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IN VITRO EVALUATION OF ANTIMICROBIAL PROPERTIES OF *Carica papaya*

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ABSTRACT

In the present study Cold aqueous, hot aqueous, 70 % Ethanol, 80 % Methanol and Acetone extracts of *Carica papaya* (dry leaf, green leaf, root, stem, ripe pulp, unripe pulp, ripe peel, unripe peel and seed) in different concentrations were evaluated for their antimicrobial properties against pathogenic microorganism such as *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherchia coli* and fungal pathogenic microorganism *Candida albicans, Aspergillus niger* and *Trichophytom rubrum* using agar well diffusion method. All the extracts were effective against used pathogens but hot aqueous extract of ripe peel showed best antibacterial activity forming a zone of inhibition of 13 mm against the *Escherchia coli*, which was equal to the zone of inhibition formed by the standard antibiotic Tetracycline (500 µg/ml). 80 % methanol extract of unripe pulp shows an average zone of inhibition of 18 mm against the *Candida albicans*, which was nearly equal to the zone of inhibition of Fluconazole.

Keywords: *Carica papaya*, Antimicrobial Properties, Agar well Diffusion, Tetracycline, Fluconazole

INTRODUCTION

Carica papaya is the sole species in the genus *Carica* of the plant family *Caricaceae*. It is native to the tropics of the Americas, and was first cultivated in Mexico **[1]**. The papaya is a large tree with a single stem growing from 5 to 10 metres tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is

conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 centimetres diameter. The flowers are similar in shape to the flowers of the *Plumeria*, but are much smaller and wax-like. They appear on the axils of the leaves, maturing into the large 15–45 centimetres long. Papaya fruit is a rich source of nutrients such as provitamin A carotenoids, vitamin C, vitamin B complex, dietary minerals and dietary fibre. Papaya skin, pulp and seeds also contain a variety of phytochemicals, including natural phenols. Danielone is a phytoalexin found in the papaya fruit and showed high antifungal activity against *Colletotrichum gloesporioides*, **[2]** pathogenic fungus of papaya. The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper. Papaya leaves are made into tea as a treatment for malaria.

Table 1: Classification of *Carica papaya*

Kingdom	***Plantae***
Order	***Brassicales***
Family	***Annonaceae***
Genus	***Carica***
Species	***Carica papaya***

Herbalism is the study and use of medicinal properties of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, herblore, and phytotherapy. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Pharmacognosy is the study of medicines derived from natural sources. The use and over use of synthetic antibiotics has led to increase in drug resistance bacteria. Prescription antibiotics tend to wipe out good and bad bacteria, so that the immunity of the body decreases. Natural/Herbal antibiotics are "smart" in a way that typical antibiotics are not. Natural/Herbal antibiotics have no side effects on the human body. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-norcotic, having no side-effect, easily available at the affordable price and sometime the only source of health care available to the poor **[3]**.

Looking at the importance of herbal antimicrobials and having an idea of antimicrobial nature of *Carica papaya* as has been reported by **[4, 5]** the present study was designed in order to decipher the antimicrobial properties of *Carica papaya* with following objectives in mind:

- Collection of plant samples [Leaves (green and dry), stem, root, fruit

(ripe and unripe), peel (ripe and unripe) and seeds].

- Collection of pathogens against which activity was to be checked. [Bacterial pathogens (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) and Fungal pathogen (*Aspergilus niger, Candita albicans, Trichophyton rubrum*).
- Extraction of antimicrobial components (secondary metabolites) using solvent extraction procedure.
- Assessment of antibacterial and antifungal properties of extracted metabolites.

MATERIALS AND METHODS

Plant Samples

Carica-papaya leaves (green and dry), stem, root, fruit (ripe and unripe), peel (ripe and unripe) and seeds were collected from various places in Lucknow after proper identification by the experts at **MRD LifeSciences, Lucknow**. Samples were washed with tap water and later with distilled water, sun dried and ground into powder by the help of a grinder.

Pathogens

Bacterial and Fungal pathogens available at **MRD LifeSciences (P) Ltd., Lucknow,** availed from IMTECH, Chandigarh were sub-cultured and used throughout the study.

Preparation of Plant Extracts

Leaves (green and dry), stem, root, fruit (ripe and unripe), peel (ripe and unripe), seeds of *Carica papaya* were used for extraction of antimicrobial components by using solvents such as cold aqueous, hot aqueous, 70 % ethanol, 80% methanol, Acetone. 4 gm of powdered samples were soaked in 40 ml of Distilled water, 70% ethanol, 80% methanol, Acetone. Containers were kept in dark for 3-5 days. The solutions were filtered and left in hot air oven at 50ºC till the extract got dried. The amounts of dried metabolites obtained from all extracts were dissolved in Dimethyle sulfoxide (DMSO) making extracts of different concentrations as shown in **Table 2**. For hot water extraction 4 gm powdered sample was added to 40 ml of hot water and kept in water bath at 85°C for 2 hours, solution was filtered and left in hot air oven for drying.

Table 2: Different Concentrations of Extracts

S. No	Sample	Solvent	Weight of Empty P.P. (gm)	Weight of P.P. + Metabolites (gm)	Weight of Metabolite (gm)	DMSO (ml)	Concentration (gm/ml)
1.	Dry leaves	Cold aqueous	30.32	30.51	0.19	0.48	0.39
2.	Dry leaves	Hot aqueous	27.03	27.16	0.13	0.36	0.36
3.	Dry leaves	70% Ethanol	27.87	28.15	0.28	0.66	0.42
4.	Dry leaves	80% Methanol	32.37	32.63	0.31	0.72	0.43
5.	Dry leaves	Acetone	27.28	27.44	0.16	0.42	0.38
6.	Green leaves	Cold aqueous	27.25	27.76	0.51	1.2	0.42
7.	Green leaves	Hot aqueous	29.35	29.59	0.24	0.58	0.41
8.	Green leaves	70% Ethanol	27.89	28.21	0.32	0.74	0.43
9.	Green leaves	80% Methanol	27.99	28.26	0.27	0.54	0.50
10.	Green leaves	Acetone	29.35	29.59	0.24	0.48	0.50
11.	Stem	Cold aqueous	29.79	29.89	0.10	0.30	0.33
12.	Stem	Hot aqueous	29.21	29.35	0.14	0.38	0.36
13.	Stem	70% Ethanol	29.94	30.09	0.15	0.30	0.50
14.	Stem	80% Methanol	29.93	30.07	0.14	0.28	0.50
15.	Stem	Acetone	29.35	29.36	0.01	0.12	0.08
16.	Root	Cold aqueous	32.72	32.80	0.08	0.26	0.30
17.	Root	Hot aqueous	32.49	32.51	0.02	0.14	0.14
18.	Root	70% Ethanol	27.86	27.90	0.04	0.18	0.22
19.	Root	80% Methanol	32.50	32.54	0.04	0.18	0.22
20.	Root	Acetone	27.89	27.90	0.01	0.12	0.08
21.	Ripe pulp	Cold aqueous	28.23	28.32	0.09	0.28	0.32
22.	Ripe pulp	Hot aqueous	27.25	27.76	0.51	1.2	0.42
23.	Ripe pulp	70% Ethanol	32.35	34.11	1.76	3.5	0.50
24.	Ripe pulp	80% Methanol	32.50	32.54	0.04	0.18	0.22
25.	Ripe pulp	Acetone	27.99	28.04	0.05	0.10	0.50
26.	Ripe peel	Cold aqueous	31.62	32.05	0.43	0.86	0.50
27.	Ripe peel	Hot aqueous	29.46	29.82	0.36	0.82	0.43
28.	Ripe peel	70% Ethanol	32.35	32.82	0.47	0.94	0.50
29.	Ripe peel	80% Methanol	28.33	28.69	0.36	0.72	0.50
30.	Ripe peel	Acetone	27.86	27.91	0.05	0.10	0.50
31.	Seed	Cold aqueous	30.03	30.14	0.11	0.40	0.27
32.	Seed	70% Ethanol	29.82	29.94	0.12	0.24	0.50
33.	Seed	80% Methanol	32.72	32.80	0.08	0.36	0.22
34.	Unripe pulp	Cold aqueous	27.84	28.00	0.16	0.32	0.50
35.	Unripe pulp	70% Ethanol	30.91	31.20	0.29	0.58	0.50
36.	Unripe pulp	80% Methanol	30.42	30.69	0.27	0.54	0.50
37.	Unripe peel	Cold aqueous	30.93	31.09	0.16	0.32	0.50
38.	Unripe peel	70% Ethanol	28.42	28.77	0.35	0.70	0.50
39.	Unripe peel	80% Methanol	29.92	30.37	0.45	1.00	0.45

Antibiogram Analysis of Extracts

Antibacterial Properties

For assessment of antibacterial properties of extract, after solidification of nutrient agar plates 25 µl Bacterial pathogens were spread on respective plates and wells of 8 mm diameter were bored by the help of sterile borer. 50 µl of antimicrobial extract, autoclaved DMSO and standard antibiotic tetracycline (0.5mg/ml) were loaded into the wells. Plates were incubated at 37 ºC for 24 hrs, and observed for Zone of inhibition.

Antifungal Properties

For assessment of antifungal properties of extracts, after solidification of potato dextrose agar plates 25 µl fungal pathogens were spread on respective plates and wells of 8 mm diameter were bored by the help of sterile borer. 50 µl of antimicrobial extract, autoclaved DMSO and standard antifungal drug Fluconazole (5 mg/ml).were loaded into the wells. Plates were incubated at 37 ºC for 24 hrs, and observed for Zone of inhibition.

RESULTS

Antibacterial Properties

All the extracts were assessed for their antibacterial properties by agar well diffusion method, results of the same can be seen in the **Table 3-11** below.

Table 3: Antibacterial Properties of Dried Leaves Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	***S. aureus***	***E. coli***
Aqueous	**00**	**00**	**00**
Hot aqueous	**13**	**12**	**12**
70% Ethanol	**00**	**00**	**12**
80% Methanol	**15**	**15**	**17**
Acetone	**13**	**13**	**13**
TETRACYCLINE	**27**	**29**	**28**

Table 4: Antibacterial Properties of Green Leaves Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	00	00	12
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
Acetone	00	00	00
TETRACYCLINE	27	29	28

Table 5: Antibacterial Properties of Stem Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	00	12	00
Hot aqueous	16	16	15
70% Ethanol	20	28	20
80% Methanol	18	24	20
Acetone	12	00	00
TETRACYCLINE	27	29	28

Table 6: Antibacterial Properties of Root Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	13	11	00
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
Acetone	12	00	00
TETRACYCLINE	27	29	28

Table 7: Antibacterial Properties of Ripened Pulp Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	00	00	00
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	11	00	00
Acetone	12	00	00
TETRACYCLINE	27	29	28

Table 8: Antibacterial Properties of Unripened Pulp Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	15	00	00
70% Ethanol	12	12	10
80% Methanol	13	13	11
TETRACYCLINE	27	29	28

Table 9: Antibacterial Properties of Ripened Peels Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	12	00	00
Hot aqueous	12	00	13
70% Ethanol	13	11	12
80% Methanol	00	12	12
Acetone	12	12	00
TETRACYCLINE	27	29	28

Table 10: Antibacterial Properties of Unripened Peel Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	00	00	12
70% Ethanol	00	12	00
80% Methanol	13	13	12
TETRACYCLINE	27	29	28

Table 11: Antibacterial Properties of Seeds Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
Aqueous	12	00	00
70% Ethanol	00	00	00
80% Methanol	13	12	14
TETRACYCLINE	27	29	28

Antifungal Properties

All the extracts were assessed for their antifungal properties by agar well diffusion method, results of the same can be seen in the **Table 12-20** below.

Table 12: Antifungal Properties of Dried Leaves Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	00	00	12
Hot aqueous	00	00	00
70% Ethanol	00	12	00
80% Methanol	12	00	00
Acetone	00	00	00
FLUCONAZOLE	27	34	32

Table 13: Antifungal Properties of Green Leaves Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	11	00	00
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
Acetone	11	00	00
FULCONAZOLE	27	34	32

Table 14: Antifungal Properties of Stem Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	00	00	00
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
Acetone	00	00	00
FULCONAZOLE	27	34	32

Table 15: Antifungal Properties of Root Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	00	00	00
Hot aqueous	00	13	00
70% Ethanol	12	00	00
80% Methanol	00	00	00
Acetone	00	00	00
FULCONAZOLE	27	34	32

Table 16: Antifungal Properties of Ripened Pulp Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	00	12	00
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
Acetone	00	00	00
FULCONAZOLE	27	34	32

Table 17: Antifungal Properties of Unripened Pulp Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	00	14	00
70% Ethanol	15	00	12
80% Methanol	12	18	00
FULCONAZOLE	27	34	32

Table 18: Antifungal Properties of Ripened Peels Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	00	13	00
Hot aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
Acetone	00	00	00
FULCONAZOLE	27	34	32

Table 19: Antifungal Properties of Unripened Peel Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Cold aqueous	00	00	00
70% Ethanol	00	00	00
80% Methanol	00	00	00
FULCONAZOLE	27	34	32

Table 20: Antifungal Properties of Seeds Extracts

SAMPLES	ZONE OF INHIBITION (In mm)		
	A. niger	*C. albicans*	*T. rubrum*
Aqueous	13	15	13
70% Ethanol	10	00	00
80% Methanol	00	14	16
FULCONAZOLE	27	34	32

DISCUSSION

Herbal medicines are valuable and readily available resources for primary health care system. Undoubtedly the plant kingdom still holds many species of the plant containing substances of medicinal values that are yet to be discovered, though large numbers of plants are constantly being screened for this antimicrobial properties but more pharmacological investigation is necessary.

Plant extracts were prepared using solvents like cold aqueous, hot aqueous, 70% ethanol, 80% methanol and acetone by solvent extraction procedures as has been done earlier by **[6, 7]**.

Antibacterial and antifungal properties of prepared extracts were assessed by the help of agar well diffusion method of **Kirby Bauer** as has been used earlier by **[1, 6, 9]**.

In case of antibacterial assay of dried leaf, green leaf, ripe pulp, ripe peel, root, stem, unripe pulp, unripe peel, seeds maximum zone of inhibition of 15mm, 12mm, 12mm, 13mm, 13mm, 28mm,15mm, 13mm, 14mm against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Escherichia coli, Pseudomonas aeruginosa,*

Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli were seen respectively.

Similarly, in case of antifungal assay of dried leaf, green leaf, ripe pulp, ripe peel, root, unripe pulp, seeds maximum zone of inhibition of 12mm, 11mm, 12mm, 13mm, 13mm, 18mm, 15mm against *Trichophyton rubrum, Aspergilus niger, Candita albicans, Candita albicans, Candita albicans, Candita albicans, Candita albicans* was observed respectively. However in stem and unripe peel no zone of inhibition is seen.

Nearly all the extracts used in the study were effective against bacterial and fungal pathogens however hot aqueous extract of ripe peel and 80 % methanol extract of unripe pulp showed best results against both bacterial and fungal pathogens showing a zone of inhibition of 13mm and 18mm against the *Escherichia coli* and *Candita albicans* respectively, the zone was comparable to the zone of inhibition formed by standard drugs Tetracycline and Flucanazole used in the study. So it can be said that hot aqueous extract of ripe peel and 80 % methanol extract of unripe pulp could be used as an effective drug after proper pharmacological evaluation. Earlier water and organic extracts have been shown to be effective by **[5]**; methanol extracts by **[6]**; methanol extracts by **[7]**; cold water extracts by **[9]**.

CONCLUSION

Based on the above research work it can be concluded that *carica papaya* can be very good source for herbal drug and specially the solvent like cold aqueous, hot aqueous, 70% ethanol, 80% methanol and acetone can be explored further for the extraction of antimicrobials by more sophisticated procedures for extraction in order to increase the yield.

Further work also includes the further pharmacological investigation of the solvents extracts and also the investigation of phytochemicals responsible for antimicrobial activity.

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