

HPTLC Finger Printing Profile and Antimicrobial Evaluation of Papaya, *Carica papaya*

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ABSTRACT

Papaya is simply known as Pawpaw, belonging to the family of Caricaceae. *Carica papaya* leaves exclude a number of essential medicinal compounds. The aim of the study was to investigate the phytochemicals, HPTLC finger printing and antimicrobial activity of chemical compounds of *Carica papaya*. Antimicrobial activity of *Carica papaya* leaf extract against yeast, TBC, *E. coli*, *Pseudomonas* and *Salmonella* were examined. Aqueous leaf extract showed more inhibitory actions *in vitro*. HPTLC profiling of the chemical compounds indicated the presence of papain and chymopapain.

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KEY WORDS : *Carica papaya*, Disease, Leaf extract, Medicinal plant.

Introduction

Carica papaya is also known as papaya belongs to family Caricaceae. Papaya is large tree-like plant, with a single stem growing and arranged in twisting leaves to the top of the trunk. Papaya was originated from Central America. The plant of papaya has described with a large variety of adjectives, which confesses the structural and functional complications and high phenotypic plasticity type of enormous tropical herbs⁸. Plants and their parts such as roots, stems, shoots, leaves, flowers, fruits, seeds, and efflux form crucial constituents are used for traditional herbal drugs⁷. The chemical activity of traditional medicine which is used for the treatment of human disease by the natural compound is important in herbal formulation¹⁰. Papaya is used in improving a risk of heart disease, diabetes, cancer, digestion, wound healing and low blood pressure. Papaya leaf juice is used for boosting up platelet count in the people to reduce the effect of diseases. Papaya is a semi-woody, sole stemmed, usually latex manufacture, short-life everlasting herbs⁹. It incorporates six genera and 35 species^{3,6}. Papaya is a polygamous species and is recognized as a difficult plant even it is a male, female and hermaphroite plant².

Carica papaya leaves, fruits and seeds are entailing high level of antioxidants, vitamins A, C and E. Papain and chymopapain help to chronic anarchic conditions like arthritis and asthma. Vitamins help to boost the immune system, fight against the bacterial and viral illnesses. Papaya is a naturally tastes sweet and soft texture. It is also a good source of foliate, fiber,

copper, potassium, magnesium, pantothenic acid.

Material and Method

Collection and identification of plant material

Fresh healthy, disease free, mature, plant leaves of papaya were handpicked from the tree. Leaves were authenticated in the Department of Biological Sciences. Fresh leaves were washed properly in sterile distilled water and dried in a dark place at room temperature for a few days. Dried plant samples were ground in grinder and stored in an airlift container protected from sunlight until required for analysis.

Preparation of Plant extract

2g powder sample was extracted with 50ml of water and ethanol through rotator for 4 hours. For microbial activity - 2g sample was extracted with 25ml distilled water. Over the rotary shaker, the samples were shaken for 4 hours. For HPTLC - 2g sample was extracted with 50ml methanol through the rotary shaker, shook for 6 hours. Every time the extract was filtered through filter paper to remove unrestricted substance.

Phytochemical Qualitative Analysis

Phytochemical constituents were determined qualitatively using standard procedures⁵ with slight modification.

Test for Carbohydrate -Fehling test, Alkaloid and Dragendoff test were performed.

Test for Protein - Ninhydrin test was performed.

Test for Steroids and Tannin - the methodology was performed⁴.

TABLE-1: Microbial Activity

Media	Specific Pathogen
EMB Agar	<i>E. coli</i>
Soyabean Casein Digest Agar	<i>TBC</i>
Potato Dextrose Agar	<i>Yeast & Mould</i>
Cetrimide Agar Base	<i>Pseudomonas</i>
Violet Red Bile Glucose	<i>Staphylococcus</i>
Salmonella Agar	<i>Salmonella</i>

Test for Flavonoids and Glycosides - Alkaline reagent test was followed¹⁰.

Test for Saponins - Foam test was done¹.

Test for Phenol - Ferric Chloride solution.

Microbial Activity

Six different media were prepared for microbial activity against six pathogens mentioned in Table-1.

HPTLC (High Performance Thin Layer Chromatography)

HPTLC is a sophisticated, automated and used for separation of components present in mixture both quantitatively as well as qualitatively. For medicinal plant sufficient quantity of Methanol extract after dissolving in methanol was passed through 8µ millipore membrane filter unit. About 5µl was applied on HPTLC plates with WINCATES software. After development of plates in appropriate mobile phase solvent system (Toluene: Ethyl acetate: Formic acid) the plates were photo documented at ultraviolet light at 254 nm and 366 nm. The Rf values and color of the band were recorded. The overall experiments were repeated thrice.

Results and Discussion

Phytochemical tests

The phytochemical constituents of *Carica papaya* were estimated in aqueous and alcoholic extracts. In *Carica papaya*, Phenol, Carbohydrate, Protein, Tannin, Alkaloid, Glycoside, Flavonoids and Steroids were present in both the extracts whereas Saponin and Reducing sugar were almost absent (Table-2).

Antimicrobial Activity

The antimicrobial activity was evaluated against six

TABLE 2: Phytochemical constituents of *Carica papaya* leaf extract

Phytochemicals	Alcoholic	Aqueous
Carbohydrate	+	+
Protein	+	+
Phenol	+	+
Saponin	-	-
Tannin	+	+
Alkaloid	+	+
Flavonoids	+	+
Glycosides	+	+
Steroids	+	+
Reducing sugar	-	-

Key: + Present, - Absent

pathogens and the findings are mentioned in Table-3.

HPTLC fingerprints profile of Papaya leaf

The presence of bio-active stuff in papaya has been expressed to bestow resistance to plants against bacteria, fungi and pests. The antimicrobial activity of

TABLE-3: Microbial activity of Papaya extract

Name of Pathogen	Result
<i>Yeast & Mould</i>	200g
<i>TBC</i>	400g
<i>Staphylococcus</i>	Absent
<i>Salmonella</i>	Absent
<i>E.coli</i>	Absent
<i>Pseudomonas</i>	Absent

TABLE-4: R_f values of HPTLC Fingerprints profile of Papaya leaf after Derivatization

S. No.	R_f value	245 nm	366 nm before derivatization	366 nm after derivatization
1	R_{f1}	0.20 black	0.11 (sky blue)	0.23 (sky blue)
2	R_{f2}	0.44 black	0.22 (sky blue)	0.46 (sky blue)
3	R_{f3}	0.94 black	0.32 (sky blue)	0.65 (grey)
			0.45 (sky blue)	0.92 (red)
			0.57 (red)	
			0.66 (purple)	
			0.76 (red)	
			0.89 (red)	

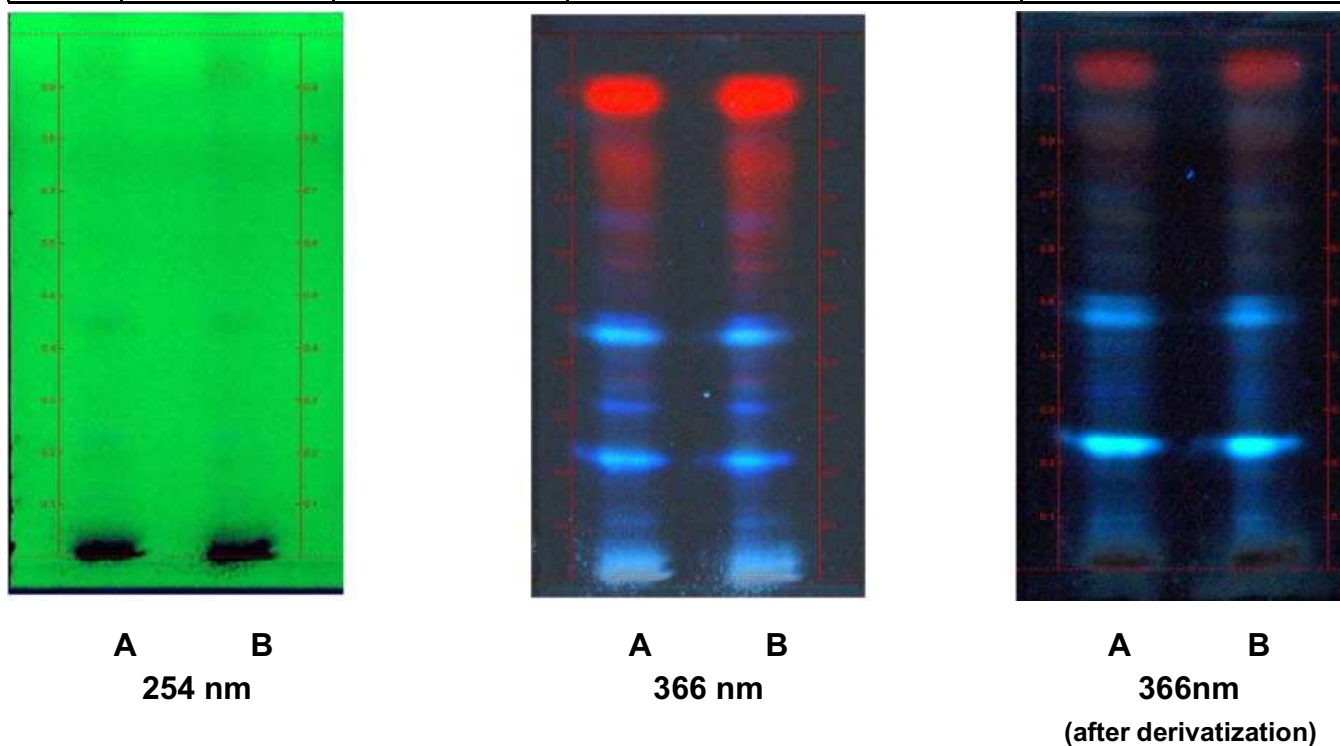


Fig.1: HPTLC fingerprints profile of Papaya leaf

papaya extract could be imputed to different bio-active compounds, which was authenticated by the phytochemical analysis, showing the presence of alkaloids, glycoside, flavonoids, protein, tannin and steroid but absence of saponin and reducing sugar (Table-2). The presence of ascorbic acid in *Carica papaya* leaves indicates that the plant could be used in

herbal medicine for the treatment of common cold and other diseases like prostate cancer. HPTLC fingerprint profile of the test solution is depicted in (Fig.1) indicates the presence of different types of phytochemicals like Papain and Chymopapain. The fingerprint profile would serve as a reference standard of the authentic sample. The TLC plate was examined under 254nm, 366nm, and

after derivatization 366nm. The R_f values and colors of the bands captured.

Conclusion

The screening of phytochemical constituent's results demonstrated the presence of various bio-active metabolites. The antimicrobial activity results showed the inhibitory activity of *Carica papaya*, the demonstration of antimicrobial activity of *Papaya* against the test organisms has provided a scientific basis for its local usage as a medicinal plant also. It is an indication that

the plant is a potential source for production of drugs with a broad spectrum activity. The phytochemicals of papaya are to be observed as potential sources of antimicrobials and can be made available commercially for its medicinal value. This study demonstrated that the herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. Using different purification, isolation and characterization methods, antimicrobial products can be obtained and thus the activity of antimicrobial compounds can be improved for further pharmaceutical uses.

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