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Research article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF A MEDICINAL PLANT STERCULIA VILLOSA

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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. Sterculia villosa which is a medicinal plant in our usage was used and compoundswas separated through column chromatography and screened these compounds on several pathogenic bacteria. The inhibition of *Staphylococcus aureus* was done by using Sterculia villosa after isolation. Maximum inhibitory regions were observed with the treatment of Sterculia villosa on Pathogenic bacteria. We have measured the content in control, Carrier control and experiemental groups and found reduction in experiemental groups.
Key words: Anti microbial activity, Column chromatography, *staphylococcus aureus, Sterculia villosa*.

INTRODUCTION

Infectious disease is one of main causes of death accounting for approximately one-half of all deaths in tropical countries [1]. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States [2, 3]. Besides, an increase in antibiotic resistance in nosocomial and community acquired infections has been found common now-a-days with the most dramatic increases are occurring at the age of 25–44 year [4]. Several research programs also showed results of drug resistance to human pathogenic bacteria from all over the world. Due to these facts, in recent times extensive attention has been made on finding alternative antimicrobials from natural source. It is scientifically proved that free radicals induce oxidative damage to biomolecules [5]. This damage causes cancer, aging, neurodegenerative diseases, atherosclerosis, malaria and several other pathological events in living organisms. Antioxidants which scavenge free radicals are known to possess an important role in preventing these free radical induced-diseases [6]. There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and diseases [7-9]. A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade [10]. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids. Synthetic antioxidant such as tert-butyl-1- hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), propylgallate (PG) and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase shelf life, especially lipid and lipid containing products by retarding the process of lipid peroxidation [11]. However, BHT and BHA are known to have not only toxic and carcinogenic effects on humans, but abnormal effects on enzyme system. Therefore, the interest in finding antioxidant from natural source with minimized side effects has greatly increased in recent years [12-15]. Therefore, there is a need for more effective, less toxic and cost effective compounds from natural sources [16]. Several medicinal plants with ethno-botanical uses have been used traditionally in the treatment of diseases and have been exploited for different desired traits.

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 Krishna Devaraya 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, a noted with the date of collection, locality, and their medicinal uses were recorded.

Preparation of Plant Extract

Extraction

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 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.

Introduction to Column Chromatography

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.

Materials

Plant material or fresh samples

Mortar and Pestle

1.5 mL clear centrifuge tubes for collection, 2 mL tubes for extraction

Column with stopcock – approx 0.7 cm diameter,10 cm length.

Ring stand and clamp for holding column.

Slurry: You will use Phenyl Sepharose or fine grade play Sand or Silica powder. (Stationary phase)

Equilibration Buffer (potassium Phosphate, EDTA, ammonium sulfate, pH 6.5)

Procedure

Weigh 1g of plant sample. 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. Discard outflow. 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. Discard outflow. The compounds bind to column in presence of equilibrium buffer. (Clean up. If you have sand slurry just dump the sand from the column into trash. If using Phenyl Sepharose, which is very expensive, “wash” the column with 5mL of 8m M NaOH and then collect and save the phenyl sepharose slurry).

Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures.

Test Microorganisms and Growth Media

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

For testing of the antibacterial cultures which were mentioned above the following medium is used.

Nutrient Agar medium (NAM)

Beef extract	--	500mg
Sodium chloride	--	500mg
Peptone	--	1gm
Ph	--	7.0 – 7.2
Distilled water	--	100ml
Agar	--	2gm

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^{o}c.

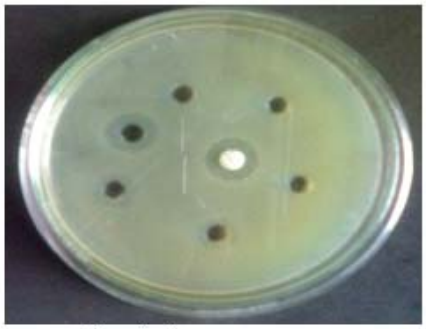
Testing of antimicrobial activity

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 inocculum (containing suspension) of P.aeruginosa and Staphylococcus 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^{o}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. (Figure-1).

Measuring the diameter of inhibition zone

The inhibition zones were lead after 1 day at 37ºc 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 (Table-1,2).

Pseudomonas aeruginosa



Staphylococcus aureus

Aspergillus niger

Figure-1: Test Microorganisms and Growth Media Antimicrobial activity *Sterculia villosa* leaf extract

Table-1: Inhibitory Activity of Plant Extracts on Microorganisms

Plants	**Zone of inhibition (MIC)**		
	Pseudomonas *aeruginosa* (-ve)	*Staphylococcus aureus* (+ve)	*Aspergillus niger*
Sterculia villosa	0.9	2.2	1

Table-2: Phytochemical Screening of Plant Leaves in Different Extracts *Sterculia villosa*

S.No	**Secondary metabolites**	**Hexane**	**Ethyl acetate**	**Ethanolic**	**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 Sterculia villosa 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 Sterculia villosa 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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