and steroidal compounds which are more effective in higher concentration inhibited the growth of all fungi[35-36]. Even in hospitals, majority of disinfectants such as phenols, lysol, cresols used are belonging to phenolic groups. Thus recent findings of antimicrobial activity against *P. aeruginosa*, *P. vulgaris*, *S. typhi*, *V. parahaemolyticus* and *V. vulnificus* revealed the medicinal potential values. The solvent extracts such as petroleum ether and ethanol and all the aqueous extracts proved to possess the antiobiotica potential against various pathogens causes abdominal pain, diarrhea, fever, nausea, septicaemia, urinary tract infections and vomiting (*E. coli*), wound and septicaemia infections (*P. vulgaris* and *P. aeruginosa*), typhoid fever (*S. typhi*) and diarrheal infections (*Vibrio* species). Further, inhibition zone on against the growth of the fungal pathogen causes skin related diseases (*C. albicans*) and aspergillosis and respiratory tract infections (*A. flavus*) respectively. The phytochemical compounds which are responsible for the significant inhibitory activity against various human infections should be isolated and identified to develop a new lead of therapeutic interest.

CONCLUSION

From the present study, it is concluded that the selected ethnomedicinal plant *Hugonia mystax* L. has proved its medicinal potential against various human ailments. Furthermore, it is necessary to isolate the bioactive molecules responsible for the activities to develop novel leads of pharmaceutical interest.

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