

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF A MEDICINAL PLANT HILDEGARDIA POPULIOLIA

Ch.Subba Lakshmi^{1*} and T.Pullaiah²

Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh.

ABSTRACT: An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. *Staphylococcus aureus* is the most common cause of staph infections such as pneumonia, meningitis, osteomyelitis endocarditis, Toxic shock syndrome (TSS), and septicemia. Antimicrobials include not just antibiotics, but synthetically formed compounds as well. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found, thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means. Hence an attempt was made to identify some bioactive compounds which are having antibacterial properties from natural resources. Hildegardia Populiolia which is a medicinal plant in our usage was used and compounds were separated through column chromatography and screened these compounds on several pathogenic bacteria. The inhibition of *Staphylococcus aureus* was done by using Hildegardia Populiolia after isolation. Maximum inhibitory regions were observed with the treatment of Hildegardia Populiolia on Pathogenic bacteria. We have measured the content in control, Carrier control and experimental groups and found reduction in experimental groups.

Key words: anti microbial activity, column chromatography, staphylococcus aureus, Hildegardia Populiolia

INTRODUCTION

Hildegardia populifolia is a species of flowering plant in the Sterculiaceae family, found only in the Eastern Ghats of Andhra Pradesh and Tamil Nadu in India. It is a smooth-barked deciduous tree. The leaves are long petiolate, large, orbicular, palmately 7-nerved, deeply cordate at the base, acuminate at the apex, and with entire margins. Individual plants are polygamous, but the sources available to me do indicate whether they are andro-, gyno or trimonoecious. The scarlet flowers are borne in April and they form axillary and terminal panicles, which are shorter than the leaves. The flower buds are oblong, the calyx is small, scarlet, with the sepals linear-spathulate and divided nearly to the base. It is downy externally. There are 10 stamens. The hispid ovary is ovoid, tapering to a short style, with a 5-lobed stigma. The fruit is apocarpous, composed of 5 follicles. These follicles are up to 10 cm long by 5 cm broad, obliquely lanceolate in outline, membranous, inflated, and strongly veined, with 2 seeds. The seeds are ovate-oblong, pale brown, and 15-20 mm long. [1]. The stems of Hildegardia populifolia have been used as a source of fibre, and the bark as a herbal medicine. Papers have been published on the fibres and their use as component in fibre-resin and fibre-plastic composite materials [2, 3, 4, 5, 6, 7, 8].

MATERIAL AND METHODS

Collection of Plant Materials

The fresh and healthy leaves of the plants were collected. The plant specimens were identified in department of Botany Sri Krishnadevaraya University, Anantapur. Plant parts were collected on the basis of the information provided in the ethnobotanical survey of India. Each specimen/plant material was labeled, numbered, and noted with the date of collection, locality, and their medicinal uses were recorded.

Preparation of Plant Extract

The extraction of the plant leaves was carried out using known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with ethyl alcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The ethyl alcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2 to 4°C. This crude extracts of ethylalcohol was used for further investigation for potential of antimicrobial properties.

Isolation of Compounds:

Chromatography is a process by which individual components of a mixture can be separated. Chromatography has a mobile phase (liquid) and stationary phase (solid). The mixture components interact differentially with the mobile and stationary phases to effect the separation of components.

Procedure

Place weighed sample into the mortar and add enough equilibration buffer to cover the beet. Grind with Pestle as much as possible getting the smallest pieces of plant you can. Place ground liquefied plant material in a 2 mL centrifuge tube. Centrifuge at 500-1000 rpm for 5 minutes to form pellet of plant solids plus liquid on top (supernatant). Transfer the supernatant (the top liquid, solid plant material should be stuck at bottom) into a fresh 2 mL tube. Discard tube with pellet. Set up a series of clean empty micro centrifuge tubes in a rack and number them in the order they will be used. Position the column on a stand above the test tube rack. Close the column stopcock; add 4ml of equilibrium buffer. Open the stopcock as you add ~ 2 mL of your Phenyl Sepharose or Sand slurry or Silica powder, depending on your assignment. The bed height should be about 3-4 cm high. DO NOT let your slurry/sand run dry. If you run out of Equilibration Buffer, just add more to keep slurry/sand wet/silica powder. Continue to run equilibration buffer through the column until the solid material is completely level and settled down into compact bed. Once the meniscus of the equilibration buffer has reached the top of the bed, close the stopcock and add 500 mL of plant extract/sample/material. Drizzle this extract gently and slowly down the side of the column so that you do not disturb the bed. Open stopcock and allow the sample to flow into the bed. When the top meniscus of the sample touches the top of the bed, close the stopcock. The compounds bind to column in presence of equilibrium buffer.

Test Microorganisms

The following microorganisms *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 424) and fungal strain *Aspergillus niger* (MTCC 282) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium, respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the fungi were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Antimicrobial Activity

Determination of zone of inhibition method

Preparation of Discs:

Whatman No: 1 filter paper discs of 6mm diameter are prepared and autoclaved by keeping in a clean and dry Petri plate. The filter paper discs were soaked in plant extracts for 6 hours are taken as test material. After 6 hours the discs were shade dried. The concentrations of plant extracts per disc are accounted for 0.1 grams/1ml. Subsequently they are carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, benzene, distilled water are prepared and used as control.

Preparation of media:

Medium for bacterial cultures:

The medium was steamed for 30 min neutralized at 37°C and steamed for half an hour and filtered. The medium was sterilized at 15 lbs for 20 min at 121°C.

Testing of antimicrobial activity:

To test the antimicrobial activity on agar plates, LB agar medium was prepared using the ingredients mentioned above. The medium was sterilized at 121°C for 30 min's. The agar test plates were prepared by pouring about 15ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inoculum (containing suspension) of *P.aeruginosa* and *Sta.aureus* was poured to the respective plates separately containing solidified agar media. Six replicates were maintained. The prepared sterile whatman no :1 filter paper discs of 6mm diameter were impregnated with the extracts and shaken thoroughly and this test plates incubated for a period of 48 hrs in BOD at 37°C for the development of inhibitory zones and the average of 2 independent readings for each organism in different extracts were recorded. The control Petri plates and also maintained above respective cultures

Measuring the diameter of inhibition zone:

The inhibition zones were lead after 1 day at 37°C for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. 7 paper discs placed in 1 Petri plate.

Test Microorganisms and Growth Media

Antimicrobial activity of *Hildegardia populifolia* leaf extract

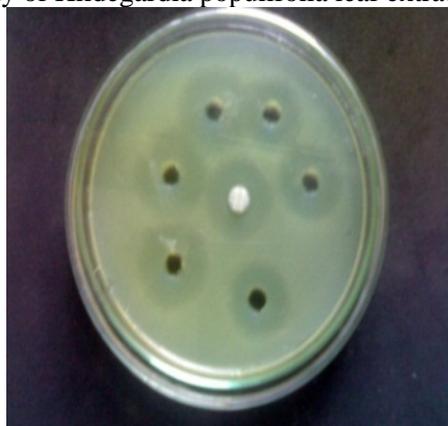


Fig 1: *Pseudomonas aeruginosa*

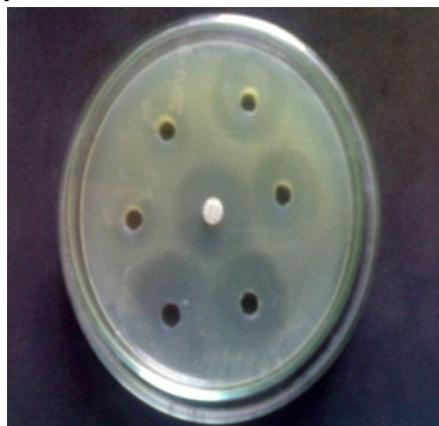


Fig 2: *Staphylococcus aureus*



Fig 3: *Aspergillus niger*

Table 1: Well plate and disc diffusion method of *Hildegardia populifolia*

Plants	Zone of inhibition (MIC)		
	<i>Pseudomonas aeruginosa</i> (-ve)	<i>Staphylococcus aureus</i> (+ve)	<i>Aspergillus niger</i>
<i>Hildegardia populifolia</i>	2.4	1.6	1.7

Table 2: Phytochemical Screening of Hildegardia populifolia leaves in different Extracts

S.No	Secondary metabolites	Hexane	Ethyl acetate	Ethanollic	Aqueous
1	Steroids	+	+	+	+
2	Triterpenes	-	+	+	-
3	Saponins	+	-	-	+
4	Tri terpinoidal saponins	-	-	-	-
5	Alkaloids	+	+	+	+
6	Carbohydrates	+	+	+	+
7	Flavonoids	+	+	+	+
8	Tannins	+	+	+	+
9	Glycosides	+	+	+	+
10	Polyphenols	+	+	+	+

CONCLUSION

In the present study it was found that Hildegardia Populiolia a plant has an excellent antimicrobial activity. The pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and fungus *Aspergillus niger* were inhibited in presence of the extracts of Hildegardia Populiolia from ethanolic extract. Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria.

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