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PHYTOCHEMICAL AND MICROBIOLOGICAL EVALUATION OF DIFFERENT CHEMICAL EXTRACTS OF PAPAYA SEEDS ON CLINICAL ISOLATES OF (FGSH HOSPITAL) ISLAMABAD

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ABSTRACT

The current study was aimed to evaluate the antibacterial activity of different concentrations of aqueous and non-aqueous extracts of Carica papaya seeds. Dried and grinded papaya seeds (5g), respectively mixed with 95ml of extraction solvent (water, methanol, acetone and ether) for 24 hours were used for this study. Extraction was done by maceration and Soxhlet method. The extract was then decanted and filtered through a Whatman filter paper. Antimicrobial activities of the each extract were determined using agar well diffusion method using fresh clinical isolates of Escherichia coli and Staphylococcus aureus and their MIC and zone of inhibition were determined. The results were evaluated statistically using paired sample t-test. Aqueous extract of Carica papaya exhibits greater antimicrobial activities against the clinical isolates of Escherichia coli and Staphylococcus aureus as compared to the non-aqueous plant extracts. MIC of aqueous extract against Escherichia coli was 10^{-3}mg/mL, whereas methanolic extracts shows 10^{-4}mg/mL MIC. Similarly MIC of aqueous extract against Staphylococcus aureus was 10^{-4}mg/mL as compared to MIC of methanolic extract 10^{-6}mg/mL. There was statistically significant difference between the zone of inhibition of aqueous extracts of the plant material against Staphylococcus aureus and Escherichia coli ($P < 0.001$). Preliminary phytochemical analyses reveal that the extracts contain alkaloids, tannins, saponins and phenols. Carica papaya may be used for the treatment of infection caused by Escherichia coli and Staphylococcus aureus.

Key Words: Carica Papaya, Phytochemical evaluation, Agar well diffusion method, Extraction, Maceration

INTRODUCTION

Papaya (*Carica papaya* L.) is a member of the family Caricaecae. This plant family has four genera including Jarilla, Cylicomorpha, Cylicomorpha and Carica. *Carica papaya* L. is a common papaya and extensively grown over the world. The plant is herbaceous, soft tissue and fast growing. Common names include papaya, papaw or pawpaw, papeeta (Pakistan), papayer (French), melonenbaum (German), lechosa (Spanish), mamao, mamoeiro (Portuguese), mugua (Chinese) and malakol (Thailand).[1] Papaya is a fruit plant with a soft stem, commonly and erroneously referred to as a tree. The plant is properly a large herb growing at the rate of 1.8-3 m in the first year and reaching 6-9 m in height. The cluster of leaves at the apex and along the upper part of the stem made up the foliage of the tree. The leaves contain copious white milky latex.The papaya leaves (per 100 g), contains 74 calories, 77.5 g H2O, 7.0 g. protein, 2.0g fat, 11.3 g total carbohydrate, 1. 8 g fiber, 2.2 g ash, 344 mg Ca, 142 mg P, 0.8 mg Fe, 16 mg Na, 652 mg K, 11,565 ug β-carotene equivalent, 0.09 mg thiamine, 0.48 mg riboflavin, 2.1 mg niacin, and 140 mg ascorbic acid, as well 136 mg vitamin E. The papaya seeds contain balance-nutrients which consist of protein (24.3%), fatty oil (25.3%) and total carbohydrate (32.5%). Although it contains significantly high level of unsaturated fatty acids, papaya seeds seem not to be good oil seeds.Papaya's seeds are used generally as antic parasitic agent by human. Flowers are borne on

modified cymose inflorescences, which appear in the axils of the leaves. The type of inflorescence depends upon the sex of the tree. Papaya fruit is berry, melon-like, oval to nearly round, pyriform, or elongated club shape. Fruit from female trees are spherical and those from hermaphroditic trees can show diverse shapes explain depending upon modifying factors affecting flower morphology during ontogeny.[2]A number of small, dark brown seeds, each with a mucilaginous sarcotesta are attached to the walls inside the fruits. Papaya fruit development from pollination to ripeness takes 168 to 182 days. The variation ranges from 173 day to 282 days from fruit set to ripeness depend on the temperature.[3] Papaya fruits can be eaten both in green and ripen stage. Ripe papaya is most commonly eaten fresh. The flesh is often cubed or shaped into balls and served in fruit salad.[4]

Extracts are the preparations of crude drugs which contain all the constituents which are soluble in the solvent used in making the extract. The solvent used for extraction must diffuse into the cell to dissolve the desired compounds and the solution must pass the cell wall in the opposite direction and mix with the surrounding liquid. An equilibrium is and the speed with which this equilibrium is established depends on temperature, PH, particle size and the movement of the solvent.[5]Several approaches can be employed to extract the plant material. Although water is used as an extractant in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubility of plant constituents.[6]

The present research study was aimed at preparation of aqueous and non-aqueous extracts of *Carica papaya* Linn. Seeds, its phytochemical evaluation and antibacterial activity against clinical bacterial isolates

MATERIAL AND METHODS

General Solutions: 90% ethanol, Acetone, Chloroform, ethyl ether, Nutrient agar,70% methanol and nutrient broth.

Collection and preparation of plant: The plant *Carica papaya* was collected in a sterile polythene bag, rinsed, sundried and made into a powdery form before use. Plant materials were collected from in and around Rawalpindi, Pakistan. The seed, fruit peel and leaves, were collected, washed in tap water, rinsed in sterile distilled water and dried for 5 days at 100^{o}C. The dried plant parts were blended to powder with a clean mortar and pestle and stored in airtight glass containers kept in laboratory cupboard, until required for preparation. The seeds were separately extracted with Chloroform, acetone, diethyl ether, methanol and hot water.

Preparation of extracts: Extraction of seeds powder was done by maceration and Soxhlet Extraction. In maceration, 20g powdered seeds were soaked in 100 mL water and 20 g powdered seeds were soaked in 100 mL methanol for 5 days in closed container at room temperature with occasional shaking or stirring. The extract was then repeated from the plant particles by straining. The process is repeated for once or twice with fresh solvent. Finally the last residue of extract was pressed out of the plant particles using a mechanical press and then filtered using Whatmann filter paper.

Soxhlet Extraction: Papaya seeds were chopped into powder, thoroughly rinsed in water for a few hours and oven dried at 45 ° C for 3 days to obtain a constant weight. The dried plant material was then blended into fine powder. 50 grams of this powder was weighed and extracted with 250 ml of 70 % methanol using Soxhlet extractor. This extraction procedure was done for 24 hours for each solvent type.

Drying: The filtered methanol extract was then evaporated to dryness at 45°c while the aqueous extract at 100^{o}c using air dryer. The residues obtained were reconstituted in 70% methanol and water at stock concentration of 10mg/ml respectively. The extract solution were then stored in the refrigerator.

Phytochemical screening for extract: Phytochemical screening of the extracts were carried out by routine confirmatory test for the presence of alkaloids, tannins [7] flavonoids, glycosides, reducing sugars and saponins[8]

Preparation of different concentrations of the extracts: The stock (10mg/ml) was prepared by reconstituting 1g of the extracts in 100ml of their respective solvent. 1 mL of the stock solution was transferred to another test tube and it was diluted up to 10 mL with the solvent. 1ml of this dilution was transferred to a new test tube and diluted to 10 mL. Similarly six other test tubes were prepared each having dilution factor 10 with both water and methanol. At the end 16 test tubes were prepared out of which 8 were aqueous and the rest of the 8 were methanol extracts.

Table 1: Stock Solution Concentration for Dilutions (10mg/ml)								
Dilutions	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**
mg/mL	10	1	.1	.01	.001	.0001	.00001	.000001
10^{X}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}

Microorganism preparation/growth: The test organisms were clinical isolates *Staphylococcus aureus* and *Escherichia coli*. They were obtained from the Department of Microbiology FGSH Hospital Islamabad, Pakistan. The organisms were collected on sterile agar plates and incubated at 37°c for 48hours.

Minimum inhibitory concentration: Inoculum containing nutrient broth test tubes was prepared its turbidity was compared against McFarland solution. 1mL of each concentration of the extract was added to each test tube and was incubated for 24 hours. Next day the turbidity of each tube was determined, the minimum concentration of the extract which produces clear solution in the test tube was declared minimum inhibitory concentration. The minimum inhibitory concentration of both aqueous and non-aqueous extracts was determined.

Antimicrobial Assay of Extracts: The agar well diffusion method was used to determine the antibacterial activity of the plant extracts. One milliliter of the different standardized organisms were introduced separately and thoroughly mixed with 30milliliters of molten nutrient agar each in a sterile Petri dish and allowed to set then labeled. A sterile 5mm borer was then used to punch 5 holes in the inoculated agar and the agar was then removed. Two upper wells that were formed were filled with methanolic concentrations of the extract which were labeled accordingly; and lower two were filled with aqueous concentrations of the extract which were labeled accordingly; while the 5thwell contained the extractant i.e. the solvent used for the extraction to serve as control. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°c for 48hours. After incubation, the diameter of the zones of inhibition around each well were measured.[9]

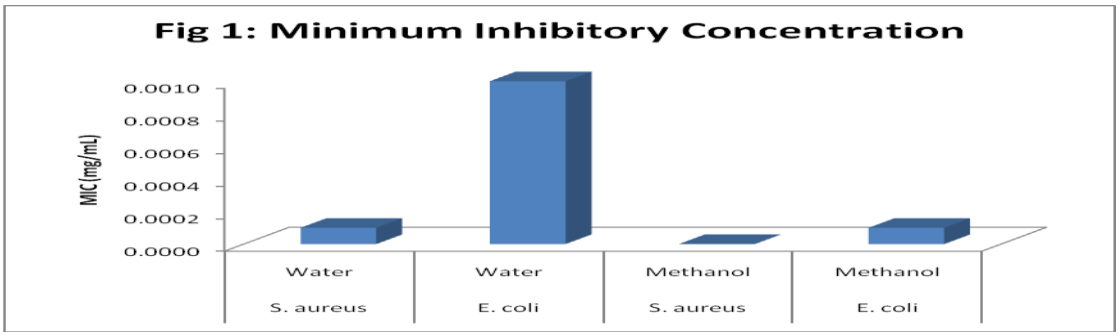
RESULTS AND DISCUSSION

Minimum Inhibitory Concentration (MIC) of the Extracts: The results reveal that all extracts are potent antimicrobials against the pathogenic organisms studied. The low MIC value observed for *Staphylococcus aureus*is a good indication of high efficacy against this bacterium. The results of MIC are summarized in the table 2.

Zone of Inhibition: The antibacterial activity was screened from the zone of inhibition. The diameter of inhibition zones for each of the samples were measured with scale in mm. The results of antibacterial sensitivity of various solvent extracts of *Carica papaya* seeds by well diffusion method are depicted below in the graph and table.

Table 2: Minimum Inhibitory concentration

Organism	Solvent	MIC (mg/mL)
Staphylococcus aureus	Water	10^{-4}
Escherichia coli	Water	10^{-3}
Staphylococcus aureus	Methanol	10^{-6}
Escherichia coli	Methanol	10^{-4}



Microbial Strains	Table 3: Extracts Concentration & Zone of Inhibition			
	Escherichia coli		Staphylococcus	
	mg/ml	mm	mg/ml	Mm
Aqueous	10^{-3}	8.1±0.5	10^{-4}	7.3±0.5
Non Aqueous	10^{-4}	10.6 ±0.5	10^{-6}	9.0±0.5

The results revealed that the aqueous extracts have greater zone of inhibition as compared to non-aqueous extracts when compared with the control.

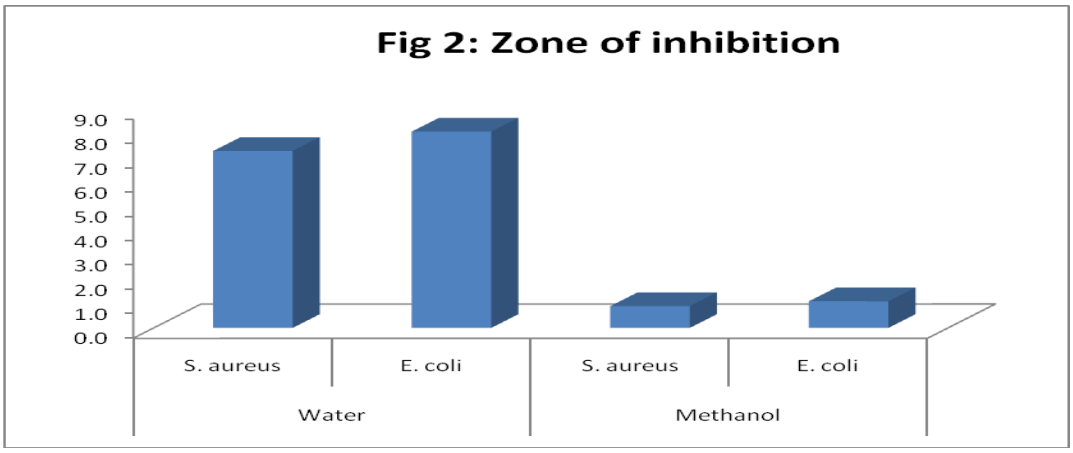


Table 4: Independent Samples T- Test									
Zone of Inhibition	Levene's Test		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% CI Lower	95% CI Upper
Equal variances assumed	.000	1.000	8.0	8	.000	.80000	.10000	.56940	1.03060
Equal variances not assumed			8.0	8.0	.000	.80000	.10000	.56940	1.03060

As the two tail significance value ($p< 0.001$) is less than 0.05 therefore it is concluded that there is statistically significant difference between the zone of inhibition of aqueous extracts of papaya against *Staphylococcus aureus* and *Escherichia coli*. The results of this study reveal that the aqueous extracts were more effective than organic extracts and possess highest antimicrobial activity. This may be due to the better solubility of the active components in aqueous solvents. Among the Gram-positive and Gram-negative bacteria tested against the seed extract of C. papaya, the Gram-negative bacteria were more susceptible to the extracts. The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of various ailments.

Phytochemical Constituents of *Carica papaya* Seed Extract: Water fraction had soft gummy texture. The colors of the methanolic extracts were both yellow green while that of the hot water was brown. The results of phytochemical screening of methanol and water extracts and fractions of C. papaya revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins. These metabolites have been reported to possess.

Table 5: Phytochemical constituents of *Carica papaya* seed Extract/fractions							
Extracts	Alkaloids	Flavonoids	Glycosides	Reducing sugar	Saponins	Steroids	Tannins
Methanol	+	+	-	-	-	+	+
Water	-	-	-	-	+	-	+
Key: + = Present, - = Absent							

CONCLUSIONS

It is concluded that the demonstration of antimicrobial activity against both gram-negative and gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The fact that the extracts were active against both Gram-negative and Gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. From the results of this work, it can be concluded that *Carica papaya* Linn. has the potential for the production of drug for the treatment of infections caused by *Staphylococcus aureus* and *Escherichia coli*.

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