

Phytochemical analysis and antibacterial activity of the *Hugonia mystax* aerial parts

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Abstract

Background: The microorganisms, in particular, bacterial strains have been getting resistance to antibiotics due to indiscriminate use of them against different diseases. Hence, there is a need to isolate new antibiotics from natural sources which have less cost, more efficacy, and less side effects. **Aim:** *Hugonia mystax* has been using in a traditional medicine. On the basis of the ethno medical, folkloric information the author selected the *H. mystax* aerial parts to study its phytochemical constituents and capacity of its antibacterial activity to provide the scientific evidence to its traditional use. **Materials and Methods:** Phytochemical analysis was performed using standard test procedures and antibacterial activity was tested using cup plate method. **Results:** Qualitative phytochemical screening of *H. mystax* extracts exposed the presence of different phytochemical constituents in it like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins, saponins, and carbohydrates. The extracts gave negative results for the amino acids and oils. The selected plant extracts showed the antibacterial activity along with the standard drug rifampicin. Ethyl acetate extract showed more activity on tested microorganisms compared to hydroalcoholic and hexane extracts. Three extracts showed more antibacterial activity on Gram-negative bacteria than Gram-positive bacteria. **Conclusion:** The further research is need to evaluate more pharmacological activities and in isolation of bioactive compounds.

Key words: Aerial parts, *Hugonia mystax*, phytochemical analysis and antibacterial activity

INTRODUCTION

The infectious microorganisms, in particular, bacterial strains have been getting resistance to antibiotics due to indiscriminate use of them against different diseases. The present using antibiotics are synthetic drugs, expensive and inadequate for the treatment of diseases, and also these are causing different side effects to people.^[1-4] Therefore, there is need to search new infection-fighting drugs to control microbial infections^[5] with low cost and less side effects. A major part of the world population mainly in the developing countries still uses traditional, folk medicine^[6,7] to avoid synthetic drugs treatments.

The use of medicinal plants as a source for relief from different diseases was used in ethnomedicine around the world as old as mankind.^[8,9] The potential use of medicinal plants as a source for new drugs is still largely unexplored, and only a small percentage has been investigated to know

their phytochemical components and pharmacological activities.^[10] Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs, and they possess varied medicinal properties.^[11-14] Considering the vast potentiality of plants as sources for antimicrobial drugs, a systematic investigation was undertaken to screen the phytochemical analysis and antibacterial activity of *Hugonia mystax* aerial parts which has been using in traditional medicine, i.e., useful in fever, verminosis and vitiated conditions of vata, externally as a past for inflammations and used swelling due to viper bite.^[15-18]

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MATERIALS AND METHODS

Chemicals and Drugs

All chemicals and solvents were analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic., Mumbai.

Collection of Plant Material and Preparation of Extracts

The plant material was collected near Kailasagiri, Visakhapatnam at Andhra Pradesh, India, during the month July 2012. The authentication of the plant was done by Rtd. Prof. M. Venkaih, Department of Botany, Andhra University, Visakhapatnam. The plant material was shade dried and powdered. The powder was used separately extracted using maceration process with hexane, ethyl acetate and hydroalcoholic (ethanol (70%v/v)) were concentrated to dryness under vacuum using rotavapor.

Phytochemical Analysis^[19-21]

Phytochemical studies were carried out for hexane, ethyl acetate and hydroalcoholic extracts of *H. mystax* aerial parts to detect the presence of different phytochemical constituents such as steroids, terpenoides, tannins, flavanoids, saponins, glycosides, amino acids, etc. by using standard procedures.

Quantification of Phenolic and Alkaloidal Content^[21-24]

Total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent. Folin-Ciocalteu colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765 nm. The intensity of the light absorption at that wavelength is proportional to the concentration of phenols. By using standard gallic acid calibration curve, measure the concentration of phenolic content in gallic acid total equivalents using unit's mg/g.

Total alkaloidal content

The plant extract (1 mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 ml of this solution was transferred to a separating funnel, and then 5 ml of BCG (Bromocresol Green) solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in

chloroform was measured at 470 nm. All the experiments were performed thrice, and the results were averaged and reported in the form of mean \pm standard error of mean.

Determination of Zone of Inhibition by Cup-Plate Method^[25,26]

The cup-plate method is based on measuring the diameter of zone of inhibition of microbial growth surrounding the cups containing various dilutions of extracts. The nutrient agar media was inoculated (1 ml culture) with the test organism at a temperature of 45°C and was poured into sterile petri-plates by pour plate method. After solidification, a sterile metal borer was used to prepare the wells of 4 mm diameter in the agar plates. Then, the test samples (100 μ l) and the standards (50 μ l) were added into the wells using a micropipette. All the nutrient agar plates were incubated at 28°C for 48-72 h. The presence of definite zone of inhibition of any size around the well indicates antimicrobial activity. The solvent controls were tested simultaneously to assess the activity of dimethyl sulfoxide which was used as a vehicle. The experiment was performed thrice. The diameter of the zones was measured and recorded.

RESULTS

Qualitative Phytochemical Screening of *H. mystax*

Qualitative phytochemical screening of *H. mystax* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins, saponins, and carbohydrates. The extracts gave negative results for the amino acids and oils.

All extracts revealed the presence of steroids, terpenoids, phenols, alkaloids, carbohydrates and glycosides and gave negative results to amino acids and oils. The hydroalcoholic and ethyl acetate extracts revealed the presence of saponins, flavonoids and tannins but the hexane extract gave negative results. The results were given in Table 1.

The quantified phenolic contents of *H. mystax* extracts were ranging from 12.82 ± 2.62 to 36.42 ± 1.46 (mg/g). The hydroalcoholic extract has more phenolic content, i.e., 36.42 ± 1.46 (mg/g) than other extracts. The quantified alkaloid content was ranging from 11.44 ± 3.28 to 30.02 ± 2.12 (mg/g). The hydroalcoholic extract has more alkaloid content, i.e., 30.02 ± 2.12 (mg/g) than other extracts. The results were given in Table 2.

Antibacterial Activity of *H. mystax*

The antibacterial activity of the different extracts of *H. mystax* was observed using cup-plate method at a different concentration, i.e., 100, 200, 400 and 800 μ g/100 μ l by

Table 1: Nature of phytoconstituents in different extracts of *H. mystax*

Phytochemical constituents	<i>H. mystax</i>		
	Hexane extract	Ethyl acetate extract	Hydro alcoholic extract
Phytosterols	+	+	++
Terpenoids	+	++	+
Glycosides	+	+	++
Saponins	-	+	+
Flavonoids	-	+	+
Tannins	-	+	+
Carbohydrates	+	+	++
Alkaloids	+	+	++
Amino acids	-	-	-
Oils	-	-	-
Phenols	+	+	++

+, ++: Present, -: Absent, *H. mystax*: *Hugonia mystax***Table 2:** Total phenolic and alkaloid contents (mg/g) of *H. mystax* extracts

Name of the extract	Total phenolic content (mg/g)	Total alkaloid content (mg/g)
Hexane	12.82±2.62	11.44±3.28
Ethyl acetate	24.68±1.22	22.38±1.66
Hydroalcoholic	36.42±1.46	30.02±2.12

H. mystax: *Hugonia mystax*

measuring the diameter of zone of growth inhibition against different microorganisms using rifampicin as standard.

The different extracts of *H. mystax* inhibit the growth of the tested microorganisms. Three extracts at lower concentration (100 µg/100 µl) do not show the effect on maximum tested bacteria but as the concentration of extracts increases the effectiveness of the action on microorganisms increased [Table 3] respectively.

Hexane extract does not show any zone of inhibition on *Staphylococcus epidermidis* and *Klebsiella pneumoniae* at 200 µg/100 µl and showed more zone of inhibition on *Salmonella typhi* i.e. 14mm at 800 µg/100 µl. Ethyl extract showed the zone of inhibition on almost every tested concentration except on *Bacillus megaterium* at 100 µg/100 µl and showed more zone of inhibition on *K. pneumoniae*, i.e., 17 mm. Hydro-alcoholic extract does not show any zone of inhibition on *S. typhi* and *K. pneumoniae* at 100 and 200 µg/100 µl and showed more zone of inhibition on *Lactobacillus acidophilus*, i.e., 15 mm.

Ethyl acetate extract showed more activity on tested microorganisms compared to hydro-alcoholic and hexane extracts. Three extracts showed more antibacterial activity on Gram-negative bacteria compared to Gram-positive bacteria.

DISCUSSION

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens and

Table 3: Antibacterial activity of *H. mystax* extracts

Name of the extract	Dose (µg/cup)	Zone of inhibition* (Diameter in mm)					
		Gram-positive			Gram-negative		
		<i>S.e</i>	<i>B.m</i>	<i>L.a</i>	<i>E.c</i>	<i>S.t</i>	<i>K.p</i>
Hexane extract	100	-	7	-	-	7	-
	200	-	8	7	8	9	-
	400	7	10	9	10	11	7
	800	9	12	11	12	14	9
Ethyl acetate extract	100	8	-	7	7	8	8
	200	10	8	9	8	10	11
	400	12	10	12	11	13	14
	800	15	13	16	15	13	17
Hydro-alcoholic extract	100	7	7	-	7	7	-
	200	9	8	8	8	8	-
	400	12	10	11	10	9	8
	800	14	13	15	13	11	10
Rifampicin	50	24	26	22	20	22	24
DMSO		-	-	-	-	-	-

*Values includes the cup diameter (4 mm). *S.e*: *Staphylococcus epidermidis*, *B.m*: *Bacillus megaterium*, *L.a*: *Lactobacillus acidophilus*, *E.c*: *Escherichia coli*, *S.t*: *Salmonella typhi*, *K.p*: *Klebsiella pneumonia*, -: No activity, DMSO: Dimethyl sulfoxide

therefore considered as a potential source for different classes of antimicrobial substances.^[27-31] The development of drug resistance in human pathogens against commonly used antibiotics is resulting from the excessive and inappropriate use of antimicrobial agents (antibiotics), which, in turn, lead to potentially serious public health problem and it has necessitated a search for new antimicrobial substances from natural sources.

Plants are used in traditional medicine; they contain a wide range of substances those can be useful in the treatment of chronic as well as acute infectious diseases caused by different microorganisms. The substances that can either inhibit the growth of micro-organisms or kill them are considered as bioactive molecules for developing new drugs for the treatment of various infectious diseases.^[32]

Qualitative phytochemical screening of *H. mystax* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins, saponins, and carbohydrates. The plant extracts have the more phenolic and alkaloid compounds.

The medicinal properties of plants are due to the on its own or combinations of different secondary metabolites. Different plants parts would have different secondary metabolites that would often be taxonomically distinct in individual plants resulting in unique medicinal properties.^[33,34] Secondary metabolites are generally produced for defense against predators, pathogens or competitors or for protection and are responsible for most of the biological activities.

Many scientists reported that different phytochemical compounds (Secondary metabolites) having antimicrobial activity like phenols, alkaloids, steroids, terpenoids, saponins, flavonoids, tannins and glycosides.^[35-37] Among the tested extracts of *H. mystax*, ethyl acetate extract showed more inhibition activity on tested microorganisms compared to hydroalcoholic and hexane extracts but the hydroalcoholic extract have the more phenolic and alkaloid contents. Based on the obtained results, the more active bio-molecules may be present in the ethyl acetate extract. Standardization of selected plant extracts can be carried out by obtaining a chemical fingerprint/profile or through chromatographic techniques are more commonly used for standardization in terms of one or more marker compounds. Use of isolated compounds can be used as drugs for treat more diseases using its higher concentrations. Hence, further research is need to evaluate the *H. mystax* for its more pharmacological activities and isolation of the bioactive compounds.

CONCLUSION

In this research, based on folklore reports plant, i.e., *H. mysatx* was selected to identify for its phytochemical constituents and antibacterial activity with different extracts. In the phytochemical analysis, different extracts showed

positive and negative results for different phytochemicals present in them. Then, the same extracts are tested for antibacterial activity at different concentration on selected bacterial strains those are pathogenic. The tested extracts showed different zone of inhibition on tested strains. The effectiveness of the extracts increasing on strains as their concentration increases. Based on the obtained results, it may conclude that the ethyl acetate extract of *H. mystax* has the more antibacterial potential bioactive molecules compared to other extracts.

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