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Antibacterial and Phytochemical Analysis of *Carica papaya* L.

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A B S T R A C T

Plants are used medicinally in different countries and are sources of many potent and powerful drugs. *Carica papaya* belongs to 'Caricaceae' family and it is commonly known as Papaya. *Carica papaya* is used in ayurvedic medicines from very long time. *Carica papaya* is used as anti-inflammatory, antioxidant, diuretic, antibacterial, vermifuge, hypoglycemic, antifungal activity, antihelmenthic and anti-immunomodulatory etc. In the current study, aqueous extract acetic acid extract of leaves of *Carica papaya* plants were used to study antibacterial properties and phytochemical screening. Aqueous extract showed maximum antibacterial activity than the acetic acid extract against the test organisms. Phytochemical analysis confirmed the presence of carbohydrates, sugars, fats, proteins, amino acids, steroids, glycosides, flavonoids, tannins and phenolic compounds, organic acid (citric acid), vitamin 'C' etc. TLC helped in confirmation of presence of different constituents depending on the polarity of the constituents which are exhibited as number of resolved bands. Acetic acid extract showed highest amount of amino acids, fatty acid, glycosides and vitamin 'C'.

***Key words*:** *Carica papaya*, Antimicrobial activity, Phytochemical analysis, Thin layer chromatography

Ayurveda, the Indian system of medicine, is attainment superior attention and popularity in many parts of the world. The disease protective and health primitive approach of Ayurveda, which takes into consideration the entire body, mind and spirit while dealing with the maintenance of health promotions [1]. The growth of bacteria, yeast, and mould in foods and food products results in waste products and is costly as well as sometimes hazardous. Many different bacterial and fungal species can spoil food products or produce toxins or both. Several food preservation systems such as heating, refrigeration and addition of antifungal compounds can be used to reduce the risk of outbreaks of food poisoning [2]. Plants have always been a source of natural products for the treatment of various diseases [3]. Around 70 to 80% of the world populations, particularly in developing countries, rely on-conventional medicine in their primary healthcare as reported by the World Health Organization [4].

Plant based medicines have an advantage over synthetic drugs in having low human toxicity. In addition, chemical diversity of secondary plant metabolites that result from plant evolution is equal or superior to that found in synthetic combinatorial chemical libraries. The antimicrobial activities of these plants for the treatments of multidrug resistance against the pathogenic bacteria as claimed by traditional healers and much more research need to extract the value-added food preservative agents for selected microbes are being investigated [5]. Different parts of this plant are used for several conditions anti-helminthic, anti-fertility, anti- implantation, abortifacient, purgative, antihypertensive, antibacterial, antioxidant, anti-inflammatory, ulcer healing, diuretic and platelet count increasing activity. Because of these activities.

The papaya, *Carica papaya* L., is a member of the small family Caricaceae allied to the Passifloraceae [6]. Vast applications *Carica papaya* are found making it a green treasure in medicinal field. Papaya extract may be used for the treatment of gastroenteritis, urethritis, wound infection and otitis media [7]. In this study, aqueous and acetic acid extract of *Carrica papaya* leaf were tested for the antibacterial activity. Aqueous extract showed maximum antibacterial activity than the acetic acid extract. Antibacterial activity was significant against *Pseudomonas aeruginosa* as compared to *Bacillus subtilis.* Phytochemical analysis indicated presence of tannins, saponoins, glycosides and phenols in the leaf extract. It is suggested that *Carica papaya* may be recommended as useful source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost effective. Current approach can be considered for screening and dentification of active agents from natural sources which can be added to new pharmaceuticals.

MATERIALS AND METHODS

Collection of leaves

The leaves of *Carica papaya* L. are collected from At/P. Ambodi, Saswad, Pune, Maharashtra, India. The leaves dried in oven (Gallenhamp Incubator Model IH-150) at 50-60ºC. The dried leaves were cooled at room temperature. Before and after

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drying weight was recorded. Around 70 grams of dried leaves were used for extraction process.

Test organisms used

Human pathogens *Pseudomonas aeruginosa* (Gram Negative); *Bacillus subtilis* (Gram Positive) were used in this study. All cultures are maintained in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar (Himedia MM012) slants at 4ºC.

Preparation of extract

For preparation of extract "Soxhlet extractor" was used. 20 gm powder was packed in Soxhlet apparatus in filter paper. After finishing the process the extracts were kept in incubator for drying. Solvents like acetic acid distilled water used separately for extraction. Dilution of extract was carried out [8].

Antibiogram analysis of extracts

For assessment of antibacterial properties of extract, after solidification of nutrient agar plates 25 µl Bacterial pathogens (*P. aeruginosa* and *B. subtilis)* were spread on respective plates and wells of 8 mm diameter were bored by the help of sterile borer. The test quantities of specific extracts were dissolved in depending upon the solubility of the extracts. The different dilution concentration (mg/ml) of the organic extracts (Acetic acid and aqueous), accordance with control were loaded into the wells and standard antibacterial drug Cotraximol (25mcg/disc) place into medium. Plates were incubated at 37ºC for 24 hours, and observed for Zone of inhibition [9].

Phytochemical analysis

Organic and inorganic components from aqueous and actetic acid extract were detected using polar and non-polar solvents as per the solubility [10].

Thin layer chromatography

The TLC plates were prepared by using Silica gel 'G' as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 minutes and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts of acetic acid and water with respective solvent and then applied usually 1-10µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes. Development of the chromatogram After the application of the sample on the plate the plates were kept in TLC glass chamber (solvent saturated) than mobile phase was allowed to move through adsorbent phase up to 3/4th of the plate [11].

RESULTS AND DISCUSSION

Antimicrobial activity

Data depicted in (Table 1) shows the zones of inhibition (mm) against *P. aeruginosa* and *B. subtilis* for the acetic acid extract. Maximum zone obtained was 26 mm against *P. aeruginosa.* Maximum zone obtained was 26 mm against *P. aeruginosa.* (Table 2) shows the zones of inhibition (mm) against *P. aeruginosa* and *B. subtilis* for the aqueous extract. Maximum zone obtained was 55 mm against *P. aeruginosa*. Maximum zone obtained was 55 mm against *P. aeruginosa*. Hence, from the results obtained it can be interpreted that aqueous extract show effective antimicrobial activity than the acetic acid extract.

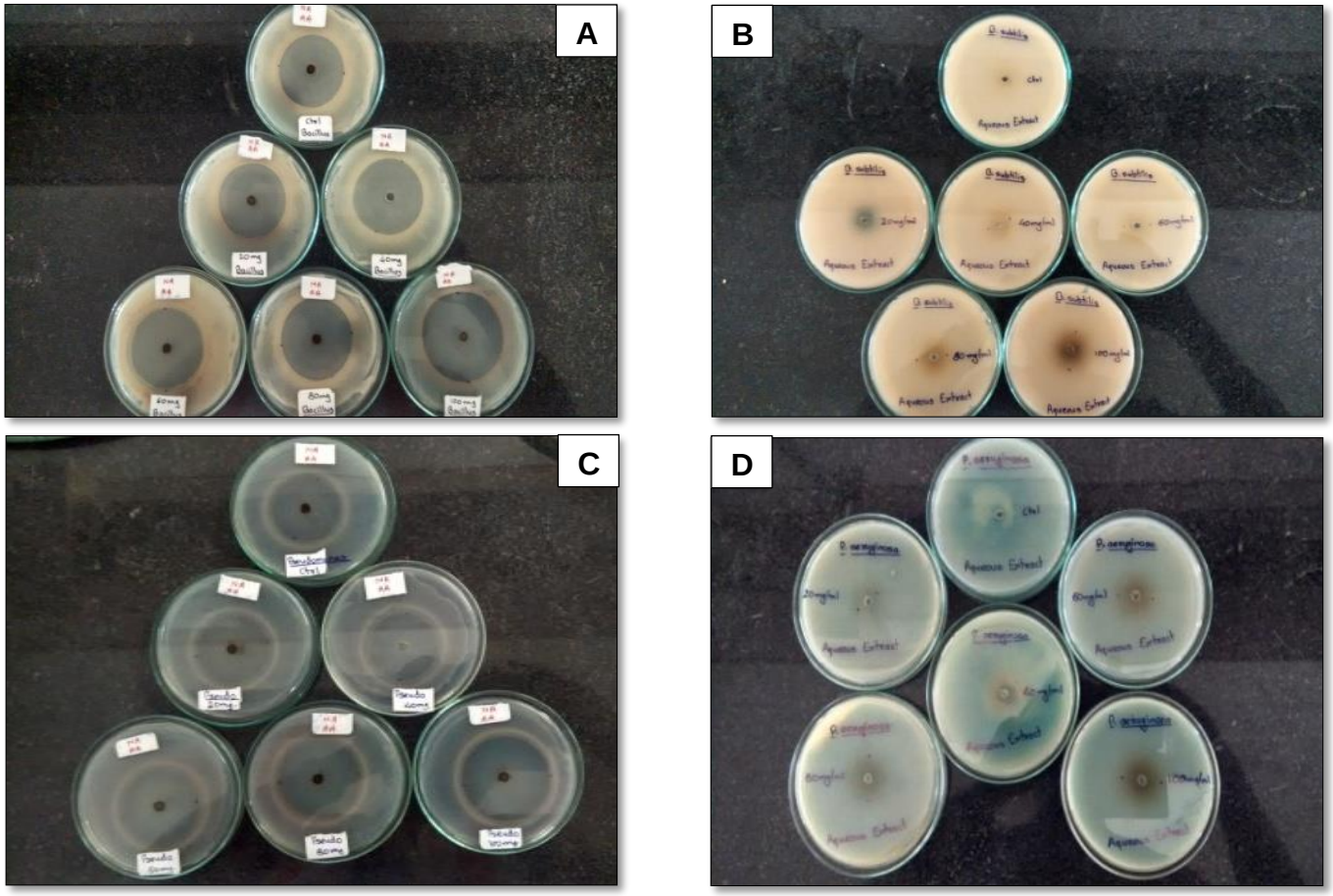

Fig 1 Antimicrobial activity of Aqueous leave extract of *Carica papaya* against (a) *P. aeruginosa* (b) *B. subtilis* (c) Antimicrobial activity of acetic acid extract of *Carica papaya* against *P. aeruginosa* and *B. subtilis* compared with (d) control by poison plate method

Table 1 Diameter of zones of inhibition (mm) against *P. aeruginosa* and *B. subtilis* for the acetic acid extract

Plant	Extract solvent (mg/ml)	Diameter of the inhibitory zone (mm)	
		P. aeruginosa	*B. subtilis*
Papaya (*Carica papaya*)	Acetic acid	10	10
	20	15	14
	40	19	17
	60	22	19
	80	24	22
	100	26	25

Table 1 Diameter of zones of inhibition (mm) against *P. aeruginosa* and *B. subtilis* for the aqueous extract

Plant	Extract solvent (mg/ml)	Diameter of the inhibitory zone (mm)	
		P. aeruginosa	*B. subtilis*
Papaya (*Carica papaya*)	Water	-	-
	20	41	23
	40	42	28
	60	44	32
	80	48	32
	100	55	44

Phytochemical analysis

Data depicted in (Table 1) shows the presence of carbohydrates, sugars, fats, proteins, amino acids, steroids, glycosides, flavonoids, tannins and phenolic compounds, organic acid (citric acid), vitamin 'C' etc. Several factors predispose bacteria to antibacterial agents, such as, previous encounters with the agents or the nature of medium used that may affect the diffusion ability of the agent. The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of certain ailments. The fact that the extracts were active against both gram-negative and gram-positive bacteria tested may be an indication of the broad-spectrum activity of the extract [12]. This observation is significant because of the possibility of developing therapeutic substances that will be active against multidrug resistant organisms. Therefore, this result shows the importance of the leaf extracts in antibiotics to control resistant bacteria that are becoming a threat to human health. The antibacterial activity is because of the phytoconstituent [6].

Table 2 Analysis of organic and inorganic components. (+) indicates presence and (-) indicates absence of the component

S. No.	Phytochemical compound	Aqueous extract	Acetic acid extract
1)	Tests for carbohydrates		
	Molisch's test (General test)	+	+
a)	Test for reducing sugars		
I.	Fehling's test	+	_
II.	Benedict's test	++	_
	Test for monosaccharides		
	Barfoed's test	+	_
b)	Test for pentose sugars		
I.	Bial's Orcinol test		
II.	Test solution + HCl + Pholoroglucinol	_	_
c)	Test for hexose sugars		
I.	Selwinoff's test (for ketohexose like fructose)	_	_
II.	Tollen's Pholoroglucinol test for galactose	++	+
III.	Cobalt-chloride test	+	+
d)	Test for gums		
	Test sample + dil. HCl +Benedict's test	_	_
2)	Tests for proteins		
I.	Biuret test (General test)	++	+
II.	Millon's test (for proteins)	+	+
III.	Xanthoprotein test (for protein containing tyrosine or tryptophan)	++	+
IV.	Test for proteins containing Sulphur	+	+++
3)	Tests for amino acids		
I.	Ninhydrin test (General test)	+	+
II.	Test for tyrosine	++	++
III.	Test for tryptophan	+	-
IV.	Test for cysteine	-	+
4)	Tests for fats and oils		
	Thick section of drug on glass slide + Sudan III reagent + After 2 min, wash with 50% alcohol + Mount in glycerin + Observe oil globules appear red	+	+
5)	Tests for steroid		
I.	Salkowski reaction	++	+
II.	Liebermann – Burchard reaction	+	++

	III.	Liebermann's reaction	+	+
6)		Tests for glycosides		
a)		Test for cardiac glycosides		
	I.	Legal's test (Test for cardenoloids)	++	++
	II.	Test for deoxysugars (Keller – Killiani test)	+	++
	III.	Liebermann's test (Test for bufadenolids)	+	+
	IV.	Raymond's test	-	-
b)		Test for anthraquinone glycosides		
		Borntrager's test for anthraquinone glycosides	+	+
c)		Test for cyanogenetic glycoside		
		Grignard reaction test	+	+
d)		Test coumarin glycosides		
	I.	Coumarin glycosides	-	+
	II.	Alcoholic extract when made alkaline show	+	-
7)		Tests for flavonoids		
	I.	Shinoda test	+	+
	II.	Sulphuric acid test	+	++
	III.	Sample residue + Lead acetate	+	-
	IV.	Sample residue + NaOH	+	+
	V.	Test solution + Zinc +Heat	+	+
8)		Tests for alkaloids		
	I.	Dragendorff's test	+	+
	II.	Mayer's test	+	+
	III.	Wagner's test	+	+
	IV.	Murexide test for purine alkaloids	-	-
	V.	Tannic acid test		
9)		Tests for tannins and phenolic compounds		
	I.	5% $FeCl_3$ solution	++	-
	II.	Lead acetate solution	+++	+
	III.	Gelatin solution	+	+
	IV.	Bromine water	-	-
	V.	Acetic acid solution	-	-
	VI.	Potassium dichromate	++	+
	VII.	Dilute iodine solution	+	+
	VIII.	Dilute HNO_3	+	+
	IX.	Dilute NH_4OH and potassium ferricyanide solution	+	+
	X.	NH_4OH + 10% $AgNO_3$ + Heat	+	+
	XI.	Dil. Potassium permanganate solution	-	-
10)		Tests for acidic compounds		
	I.	Test solution + Sodium bicarbonate		
	II.	Test solution + Litmus paper		
11)		Tests for organic acids		
		Calcium chloride test	Citric acid present	Citric acid present
		Confirmatory test for citric acid		
		2-3 ml test solution + one drop dil. NH_4OH + excess $AgNO_3$ + Boil 15 min	++	++
12)		Tests for vitamins		
a)		Tests for vitamin A	-	-
b)		Tests for vitamin C (Ascorbic acid)		
	I.	Dil. 1ml of 2% w/v solution with 5ml of water + 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside + 2ml dil. NaOH + 0.6 ml HCl dropwise stir	++	++
	II.	2ml of 2% w/v solution + 2ml water +0.1 g $NaHCO_3$+ 20mg $FeSO_4$ + shake	++	++

Thin layer chromatography

The results of TLC profiling showed the presence of flavonoid (Rf 0.86); alkaloid (Rf 0.56), flavonoid (Rf0.8) and tannin (Rf 0.92) in aqueous extract. Acetic acid extract showed the presence of alkaloid (Rf value 0.25, 0.92), flavonoid (Rf 0.82), tannins (Rf 0.84) and phenol (Rf 0.8). It was observed that among the two extracts aqueous extract was found effective in extracting maximum number of secondary metabolites [13]. Different Rf values of the compounds provides an idea about their polarity that may also help in selecting a particular solvent system for further isolation of any compound from the plant extracts using chromatographic and spectroscopic techniques. Compound showing high Rf value in less polar solvent system have low polarity while those with a low Rf value have high polarity [11].

CONCLUSION

This study has shown the antimicrobial activities and phytochemical screening of *Carica papaya* leaf extracts. Phytochemicals such as alkaloids, saponins, flavonoids, tannins and glycosides were present in both extracts. These

phytoconstituents are responsible for the antimicrobial activity of the leaf extracts. This was evidenced from the zone of inhibition in the antimicrobial activity against tested organisms used for the study. The zone of inhibition for the aqueous extracts suggests the significant degree of efficacy on test organisms. This research has confirmed the antimicrobial properties of the leaves extracts of *Carica papaya* and can be suggested that *Carica papaya* may be recommended as useful source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost effective.

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