



PHYTO-CHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF CARICA PAPAYA SEEDS EXTRACTS ON E-COLI.

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Abstract

This research work was carried out to evaluate the antimicrobial activities of Seeds of Carica papaya and their phytochemical screening. Ethanol and hot water were used to extract the active compounds from dry seeds of Carica papaya. The phytochemical analysis of the seeds extracts of the plant was also carried out to determine the active ingredients present in these extracts. The antimicrobial activities of different solvents of these extracts were determined against bacterial and fungal isolates by observing the zones of inhibition. The bacterial isolates used were Escherichia coli. Results obtained showed that the ethanolic extracts showed more antibacterial activities. The ethanolic extract of dry seed of Carica papaya showed grater activities against Yeast & E-Coli. The phytochemical analysis showed that the papaya seeds extracts contained alkaloids, saponin, tannin, terpenoid and flavonoids active ingredients.

Keywords: Seeds Extracts of Papaya, Phytochemical Analysis, Antibacterial screening.

INTRODUCTION:

Day by Day Papaya becomes youngsters diet nutritious fruit which provides Vit-E. [Table-1] It shows that papaya is an excellent source of vitamin A, vitamin C and dietary fibre and a good source of vitamin E. There is not much information about the nutritive value of papaya leaves and seeds. In recent years, the world has witnessed an increase in use of medicinal plants to cure infections and diseases. Carica papaya, commonly known as papaya or pawpaw belongs to the plant family Caricaceae. The papaya is a large, tree-like plant, with a single stem growing from 6 to 13 m tall, with spirally arranged leaves confined to the top of the trunk. It is distributed throughout Asia, Nigeria etc. It is medicinally used in the treatment of smooth upper respiratory tract ailment, psychiatric related illnesses, scorpion bites, hypertension toothache, tuberculosis, liver inflammations, arthritis and rheumatism [5]. C. papaya is one of these plants which are greatly used by the rural communities to cure illnesses in Klpapur. For this reason, study to understand the medicinal value and potential of this plant is been intensified. Plants and their secondary metabolite constituents have a long history of use in modern 'western' medicine and in certain systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine. Use of herbal medicines in developed countries has expanded sharply in the latter half of the twentieth century [1,2]. The antibacterial activity of Carica papaya seed extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted using methanol as solvents. The C. Papaya

seeds extracts were tested against Escherichia coli & Yeast by agar well diffusion method. The extract demonstrated higher activities against tested bacteria with the highest activity. demonstrated against Escherichia coli. Preliminary phytochemical analysis showed that the extracts contain alkaloids and phenols. The results justified the use of Carica papaya seed extracts in the treatment of dengue, gastroenteritis, and wound infections. Thin-layer chromatography (TLC) is a highly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction. It also permits the optimization of the solvent system for a given separation problem. In comparison with column chromatography, it only requires small quantity of the compound and is much faster as well. A thin layer chromatography uses a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The mobile phase is a suitable liquid solvent or mixture of solvents [4]. This study aims at investigating the antibacterial & Phytochemical activity of extracts of Carica papaya seeds.

MATERIAL AND METHODS:

Sample collection: The healthy fruits of Carica papaya were collected from the Unchgaon & Panhala, Kolhapur, Maharashtra, India. The seeds were washed and air dried were ground into coarse powder and stored in air tight containers for further processing. Practicals were done in ISI Std-Nikhil Analytical Lab Sangli by their expertise. [5]

Preparation of extracts: The extraction was carried out by Soxhlet extraction method. The finely powdered sample of Carica papaya SEEDS was extracted in a Soxhlet apparatus using

methanol as the solvent system not exceeding its boiling point. The extracts were then filtered using Whatman Filter paper No.1 and dried under reduced pressure using rota-vapour. The dried extracts were stored at 4°C and were further dissolved in 5% dimethyl sulphoxide (DMSO)

Phytochemical analysis Tests: Test for proteins: Xantho protein test was done for proteins. Few drops of nitric acid were added along the wall of test tube to the 1ml of extract. Formation of yellow color indicated the presence of proteins.[5]

Test for carbohydrates: About 1 ml of Fehling A and Fehling B solution were added to the extract. This was heated for 30 min and observed for the formation of brick red color which confirmed the presence of carbohydrates.

Test for resin: About 5ml of distilled water was added to the extract and observed for the turbidity.

Test for saponins : About 0.1g of sample was mixed with 5ml of distilled water and allowed to boil. Then the mixture was filtered and 2 drops of olive oil was added in 1ml of filtrate. The mixture was shaken and formation of emulsion and froth was observed. The 1ml filtrate was diluted by adding up to 4ml of distilled water. The mixture was shaken vigorously and observed for the stable froth.

Test for flavonoids: The filtrate was prepared by boiling the mixture of 0.5g of sample and 10ml of ethyl acetate for 1 min. Then the mixture was filtered and 4ml of filtrate was shaken with 1ml of 1% ammonium chloride solution. Formation of yellow color in the presence of ammonium solution indicates the presence of flavonoids. [3]

Test for phenols: About 1ml of extract was mixed with 1ml of distilled water and warmed. To this 2ml of ferric chloride solution was added. Formation of green or blue color confirms the presence of phenols.

Test for glycosides: About 0.5ml of extract was taken in test tube and 1ml of glacial acetic acid was added to it containing traces of ferric chloride. To this solution 1ml of concentrated sulfuric acid was added. Formation of reddish brown color was observed in between the two layers. In the presence of glycosides upper layer turned bluish green.

Thin layer chromatography : TLC was performed on precoated silica gel TLC plates to identify the retention factor (Rf). TLC was used to separate the phytochemical components present in the extract. The ratio of solvents were used at following ratio methanol:chloroform

(20:80), toluene: ethylacetate: acetic acid: formic acid (20:50:05:01), About 0.2 ml of aliquot was applied on TLC plates and immersed inside the solvent system. TLC plates were observed in ultraviolet chamber using 400nm long wavelength. The Rf values were calculated and based on the Rf values the components present in the extract were determined. [4,5]

RESULT:

Nikhil Analytical-Lab SANGLI [ISI-STANDARD] Reports ANALYSIS:

Phytochemical analysis: The different phytochemical tests revealed the presence of carbohydrates, flavonoids, glycosides and phenol in SEED extract of *Carica papaya*. The phytochemical tests with the *Carica papaya* SEED extract showed the presence of proteins, carbohydrates, Resins, Glycosides, Quinons & Alkaloids.

Phytochemicals Phytochemical profile :

Emblca officinalis SEEDS : 1] Proteins - +ve ; 2] Carbohydrates - +ve ; 3] Resins- +ve; 4] Saponins- -ve ; 5] Flavonoids- -ve; 6] Glycosides- +ve ; 7] Phenols - -ve; 8] Steroid- -ve; 9] Alkaloids- +ve; 10] Tannins- -ve 11] Quinons- +ve.

Antimicrobial assay: The methanol extracts of *Carica papaya* was analyzed for antimicrobial activity against the pathogens by agar-well diffusion inhibition test. Muller-Hinton agar plates were prepared and then the clinical pathogens were swabbed onto the plates. Four wells were aseptically punctured by using sterile borer and different concentrations (25µl, 50µl, 75µl and 100µl) of extracts were loaded into the wells. The plates were incubated at 37°C for 24h and the zone of inhibition was measured around the wells.[10]

Antimicrobial activity of methanolic SEED extract from *Carica papaya*

S. No. Pathogen name Zone of inhibition (mm) value

1] *Escherichia coli* -- cfu/g

2] Yeast & Mould 10 × 10² cfu/g

DISCUSSIONS: *Carica papaya* is a plant that has been reported to possess medicinal properties mainly against dengue. For this study, dry seeds of *Carica papaya* were extracted with methanol and tested for Antimicrobial activity against E-Coli & Yeast-moulds. The bioactivity of plant extracts is attributed to its phytochemical constituents. Alkaloids have been reported to possess antimicrobial properties.[1]

The cardiac glycoside also have the ability to increase the force and power of the heartbeat without increasing the amount of oxygen

needed by the heart muscles, thus increasing the efficiency of the heart and at the same time steady excess heart beats without straining the organ [2]. Alkaloids have been reported to be the most efficient therapeutically significant plant substance. Pure isolated alkaloid and their synthetic derivatives have been as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties. Alkaloids can also be used as anti-malaria agents because it contains quinine [3].

Antimicrobial activity : The results of the study showed that the SEED extracts of *Carica papaya* indicates the presence of effective antimicrobial activity, which confirms its use against infection. The analysis of antimicrobial activity was based on measurement of inhibition zones formed around the wells. This broad spectrum of activities may be significant in developing therapeutic substances that will be active against multidrug – resistant organisms. Therefore, there is need to carry out comprehensive evaluations on the bioactive compounds of different parts of *Carica papaya* in order to know those ones that possess the antimicrobial properties. *Carica papaya* seed extract showed good inhibition effect against *Escherichia coli*. Yeast & Mould 10×10^2 cfu/g. [5] The results of this study showed that the organic extracts were more effective, this may be due to the better solubility of the active

components in organic solvent [2]. The results further showed that the dried samples were more effective. The disparity between the activities of the extract and the standard antimicrobial drugs may be due to the mixtures of bioactive compounds present in the extract compared to the pure compounds contained in the standard antibiotics [3]. The high MIC observed in this study with some extracts against some test organisms might be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds [4]. The activity of extracts against test organisms provides scientific basis for the local usage of these plants in the treatment of various ailments.

CONCLUSION: The present study confirmed that the SEED extract of *Carica papaya* have great potential for antimicrobial activity against *E-Coli* & Yeast-moulds. The phyto-chemical analysis revealed that the SEED extract of both *Carica papaya* proven to be more effective against antimicrobial & antifungal activity. It doesn't have Flavonoids, Tannins, Saponins & phenols & rich in Protein, Alkaloids and carbohydrates, Quinins & Glycosides so it is used as very good nutritional and medicinal purpose.

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Table 1. Nutritive value of *Carica papaya* fruit per 100g (source: USDA National Nutrient database)

Vitamins	Nutrient Value	RDA/day for Indians
Energy	43 Kcal	2730Kcal
Water	88.06g	
Carbohydrates	10.82 g	
Protein	0.47 g	60g
Total Fat	0.26 g	
Cholesterol	0 mg	
Dietary Fiber	1.7 g	
Vitamins		
Folates	37 µg	200µg
Niacin	0.357 mg	18mg
Pyridoxine	0.038 mg	2.0mg
Riboflavin	0.027 mg	1.6mg
Thiamin	0.023 mg	1.4mg
Vitamin A	285µg	600µg
Vitamin C	60.9 mg	40mg
Vitamin E	0.30 mg	7.5 - 10mg
Vitamin K	2.6 µg	55µg

* Nutrient value of *Carica papaya* followed from USDA National Nutrient Database and Recommended Daily Allowance (RDA) referred from 'Nutrient Requirements and Recommended Dietary Allowances for Indians' followed from Indian Council of Medical Research (ICMR), Draft Guidelines 2009[A].

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