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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY ON THE FRUITS OF *HUGONIA MYSTAX* L. (LINACEAE)

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ABSTRACT

An ethnomedicinal plant, *Hugonia mystax* L., was examined 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, ethanolic and aqueous fruits extracts showed significant activity against the human pathogens such as *Streptococcus pneumoniae* causing brain abscesses, pneumonia and septic arthritis; *Proteus vulgaris*, *Pseudomonas aeruginosa* causing urinary tract infections and septicaemia; *Salmonella typhi* causing typhoid fever, *Vibrio* species causing diarrheal infections and the fungus *Candida albicans* causes urinary tract infections. The antimicrobial activity of the petroleum ether, chloroform, ethanolic and aqueous fruits extracts showed concentration-dependent activity against all the tested bacteria at various concentrations. Thus, the present findings revealed the medicinal potential of *H. mystax* to develop a drug against various human ailments.

INTRODUCTION: Medicinal plants have always playing a vital role in the therapeutic armoury of mankind [1]. WHO estimated that 80% of world populations in developing countries are totally dependent on medicinal plants for their primary health care [2]. Over 25% of prescribed medicines in industrialised countries derive directly or indirectly from plants [3]. At least 25% of the prescription drugs issued in the USA and Canada contain bioactive compounds that are derived from or modeled from plant natural products [4]. It is estimated that only 5-15% of the approximately 250,000 described high plant species have ever been in the focus of phytochemical and pharmacological investigations [5]. Only 122 compounds were identified from 94 plant species based on ethnomedicinal usage [6]. Plants have been a prime source of highly effective conventional drugs for the treatment of various diseases [7].

The genus *Hugonia* L., of family Linaceae comprise about 40 species in the world; of which two species namely *Hugonia mystax* L., and *H. ferruginea* Wight & Arn., were reported from India [8, 9]. This plant *Hugonia mystax* L. locally known as Modirakanni. Ethnobotanically the fruits used by the tribals of Kalakad Mundanthurai for the treatment of rheumatism [10]. Review of literature revealed less work on this plant. Hence, in the present study, preliminary phytochemical screening and antimicrobial activity of various extracts of the fruits of *Hugonia mystax* were reported to provide a scientific evidence to prove the ethnobotanical usages.

MATERIALS AND METHODS:

Collection of Plant material: The fruits *Hugonia mystax* L. was collected on June 2010 from the Marakanam forest of Villupuram district, Tamil Nadu, India. The collected plant material was botanically identified and confirmed by using Flora of Tamil Nadu Vols. 1-3 [11] and ´An Excursion Flora of Central Tamil Nadu, India [12]. The species confirmation was done at French Institute Herbarium, Puducherry (FIHP). The herbarium specimen was prepared and deposited at the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, for future reference.

Preparation of the Extracts: The collected fruit 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 [13]. 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^{o}C. The resulted extracts were used for preliminary phytochemical screening and antimicrobial investigation.

The fresh fruits were collected and 30 g was weighed for aqueous extraction. Then, the fruits was chopped and divided into 3 portions. Each portion was crushed by grinding with the help of mortar and pestle, transferred in to a suitable glass bottle and added 50 mL of distilled water in each of it. First glass bottle was autoclaved at 10 lbs. for 20 min. The second was boiled (100^{o}C) for 20 min. The third was mechanically shaken (200 rpm) in cold condition for two hours. The extracts were filtered off using cheese cloth and 0.45µ filter papers, transferred into sterile closed containers. The crude extract was considered as 100% extract. By adding sterile distilled water, 50% and 25% of the extract was prepared [14].

Preliminary phytochemical screening: All the extracts were subjected to preliminary phytochemical tests followed by the standard methods [13, 15].

***In vitro* antimicrobial activity:** All the three extracts (petroleum ether, chloroform and ethanol extracts) were prepared in various concentrations such as 100, 50 and 25 mg/mL respectively and used for antimicrobial activity.

Test Microorganisms: The following bacterial strains and fungal strains were used for the study of antimicrobial activity. The microbial strains of human pathogens used were procured from IMTECH, Chandigarh which include six Gram-negative bacteria, viz. *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); three Gram-positive bacteria, viz. *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).

Determination of antimicrobial activity: Agar well-diffusion method [16] (Perez *et al*., 1990) 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 in triplicates were incubated at 37°C for 18-24 hour for bacterial pathogens and at 28°C for fungal pathogens. Respective proper controls of solvent plant extracts were also maintained. The experiment was repeated thrice. Diameter of the inhibition zones and the average values were recorded.

RESULTS: The results of preliminary phytochemical screening were given in **Table 1**. From the tested all three extracts revealed the presence of phenolic compound and terpenoids in all the three extracts. Carbohydrates and flavonoids present both in petroleum ether and ethanol extracts. Likewise tannins present both in chloroform and ethanol extract. Saponins and steroids present only in ethanol extract. Alkaloids, amino acids, anthraquinones, catechins, coumarins, gum, oil & resins, proteins and quinones were absent in all the three extracts.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS ON THE FRUITS OF *HUGONIA MYSTAX* L.

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

+ = present, **- =** absent

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

Petroleum ether extract, zone of inhibition was ranged between 13-18 against gram positive bacteria, 12-18 against gram negative bacteria, *P. vulgaris* and *V. vulnificus* did shows any activity against gram negative bacteria, 12-16 against fungi. For petroleum extract did not recorded as maximum zone of inhibition.

TABLE 2: ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS ON THE FRUITS OF *HUGONIA MYSTAX* L., AGAINST VARIOUS MICRO ORGANISMS.

Tested Microorganisms	Solvent extracts									Aqueous extracts									Standard drug
	Petroleum ether			Chloroform			Ethanol			Auto claved			Boiled			Cold			
	(mg/mL)			(mg/mL)			(mg/mL)			(%)			(%)			(%)			(µg/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	18	17	15	-	-	-	31	28	25	29	25	23	27	24	23	19	18	17	35(A)
S. aureus	18	15	13	-	-	-	26	24	22	20	19	18	25	24	22	22	21	19	38(A)
S. pneumoniae	17	16	14	-	-	-	29	28	25	28	25	21	25	23	22	20	19	18	33(C)
Gram-negative bacteria																			
E. coli	15	14	13	-	-	-	26	25	23	25	24	21	23	22	21	21	20	19	33(A)
P. aeruginosa	16	14	13	-	-	-	29	28	27	26	24	23	26	24	23	25	24	23	32(A)
P. vulgaris	-	-	-	-	-	-	33	32	31	25	24	20	20	19	18	23	22	21	38(Cl)
S. typhi	17	15	12	-	-	-	28	27	25	21	20	19	24	21	20	21	20	20	37(Cf)
V. parahaemolyticus	18	16	14	-	-	-	31	30	29	23	21	20	25	24	23	17	16	15	37(K)
V. vulnificus	-	-	-	-	-	-	28	27	26	18	17	16	22	21	20	19	18	17	34(K)
Fungi																			
A. flavus	16	15	13	20	18	17	29	28	27	22	21	20	20	15	14	25	24	23	32(P)
A. fumigatus	14	13	13	-	-	-	30	29	28	25	24	23	18	17	15	24	23	21	33(P)
A. niger	15	13	12	-	-	-	25	23	20	17	16	15	17	15	14	21	18	16	32(P)
C. albicans	16	13	12	-	-	-	32	30	29	20	18	17	20	18	17	24	21	18	32(P)

(Measurement indicates the zone of inhibition). A – Ampicillin; Cl – Clotrimazole; Cf – Ciprofloxacin; K – Kanamycin; P - Penicillin

Chloroform extract did not shows any activity against tested microorganisms except *A. flavus* the maximum zone of inhibition was recorded as 20mm against 100mg/mL concentration.

For ethanol extract, the zone of inhibition recorded ranged between 22 - 31mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 31mm against *B. subtilis* at 100mg/mL; 29mm against *S. pneumoniae* at 100mg/mL; 28mm each against *B. subtilis* & *S. pneumoniae* at 50mg/mL; 26mm against *S. aureus* at 100mg/mL; 25mm each against *B. subtilis* & *S. pneumoniae* at 25mg/mL; 24, 22mm against *S. aureus* at 100, 50mg/mL concentration respectively.

In gram-negative bacteria, the zone of inhibition recorded ranged between 23-33mm. Maximum zone of inhibition was recorded as 33&32mm against *P. vulgaris* at 100&50mg/mL; 31mm each against *V. parahaemolyticus* at 100 mg/mL & *P. vulgaris* at 25mg/mL; 30 mm against *V. parahaemolyticus* at 50mg/mL; 29mm each against *P. aeruginosa* at 100mg/mL, *V. parahaemolyticus* at 25mg/mL; 28mm each against *S. typhi & V. vulnificus* at 100mg/mL, *P. aeruginosa* at 50mg/mL; 27mm each against *S. typhi* & *V. vulnificus* at 100mg/mL, *P. aeruginosa* at 25mg/mL; 26mm each against *E. coli* at 100mg/ml, *V. vulnificus* at 25mg/mL; 25mm each against *E. coli* at 50mg/mL, *S. typhi* at 25 mg/mL; 23mm against *E. coli* at 25mg/mL

concentration. In fungi, zone of inhibition recorded ranged between 20 and 32mm. Maximum zone of inhibition was recorded as 32mm against *C. albicans* at 100mg/mL, 30mm against *A. fumigatus* at 100mg/mL, *C. albicans* at 50mg/mL; 29mm each against *A. flavus* at 100mg/mL, *A. fumigatus* at 50mg/mL, *C. albicans* at 25mg/mL; 28mm each against *A. flavus* at 50mg/mL, *A. fumigatus* at 25mg/mL; 27mm against *A. flavus* at 25mg/mL; 25, 23 & 20mm against *A. niger* at 100, 50 & 25mg/mL concentration respectively.

Autoclaved extract, the zone of inhibition recorded ranged from 18-29mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 29 mm against *B. subtilis* at 100%; 28mm against *S. pneumoniae* at 100%; 25mm each against *B. subtilis* and *S. pneumoniae* at 50%; 23mm against *B. subtilis* at 25%; 21mm against *S. pneumoniae* at 25% and 20mm against *S. aureus* at 100%. In gram-negative bacteria, the zone of inhibition recorded ranged between 16-26mm. Maximum zone of inhibition was recorded as 26mm against *P. aeruginosa* at 100%; 25mm each against *E. coli* and *P. vulgaris* at 100%; 24mm each against *E. coli*, *P. aeruginosa* and *P. vulgaris* at 50%, 23mm each against *V. parahaemolyticus* at 100%, *P. aeruginosa* at 25%, 21mm each against *S. typhi* at 100%, *V. parahaemolyticus* at 50% and *E. coli* at 25% respectively, 20mm each against *S. typhi* at 50%, *P. vulgaris* and *V. parahaemolyticus* at 25% concentrations respectively. In fungi, zone of inhibition recorded was ranged from 15-25mm. Maximum zone of inhibition was recorded as 25, 24 & 23mm against *A. fumigatus* at 100, 50 & 25%; 22, 21mm against *A. flavus* at 100, 50%; 20mm each against *C. albicans* at 100% and *A. flavus* at 25% concentration respectively.

Boiled extract, the zone of inhibition recorded ranged 22-27mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 27mm against *B. subtilis* at 100%; 25mm each against *S. aureus* and *S. pneumoniae* at 100%; 24mm each against *B. subtilis and S. aureus* at 50%; 23mm each against *S. pneumonia*e at 50%, *B. subtilis* at 25%; 22mm each against *S. aureus* and *S. pneumoniae* at 25% concentration respectively. In gram-negative bacteria, the zone of inhibition recorded ranged from 18-26mm. Maximum zone of inhibition was recorded as 26mm against *P. aeruginosa* 100%; 25mm against *V. parahaemolyticus* at 100%, 24mm each against *S. typhi* at 100%, *P. aeruginosa* and *V. parahaemolyticus* at 50%, 23mm each against *E. coli* at 100%, *P. aeruginosa* and *V. parahaemolyticus* at 100%; 22mm each against *V. vulnificus* at 100%, *E. coli* at 50%; 21mm *S. typhi* and *V. vulnificus* at 50%, *E. coli* at 25%; 20mm each against *P. vulgaris* at 100%, *S. typhi* and *V. vulnificus* at 25%; In fungi, zone of inhibition recorded ranged from 14 - 20mm. Maximum zone of inhibition was recorded as 20mm against *A. flavus* and *C. albicans* at 100% concentration respectively.

Cold extract, the zone of inhibition recorded ranged 17-22mm against gram-positive bacteria. Maximum zone of inhibition was recorded as 22&21mm against *S. aureus* at 100&50%; 20mm against *S. pneumoniae* at 100%. In gram-negative bacteria, the zone of inhibition recorded ranged from 15-25mm. Maximum zone of inhibition was recorded as 25&24mm against *P. aeruginosa* at 100&50%; 23mm each against *P. vulgaris* at 100% and *P. aeruginosa* at 25%; 22mm against *P. vulgaris* at 50%; 21mm each against *E. coli* and *S. typhi* at 100%; *P. vulgaris* at 25%; 20mm each against *E. coli* at 50%; *S. typhi* at 50, 25% respectively. In fungi, zone of inhibition recorded ranged from 16 - 25mm. Maximum zone of inhibition was recorded as 25mm against *A. flavus* at 100%; 24mm each against *A. fumigatus* and *C. albicans* at 100%, *A. flavus* at 50%; 23mm each against *A. fumigatus* at 50%, *A. flavus* at 25%; 21mm each against *A. niger* at 100%, *C. albicans* at 50%, *A. fumigatus* at 25% concentration respectively.

DISCUSSION: Phytochemical screening of the petroleum ether, chloroform and ethanol extracts of fruits of *H. mystax* showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. The presence of secondary metabolites in plants produces some biological activity and the major source of pharmaceuticals, food additives, fragrances and pesticides [17-20]. A number of plant secondary metabolites have been found to be valuable pharmacological probes or biochemical tools to help target various receptors as well as to explain cellular processes and assist with the elucidation of different kinds of molecular targets [21].

From the results of antimicrobial activity, it was found that the petroleum ether and ethanol extracts exhibited maximum antimicrobial activity against the tested human pathogens.

In our study, the maximum zone of inhibition against gram negative bacteria such as *E. coli, P. vulgaris, P. aeruginosa, S. typhi and V. parahaemolyticus* and against the fungi such as *A. flavus* and *C. albicans* may be due to the presence of various classes of phytochemicals such as flavonoids, phenolic groups and steroids as suggested by previous reports [22-24]. 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 [25, 26]. 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. Evidently, the petroleum ether, ethanol and all the aqueous extracts proved to possess the antibiotic 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.

CONCLUSION: The phytochemical compounds which are responsible for the significant inhibitory activity against various human infections should be isolated, purified and identified to develop a new lead of therapeutic interest to cure various human ailments.

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REFERENCES:

1. Hostettmann K and Marston A: Twenty years of research into medicinal Plants. Results and perspectives, Phytochemistry Reviews 2002; 1: 275–285.
2. WHO: General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. World Health Organization, Geneva, 2001.
3. Newman DJ, Cragg GM and Snader KM: The influence of natural products upon drug discovery. Natural Product Reports 2000; 17 (3): 215- 234.
4. Farnsworth NR: The role of medicinal plants in drug development. In: Krogsgaard-Larsen S, Brogger-Christense, S, Kofod H.(eds.) Natural products and drug development. Balliere, Tindall and Cox, London, 1984: 8-98.
5. Rollinger JM, Stuppner H and Langer T: Virtual screening for the discovery of bioactive natural products. In: Petersen, F and Amstutz R. (eds.) Progress in Drug Research, Birkhauser Ver lag, Basel, Switzer land, Vol. 65, 2008: 213-248.
6. Fabricant DS and Farnsworth NR: The Value of Plants Used in Traditional Medicine for Drug Discovery. Environmental Health Perspectives 2001; 109(1): 69-74.
7. Verpoorte R, Kim HK and Choi YH: Plants as source of medicines. New perspectives. In: Bogers RJ, Craker LE and Lange D. (eds.), Medicinal and Aromatic Plants. Springer, Netherland, 2006: 261-273.
8. Santapau H and Henry AN: A Dictionary of flowering plants in India. Council of Scientific and Industrial Research, New Delhi, 1983:103.
9. Pullaiah T and Chennaiah E: Flora of Andhra Pradesh. Scientific Publishers, Jodhpur, India, 1997.
10. Sutha S, Mohan VR, Kumaresan S, Murugan C and Athiperumalsami T: Ethanomedicinal plants used by the tribals of Kalakad Mundanthurai Tiger Reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism, Indian Journal of Traditional Knowledge 2009; 9(3): 502-509.
11. Nair NC and Henry AN: Flora of Tamil Nadu India. Botanical Survey of India, Southern Circle, Coimbatore, Vol.1, 1983.
12. Matthew KM: An Excursion Flora of Central Tamil Nadu, India. Oxford & I.B.H Publishing Co. Pvt. Ltd., New Delhi, India, 1991.
13. Evans WC: Trease and Evans Pharmacognosy. Reed Elsevier India Pvt. Ltd., New Delhi, Fifteenth Edition 2006.
14. Sen S and Nandi P: Antibiotics from the Pteridophytes. Science and culture 1951; 16(7): 328 - 329.
15. Harborne JB: A guide to Modern techniques of plant Analysis. Kluwer Academic Publishers, U.S.A., Third Edition 1998.
16. Perez C, Paul M and Bazerque P: Antibiotic assay by agar-well diffusion method, Acta Biologiae et Medicine Experimentalist 1990; 15: 113-115.
17. Patwardhan B, Vaidhya ADB and Chorghade M: Ayurveda and Natural Product Drug discovery. Current Sciences. 2004; 86:789
18. Pal A: Production of insecticides. In: Ramawat KG, Merillon JM (eds.) Biotechnology: Secondary metabolites, Science publishers, Infield, U.S.A., Edition 2, 2007: 131-149.
19. Ramawat KG: Secondary metabolites in nature. In: Ramawat KG, Merillon JM (eds.) Biotechnology: Secondary Metabolites. Science Publishers, Enfield, CT 2007: 21

20. Ravishankar GA, Bhagyalakshmi N and Rao RS: Production of food additives. In: Ramawat KG, Merillon JM (eds.) Biotechnology: Secondary Metabolites: Plants and Microbes, Science Publishers, Enfield, CT, Edition 2, 2007: 103
21. Pan L, Blanco CDEJ and Kinghorn DA: Plant-Derived Natural Products as Leads for Drug Discovery In: A.E. Osbourn and V. Lanzotti (eds.), Plant-derived Natural Products Springer Science, Business Media, LLC, 2009.
22. Taleb-Contini SH, Salvador MJ, Watanabe EE, Ito IY and Oliveira DCR: Antimicrobial activity of Flavonoids and steroids isolated from two Chromolaena species. Brazilian Journal of Pharmaceutical Sciences 2003; 39(4): 403-408.
23. Bylka W, Matlawska I and Pilewski NA: Natural Flavonoids as Antimicrobial Agents. Jana 2004; 7(2):24-31.
24. Özçelik B, Orhan D, Özgen S and Ergun F. Antimicrobial Activity of Flavonoids against Extended-Spectrum-Lactamase (ESβL)-Producing *Klebsiella pneumonia*. Tropical Journal of Pharmaceutical Research 2008; 7 (4): 1151-1157.
25. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews 1999; 12(4): 564–582.
26. Subhisha S and Subramoniam A. Antifungal activities of a steroid from *Pallavicinia lyelii, a* liver wort. Indian Journal of Pharmacology 2005; 37(5): 304-308.

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