Journal of Research in Medical and Dental Science
2023, Volume 11, Issue 1, Page No: 185-188
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Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

HPLC Analysis and Antimicrobial activity of *Artocarpus heterophyllus*

Jeane Rebecca Roy[1*], Janaki CS[1], Balaji TK[2]

[1]*Department of Anatomy, Bhaarath Medical College and Hospital, Chennai, India*
[2]*Department of Anatomy, Chettinad Academy of Research and Education, Chennai, India*

ABSTRACT

Artocarpus heterophyllus Lam. is commonly known as Jack fruit, which has many edible and medicinal properties. The raw as well as ripe fruits are edible in various forms. The fruit, bark, leaves, and roots are used for the treatment of various ailments traditionally and ethno pharmacologically. It possesses antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, immune-modulatory, antiviral, anthelmintic, wound-healing, and antineoplastic activities. Sreeja Devi, et al. has done the phytochemical and antioxidant profiling of various parts of jack fruit. Khan, et al. has reported the antibacterial role of jack fruit. Sreeletha, et al. also has reported the antimicrobial and antioxidant roles of jack fruit. Biworo, et al. and Rao, et al. have reported the anti-diabetic role of jack fruit. Morrison et al, 2021 have reported the anticancer role of this fruit.

Key words: *Artocarpus heterophyllus*, Antimicrobial activity, Anticancer

HOW TO CITE THIS ARTICLE: Jeane Rebecca Roy, Janaki CS, Balaji TK, HPLC Analysis and Antimicrobial activity of Artocarpus heterophyllus, J Res Med Dent Sci, 2023, 11 (1): 185-188.

Corresponding author: Jeane Rebecca Roy

e-mail✉: editor.pubs@gmail.com

Received: 08-December-2022, Manuscript No. JRMDS-22-68900;

Editor assigned: 10-December-2022, PreQC No. JRMDS-22-68900(PQ);

Reviewed: 26-December-2022, QC No. JRMDS-22-68900;

Revised: 30-December-2022, Manuscript No. JRMDS-22-68900(R);

Published: 06-January-2023

INTRODUCTION

Artocarpus heterophyllus Lam. is commonly known as Jack fruit, which has many edible and medicinal properties. The raw as well as ripe fruits are edible in various forms. The fruit, bark, leaves, and roots are used for the treatment of various ailments traditionally and ethno pharmacologically. It possesses antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, immune-modulatory, antiviral, anthelmintic, wound-healing, and antineoplastic activities [1,2]. Sreeja Devi, et al. [3] has done the phytochemical and antioxidant profiling of various parts of jack fruit. Khan, et al. [4] has reported the antibacterial role of jack fruit. Sreeletha, et al. [5] also have reported the antimicrobial and antioxidant roles of jack fruit. Biworo, et al. [6] and Rao, et al. [7] have reported the anti-diabetic role of jack fruit. Morrison, et al. [8] has reported the anticancer role of this fruit.

MATERIALS AND METHODS

The leaves of *Artocarpus heterophyllus* was collected locally, shade dried and powdered. The powder was soaked in methanol for two days and filtered. The filtrate was dried to obtain extract powder which was used for various tests as per standard protocols [9].

Phytochemical analysis: Qualitative

The following tests were performed on the methanol extract of *Artocarpus heterophyllus* leaves to detect various phyto-constituents present in them.

Alkaloids-Dragendorff's test

Solvent free extract (50 mg) was stirred with few mL of dilute hydrochloric acid and filtered. To a few mL of filtrate, 1or 2 mL of Dragendorff's reagent was added. A prominent yellow precipitate indicates the test as positive.

Carbohydrates-Fehling's test

The extract (100 mg) was dissolved in 5 mL of water and filtered. One mL of filtrate was boiled on water bath with 1 mL each of Fehling solutions I and II. A red precipitate indicated the presence of sugar.

Glycosides-Borntrager's test

The extract (50 mg) was hydrolyzed with concentrated HCl for 2 h on a water bath, filtered and to the 2 mL of filtrate hydrolysate 3 mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Saponins-Foam test

The extract (50 mg) was diluted with distilled water and

made up to 20 mL. The suspension was shaken for 15 min. A two cm layer of foam indicated the presence of saponins.

Proteins-Biuret test
An aliquot of 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

Amino acids-Ninhydrin test
Two drops of ninhydrin solution were added to two mL of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

Phenolic compounds–Ferric chloride test
The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Fixed oils
A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Terpenoids
To 1 mL of extract, 2 mL of trichloroacetic acid (TCA) was added and the formation of yellow to red precipitate showed the presence of terpenoids.

Phytochemical analysis: Quantitative

Determination of total phenolic content
The method of Singleton, et al. [10] was employed for this estimation. The plant powder (2 g) was soaked in methanol and kept in the orbital shaker for 24 hrs. The residues were then filtered and the filtrate was evaporated. The extract was centrifuged at 10,000 rpm for 15 min at 4°C. 20 µL of the extract was made up to 3 mL using distilled water. 0.5 mL of Folin-Ciocalteu's phenol reagent was added to the tubes and placed in the incubator for 3 min at 45°C. After 3 min, 2 mL of 20% Na2CO3 was added to the tubes and kept for incubation after which, absorbance was measured at 650 nm. The total phenol content in the sample was calculated using the formula,

C (GAE)=cxV/M

Where, C=Concentration of sample from the curve obtained (mg/mL), V=Volume used during the assay (mL) and M=Mass of the sample used during the assay (g)

Determination of total flavonoids
Flavonoid contents were determined by slightly modified spectrophotometry method of Karadeniz, et al.[11]. One g of dry powder was weighed and ground with 200 mL of 80 % aqueous methanol in a mortar and pestle. The ground sample was filtered and a clear filtrate was obtained. The aliquot of the sample (0.5 mL) was taken in a test tube, add 3 mL of distilled water and 0.3 mL of 5% sodium nitrite were added. The solution was vortexed and allowed to stand at room temperature for 5 min. and 0.6 mL of 10% aluminium chloride was added to the solution. After 6 min, 2 mL of 1 M sodium hydroxide was added to the test tube. The solution was made up to 10 mL with distilled water. The absorbance was read at 510 nm. The total flavonoid content was calculated as quercetin equivalent (mg QE/g) using the formula,

X=(A.M0/A0.M)

Where, A=Absorption of sample, A0=Absorption of standard (quercetin),

M=Weight of sample (mg/mL) and M0=Weight of quercetin in solution (mg/mL) [12].

HPLC Analysis
HPLC analysis of methanolic extract of the samples was carried out with Chromatographic system (YL 9013, Japan) consist of an auto sampler (YL 9528) with 100µl fixed loop and an YL 9163 UV-Visible detector. The separation was performed on a CTO-10A column at an ambient temperature, a CBM-10A interface and a LC-10 Workstation. The mobile phase consists of methanol: water (70:30 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 mL/min. The samples were run for about 20 min each and the detection was done at 274 nm by UV detector. All the chromatographic data were recorded and processed using autochro-3000 software.

Antimicrobial activity
Antimicrobial assay of different samples was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU/ml. MHA plates was cultured with standardized microbial culture broth. Each well was filled with varying concentrations from 150-200 µg/

Table 1: Means, (SD) and Tukey's analysis of different pulp capping materials.

Group	the shear bond strength (MPa)			
	Mean (SD)	F, (P value)	minimum	Maximum
Theracal LC	24.3490 (.36068)a	335.714 (.000)	23.87	24.82
Light cured calcium hydroxide	34.4901 (4.74169)b		29.83	39.14
Chemical cured calcium hydroxide	3.8312 (.43500)c		3.26	4.38
biodentine	10.1010 (.20798)d		9.79	10.42
In each column mean values with different superscript letters are significantly different ((P<0.05)				

ml of the samples with positive control as streptomycin 25 mcg and negative/solvent control as 100% DMSO, respectively. The plate was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the tested samples. The zone of inhibition (ZOI) was observed and measured in mm.

RESULTS AND DISCUSSION

The results of qualitative analysis of methanol extracts of *Artocarpus heterophyllus* (Jack fruit) leaves are depicted in Table1. It was found that except for terpenoids, the presence all other phyto-constituents studied indicated very good presence in the methanol leaf extract of jack fruit. There was a total lack of amino acids and a meagre presence of terpenoids. Indicates the presence or absence of phyto-constituents in the methanol extract of *Artocarpus heterophyllus* leaf extract (Table 1). The methanol extract of *Artocarpus heterophyllus* jack fruit leaves indicated the 47.5 mg/g and 120 mg/g of phenols and flavonoids, respectively as shown in Table 2.

Table 1: Presence or absence of phyto-constituents in the methanol extract of *Artocarpus heterophyllus* leaf extract.

Phytocomponent	Degree of Presence
Alkaloids	+++
Terpenoids	+
Saponins	+++
Fixed Oils	+++
Glycosides	+++
Carbohydrates	+++
Phenolic Compounds	+++
Proteins	+++
Amino acids	---
+: Phytocomponents present at lower concentration	
++: Phytocomponents present at medium concentration	
+++: Phytocomponents present at higher concentration	
---: Phytocomponents absent	

Table 2: Indicates the quantitative analysis results of methanol extract of leaves of *Artocarpus heterophyllus*.

Phytochemical	Results
Total Phenol (mg/g)	47.5
Total Flavonoid (mg/g)	120

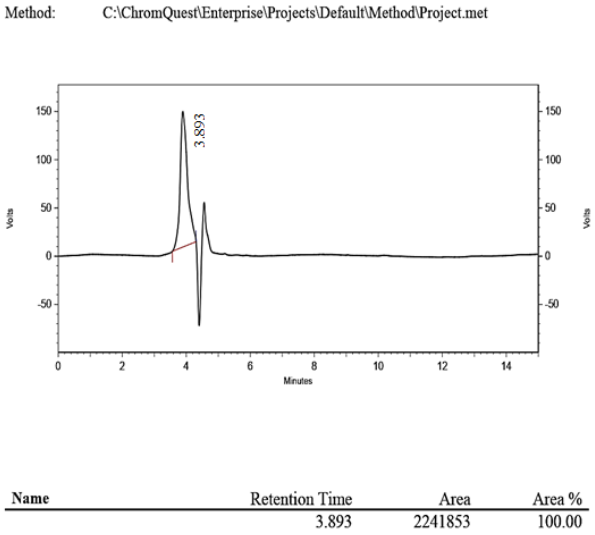

Figure 1: Represents the HPLC profile of methanol leaf extract of *Artocarpus heterophyllus*.

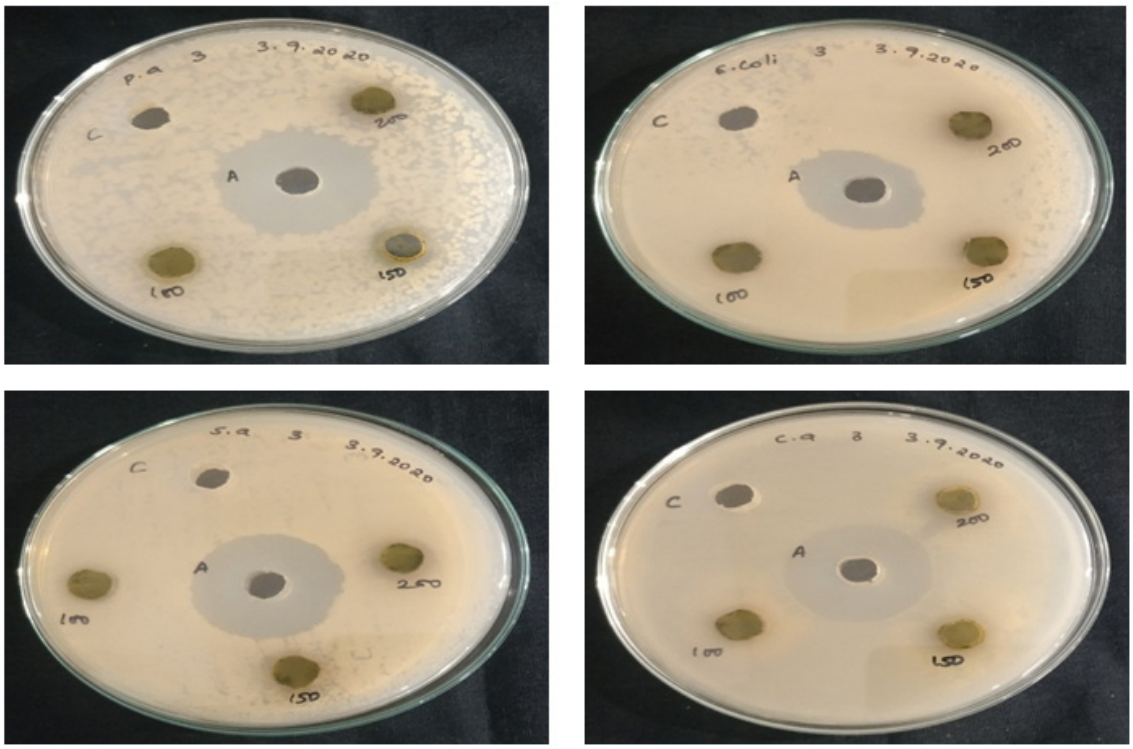

Figure 2: Indicates the antimicrobial role of methanol extract s of the leaves of *Artocarpus heterophyllus*.

Table 3: Zones of inhibition for each concentration of the sample of *Artocarpus heterophyllus* methanol leaf extract and respective antibiotics against the microorganisms.

Organisms	100 µg	150 µg	200 µg	Streptomycin 25 µg
E. coli	--	2 mm	3 mm	11 mm
PA	--	--	2 mm	11 mm
SA	--	2 mm	3 mm	11 mm
CA (Fungus)	--	2 mm	4 mm	Kanamycin 25 µg 11 mm

Bacteria: *E. coli: Escherichia coli*; PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*. Fungus: CA: *Candida albicans*

HPLC Analysis

The HPLC analysis of the methanol extract of the sample was limited to 5 µg L-1 and a flow rate of 1 mL/min showed good detection of component present with low noise level with a retention time of 3. 893 in a time interval less than 5 minutes Figure 1.

Antimicrobial activity

The antimicrobial activity of the methanol extract of *Artocarpus heterophyllus* leaves is shown in Table 3 and Figure 2. From the results it is observed that at a concentration of 100 µg, the plant sample did not show any antimicrobial activity. At 150 and 200 µg concentration there was some activity but as compared to both streptomycin (for bacteria) and Kanamycin (for fungus) the activity was less. By increasing the concentrations we expect to get better antimicrobial action of this plant extract.

CONCLUSIONS

The above results indicated that *Artocarpus heterophyllus* (Jack fruit) methanol leaf extract has indicated the presence of many phyto-constituents, the presence of which could be the source of the medicinal role of this plant. It was found that there was very good presence of phenols and flavonoids which are known for their medicinal roles. The antimicrobial roles of the methanol extract were poor compared to the respective standards used. Thus it is concluded that jack fruit leaves have good medicinal priorities due to the strong presence of various important phyto-constituents but its antimicrobial activities are limited.

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