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Short Communication

Evaluation of phytochemical and antimicrobial effect of artocarpus heterophyllus leaves extract

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ABSTRACT

The present study was aimed at detecting the phytochemicals and evaluating antimicrobial activities of Artocarpus Heterophyllus known for their medicinal properties in folk medicine. Phytochemical screening was carried out on the leaves of Artocarpus Heterophyllus. The results latifoliate the presence of carbohydrates, phenolic compounds, tannins and triterpenoids. The assessment of antifungal activity was performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar PDA) against Aspergillus niger, Candida albicans, Sclorotium, Rhizopus and Microsporum. The antibacterial effect was studied by the agar direct contact method using B.cereus, S.aureus, Pseudomonas aeruginosa and Escherichia coli strains.. These phytochemicals were isolated from the plant with yields of 4.640 % of Petroleum ether extract, 2.962% of Chloroform extract, 3.759 % of Acetone extract and 12.063% of methanol extract. The results revealed that the methanolic extract exhibited significant antimicrobial activity of concentration of 100-500 µ/ml respectively against tested organisms, particularly more effective against and Escherichia coli, Pseudomonas aeruginosa and B.cerus than the other extract when compared to the standard drug (chloramphenicol).

Keywords: Artocarpus Heterophyllus Antimicrobial activity, Phytochemical Studies

INTRODUCTION

Artocarpus Heterophyllus is a plant belonging to family *Moraceae*.[1] It is a large, evergreen tree ,10-15 m in height, stem is straight rugh whereas bark is green or black,1.25cm thick, exuding milky latex[2]. Leaves are dark green, alternate, simple, glossy, large and elliptic to oval in form. Fruits are oblong cylindrical in shape. Its leaves are useful in fever, boils, wounds and skin deseases. The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent[3].The root is a remedy for skin diseases and asthma. The wood has a sedative property. Latex is used as an anti-inflammatory agent[4].

MATERIALS AND METHODS

Collection & Identification of leaves of Artocarpus Heterophyllus: Leaves of **Artocarpus Heterophyllus** were collected from Manduwala Chakrata road, Dehradun (India). Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun (BSI). Authenticated specimen no is –Acc. No. 114567.

Extraction of leaves of Artocarpus Heterophyllus in different solvents (Non-polar to-Polar): The collected plant Material was washesd with water to removed other undesirable material and dried under shade. The air-dried leaves (500 gm) of **Artocarpus Heterophyllus** were crushed. The crushed leaves extracted with different solvents of increasing polarity viz. Petroleum ether, Chloroform, Acetone and Methanol by hot percolation method using Soxlet Apparatous. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure.

Phytochemical Analysis of different extracts: Phytochemical Tests: The different extracts of

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leaves of **Artocarpus Heterophyllus** were tested for various components as follows:

A.Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dil HCl and filtered. The filtrate was then tested for following color test:

(i) Mayer's test (a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. **(b)** 5 gms of potassium iodide was dissolved in20 ml of distilled water. (a) and (b) was mixed and the volume was adjusted to 100ml with distilled water .Appearance of cream color precipitate with Mayer's reagents showed the presence of alkaloids.

(ii) Wagner's Test: 1.27 gm of iodine and 2 gm of potassium iodide was dissolved in 5 ml of water and make up the volume to 100ml with distilled water. Appearance of reddish-brown precipitate with Wagner's reagent showed the presence of alkaloids.

(iii) Hager's test: Take 20 ml of saturated solution of picric acid and add few drops of it to 2-3 ml of extract. A yellow color was observed.

B. Detection for carbohydrates and glycosides:

(i) Molisch's test: 10 gm of alpha naphthol was dissolved in 100 ml of 95% alcohol. Extract was treated with this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides of the test tube, purple or violet color appeared at the junction.

(ii) Benedicts test: The test solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed if reducing sugars were present.

(iii) Fehling's Test: 6.932 gm of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 gm of potassium sodium tartarate and 10 gm of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solution was mixed in equal volume prior to use and few drops of sample was added and boiled, a brick red precipitate of cuprous oxide was formed, if reducing sugars were present.

C. Test for sterols and triterpenoids

(i) **Salkowski test:** Extract was treated with few drops of conc. Sulfuric acid, shake well and allowed to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

D. Test for proteins and amino acids

(i) Ninhydrin test: 1gm of ninhydrin (indane1,2,3 trione hydrate) was dissolved in n-butanol and make the volume to 100ml. Extract treated with this solution gave violet colour on boiling.

(ii) Biuret test: To 3ml test solution 4% w/v NaOH and few drops of 1% w/v copper sulphate solution were added. A blue color was observed.

E . Test for sponins

(i) Foam test: 1ml of extract was diluted with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. A one-centimeter layer of foam indicated the presence of sponins.

Anti-microbial activity of different extracts: The **anti-microbial activity** of the leaves of **Artocarpus Heterophyllus** was carried out. The leaves extract was screened for anti-bacterial and anti-fungal activities.

Anti bacterial activity of leaves extract: In this study, the anti-bacterial activity was studied against the micro-organism and the bacterial cultures used in the study were:

1. Escherichia coli 2. Pseudomonas aeruginosa 3.B.cerus 4.Staphyllococcus aureus.

These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37^0 c for about 18-24 hours and then stored at 4^0 c as stock for anti bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at 37^0c overnight. To test anti bacterial activity, the well diffusion method used.

Culture media preparation: The microbiological media prepared as standard instruction provided by the HI-Media Laboratories, Mumbai. The media used for anti-bacterial activity Muller- Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121^0C at 15 psi for 15-30 minutes autoclave.

Plate preparations: 25 ml of pre autoclaved Muller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri-plates. These petri-plates were allowed to solidify at room temperature.

Well diffusion method: After the plated solidified the freshly prepared microbial growth culture suspension (about 20µl) was spread over the Muller – Hinton agar (MHA) media using L shaped sterilized glass spreader separately under the aseptic condition using laminar air flow. Then well were made in each plate with the help of borer of 8 mm diameter. In these well, about 100µl of each leaves extracts individually was loaded. This method depend upon the diffusion of leaves extracts from hole through the solidified agar layer of petri-dish to such an extent that the growth of added micro-organism is prevented entirely in a

circular area or Zone around the hole containing leaf extract.

Incubation: Petri plates were incubated for overnight at $37^0C \pm 0.5^0C$ in the incubator.

Inhibition Measurement of zone of inhibition: After incubation, the diameter of clear zone of incubation produced around the well or holes were measured in mm by ESR Tube and compared with standard drug.

RESULTS AND DISSCUSSION: Phytochemical studies reveal that methanolicnic extract was the richest extract for phytoconstituents. It contains all tested phytoconstituents viz. Carbohydrate, Phenolic compounds, Tannins and triterpenoids. Mehanolic extract showed good antibacterial activity against B.cereus and Pseudomonas aeruginosa and good anti-fungal activity against Aspergillus niger and Candida albicans in comparison to the standard drug.

Table-1: Percentage yield of different extracts Artocarpus Heterophyllus *leaves*:

Solvent system	%age yield
Petroleum Ether	4.640 %
Chloroform	2.962 %
Acetone	3.759 %
Methanol	12.063 %

Table-2: Qualitative Phytochemical Chemical Analysis of Extract of Artocarpus Heterophyllus eaves:

Test performed	Pet. Ether Extract	Chloroform Extract	Methanol Extract
Test for Alkaloids			
Mayer's test	_	_	_
Hager's test	_	_	-
Wagner's test	_	_	-
Test for Carbohydrates			
Fehling test	_	-	+
Molish test	_	_	+
Benedict test	_	-	+
Test for phenolic compounds and Tannins			
Vanillin HCL acid test	_	-	+
Dil. $Fecl_3$ test	_	-	+
Lead Acetate Test	_	-	+
Test for Sterols / Triterpenoids			
Salkowaski test	+	-	+
Test for Saponins			
Saponins Test	_	_	-
Test for Proteins and acids			
Ninhydrin test	_	_	-
Biuret test	_	_	_

Key:- (-) Absence, (+) Presence

Table-3: Antibacterial activity of different extracts of Artocarpus Heterophyllus and standard drugs

Test organism		Inhibition zone in mm					
	Pet.Ether	Chloroform	Acetone	Methanol	Standard drug		
					Ampicilline	Streptomycin	Chloram-phenicol
E. coli	1	-	9	19	20mm	18mm	20mm
Pseudomonas aeruginosa	4	7	16	10	-	18mm	18mm
Bacillus cereus	8	10	10	14	16	17mm	18mm
M.aureus	-	-	7	6	24 mm	27 mm	15 mm

Table- 4: Antifungal activity of different extract Artocarpus Heterophyllus and standard drug Clotrimazole.

Test Organism		**Inhibition zone in mm**				
	Pet. Ether	Chloroform	Acetone	Methanol	Standard drug	
					Amphotericin-B	Clotrimazole
Aspergillus niger	13	_	29	11	_	19 mm
Candida albicans	9	12	13	13	13	11
Sclorotium	6 mm	-	13	7	_	-
Rhizopus	9	-	16	11	-	-
Microsporum	6	4	18	13	-	-

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