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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITIES OF PAPAYA *CARICA PAPAYA* L.

***Premalatha, M. and Lakshmi, S.**

PG and Research Department of Botany, Arignar Anna Government Arts College, Villupuram –605 602, Tamil Nadu, India

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ABSTRACT

This study aimed to determine the antibacterial activity on *Carica papaya* leaves. The objective of the study uses bacteria to resistant or susceptible characteristic over the phytochemicals obtained from the papaya leaf by applying two different solvent systems. The study also targets the reactive oxygen species antioxidant role of the leaf extracts as a bioactive component in removing off the free radicals with various chemically obtained free radicals by in vitro study by evaluating the capability by absorption spectrum. The activity of the extracts calibrated with its activity upon the bacteria S.aureus by locating the type of phytochemicals present in the extract and its role on the bacteria been studied with studying the fingerprinting by thin layer chromatography.

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INTRODUCTION

Carica papaya L. is commonly known for its food and nutritional value throughout the world. Medicinal plants have become the focus of intense study for potential pharmacological effects. Present day 80% of individual from developed countries use traditional medicine which has compound derived from medicinal plants. *Carica papaya* has been used as remedy against a variety of diseases. *Carica papaya* is a neutraceutical plant having a wide range of pharmacological activities. The whole plant has its own medicinal value. Papaya is a powerhouse of nutrients and is available throughout the year. Leaves have been poultice into nervous pains, elephantoid growth. Papaya leaves are made into tea as a treatment for malaria.

MATERIALS AND METHODS

Collection of plant materials: Fresh papaya green leaves were obtained from home garden in Ethiraj province, Villupuram, Villupuram district, Tamil Nadu and was dried by applying the leaves over shade for about a week.

****Corresponding author:*** **Premalatha, M.,**
PG and Research Department of Botany, Arignar Anna Government Arts College, Villupuram –605 602, Tamil Nadu, India.

The dried leaves were grinded using a blender to a fine powder. The total weight of the leaf powder was measured as 408.4g.

Samples preparation and extraction: Five hundred grams of papaya samples were cleaned and washed with tap water. They were chopped into small pieces and homogenized using a blender for 2 minutes. The homogenized samples were kept in the freezer maintained at 800°C for three days. Later, all the samples were grounded into fine powder using a dry grinder and stored in a freezer at 200°C before extraction. Ten grams of samples were homogenized in 250 ml 80% (v/v) ethanol and chloroform at room temperature. The mixture was shaken using shaking incubator at 200 rpm for 120 min at 500°C. The mixture was then centrifuged at 3000 rpm for 15 min at room temperature and the supernatant was taken. This supernatant was stored at 200°C until further analysis

Test organisms: Cultures *of Staphylococcus aureus* were obtained from the Refsyn Bioscience Laboratory at Pondicherry. Purity plates of the bacterial isolates were obtained by culturing on their respective selective media. Biochemical tests were performed to reidentify and confirm the identity of the isolates. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Discrete colonies of fresh cultures of the different bacterial isolates were then picked and suspended in 5 ml nutrient broth (NB,Oxoid), in well-labeled sterile Bijou bottles, and incubated for 24 hours at 37ºC prior to antimicrobial susceptibility testing.

Culture medium: Approximate formula per liter of each medium was as following;

Nutrient agar: Hi media nutrient agar was used for preparation of stock cultures on agar slopes the basic agar culture. Peptone 5g, Sodium chloride 5g, Beef extract 1.5g, Yeast extract 1.5g, Agar 15g, Distilled water 1litre, pH 7.4.

Mueller hinton agar (MHB): Difco Muller Hinton broth was the medium used for determining the antimicrobial susceptibility testing. Beef infusion 300g, Casein hozdrolasate 17.5g, Starch 17g, Agar 17g, Distilled water 1litre, pH7.3, DPPH 0.1M in methanol, NaOH 2%, HCL1%, jones reagent 1% Chromic acid in Concentrated Sulphuric acid, H2O2 40mM and Sodium carbonate 7.5%.

Determination of antibacterial activities: The crude extracts and the fractions were tested for antibacterial activity using *Staphylococcus aureus* as test organisms by the Kirby-Bauer disc diffusion method. 90 ml of distilled water was added 1.3 g nutrient agar and dissolved. It was then autoclaved at 121ºC for 15 min for complete sterilization. The agar solution was allowed to cool and 15ml was poured into 6 sterile glass petri dishes. The plates were allowed to set and then incubate overnight at 37º C to test for sterility. Nutrient agar plates were inoculated with the organisms with the organisms and incubated at 37ºC overnight. Colonies were picked from the plates and used to inoculate nutrient broth contained in test tubes. The tubes were incubated at 37ºC overnight. Disc of Whatman No.3 filter paper were sterilized by heating in an oven for 30 minutes at 80ºC. Nutrient agar plates were inoculated with 100 µl of the concentrated extracts, fractions or ampicillin (8 µg/ml) were transferred onto the agar surface of each plate using sterile forceps. The plates were then incubated at 37ºC overnight. The effectiveness of the extract as antibiotic against the test organism was determined by measuring the diameter of zone of inhibition.

Disc diffusion method: Antimicrobial activity was determined by disc diffusion method of NCCLS (1997). Briefly, 0.1 ml of 10^8 cells per ml of a suspension of the tested microorganism (*Staphylococcus aureus* ATCC 25923) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15µl of the aqueous extracts and placed on the inoculated plates. These plates, after staying at 4ºC for 2 hours, were then incubated at 37ºC for 24 hours. The diameters of the inhibition zones were measured in millimetres. Vancomycin was used as positive control.

Minimum inihition concentration (MIC): Microdilution broth susceptibility assay was used as recommended by NCCLs for the determination of MIC (1999). All tests were performed in Mueller Hinton broth (MHB ; BBL) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v). Bacterial strain was cultured overnight at 37ºC in MHA. Test strain was then suspended in MHB to give a final density of 5×10^5cfu /ml and these were confirmed by viable counts. Geometric dilution ranging from 0.036 -72.00 mg/ml of the extracts were prepared in a 96-well microtiter plate, including one growth control (MHB+Tween 80) and one sterile control MHB+ Tween 80 +test extracts. Plates were incubated under normal atmospheric conditions at 37ºC for 24 hours. The MIC of vancomycin was determined in order to control the sensitity of the test organism. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

Determination of chemical constitutents and their activity against *Staphylococcus aureus* by their chromatography (TLC) (Marica *et al.,* 2004): The analysis was performed on precoated 20×20 cm TLC plates of silica gel 60F254 by Whatman. Ten µl of each extract was applied as spots onto TLC sheets. Six different mobile phases were selected from (Marica *et al.*, 2004) to establish the optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. The plates were developed at room temperature in a vertical separating chamber to the height of approximately 17cm from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). After drying, visualization was performed in two way: in short UV light (254nm), and spraying with sulphuric acid and then chromatograms were interpreted in long wave UV light (366nm). The Rf value was identified by comparison with Rf of standards in similar conditions. Antibacterial assay against *Staphylococcus aureus* of (Chomnawang *et al.,* 2005) was adapted for this experiment. The dried developed TLC plates were placed onto smeared agar plates then kept in an incubator at 37º C for 24 hours. The inhibition zone was then measured and analyzed. The separation of phytochemicals was analyzed using its polarity adopting thin layer chromatography. The separation technique employs different solvent as mobile phase according to its polar nature. Materials are used TLC- Silica Gel G 254 (Merck made) Mobile I: Petroleum Ether: Ethyl acetate (8:2), Mobile II: n-Hexane: Ethyl acetate: Acetic acid (2:4:0:5). A portion of the TLC was sliced without touching and made a line using pencil above 0.5cm above the plate. The sample, fermented Jackfruit and raw Jackfruit were spotted on the mid line using a capillary tube and spotting was repeated thrice. Allowed the spot to dry. TLC chamber was made and the solvent was poured and covered using a lid. The spotted plate was place onto the solvent and the mobile phase eluted until the solvent phase reaches the top of the plate. The plates were air dried and kept in iodine chamber for a minute for colour visualization. The phytochemicals and their activity on *Staphylococcus aureus* was observed and interpreted. Separation by TLC (Thin layer chromatography) gives impressive output by giving almost most of the phytochemicals. The separation of phytochemicals over the bacteria does not have the antibacterial as the organism grows by degrading one of the phtyoconstituents. With different combination and proportion of the mobile phase few variations noted as the target degrades the bioactive molecules.

RESULTS AND DISCUSSION

In the present investigation has been carried out to analysis of antibacterial activities of *Carica papaya* L. against *Staphylococcus aureus.* Papaya (*Carica papaya* L.) plants were collected from home garden in Ethiraj province, shown in (figure 1.). The leaves were collected and dried. The dried leaves were than powdered (Figure 2).

Antibacterial activity: Results of the antibacterial activity by Disk diffusion method (Chloroform and ethanol) with minimal inhibiting concentration (MIC) revealed of *Carica papaya* L. have considerable antibacterial activity against *Staphylococcus aureus* with minimal inhibiting concentration shown in figure 3 and 4. Table 1 & 2 shows the results of minimum inhibiting concentration determination on the test organism, while the MIC ranging between without dilution to with dilution (1:10, 2:10) against the test bacteria.The MIC of chloroform and ethanol extracts of leaves against *Staphylococcus aureus.* The results indicates the chloroform and ethanol extracts having a low effective and the activity are resistant there is no zone of inhibition.

Determination of chemical constituents and their activity against *Staphylococcus aureus* by thin layer chromatography. Detecting phytoconstitutent and its activity TLC: The extracted leaf extract (100/µl) was inoculated aseptically with the bacterium used the experiment, *Staphylococcus aureus* in nutrient broth and incubated at 37ºC for 24 hour (figure 8).

Figure 1. Papaya plant and its leaf

Figure 2. Papaya leaf powder

Tables 1. Represents the antibacterial activity –Chloroform

Description	Organism	Method	Observation	Interpretation
A, without dilution	*S.aureus*	Disc diffusion	No zone of inhibition	Resistant
.B,1:10 dilution	*S.aureus*	Disc diffusion	No zone of inhibition	Resistant
C,2:10 dilution	*S.aureus*	Disc diffusion	No zone of inhibition	Resistant

Table 2. Represents the antibacterial activity – Ethanol

Description	Organism	Method	Observation	Interpretation
A, without dilution	*S.aureus*	Disc diffusion	No zone of inhibition	Resistant
B,1:10 dilution	*S.aureus*	Disc diffusion	No zone of inhibition	Resistant
C,2:10 dilution	*S.aureus*	Disc diffusion	No zone of inhibition	Resistant

Table 3. Rf of the extract and the Co

Chloroform Extract (S)	Chloroform extract + *S.aureus*(Co)	Ethanol extract (S)	Ethanol extract +*S.aureus*(Co)
0.08	**	0.28	0.28
0.19	**	0.34	0.34
0.25	**	0.50	0.50
0.33	**	0.00	**
0.48	0.48	0.48	0.48
0.60	0.60	0.69	0.69
0.89	0.89	0.89	0.89
0.93	0.93	0.93	0.93
0.95	0.95	0.95	0.95

Table 4. Rf value of the extracts and its co.

Chloroform Extract(C)	Chloroform extract + *S.aureus*(Co)	*S.aureus*(S)	Ethanol extract (E)	Ethanol extract *S.aureus*(Co)	*S.aureus* (S)
0.03	*	*	0.13	0.13	*
0.06	*	*	*	**	*
0.24	0.24	*	*	**	*
0.29	0.29	*	*	**	*
0.36	*	*	0.36	0.36	*
0.40	*	*	0.41	0.41	*
0.51	*	*	0.46	0.46	*
0.54	0.54	*	*	**	*
0.73	0.73	*	0.73	0.73	*
0.94	0.94	*	0.94	0.94	*

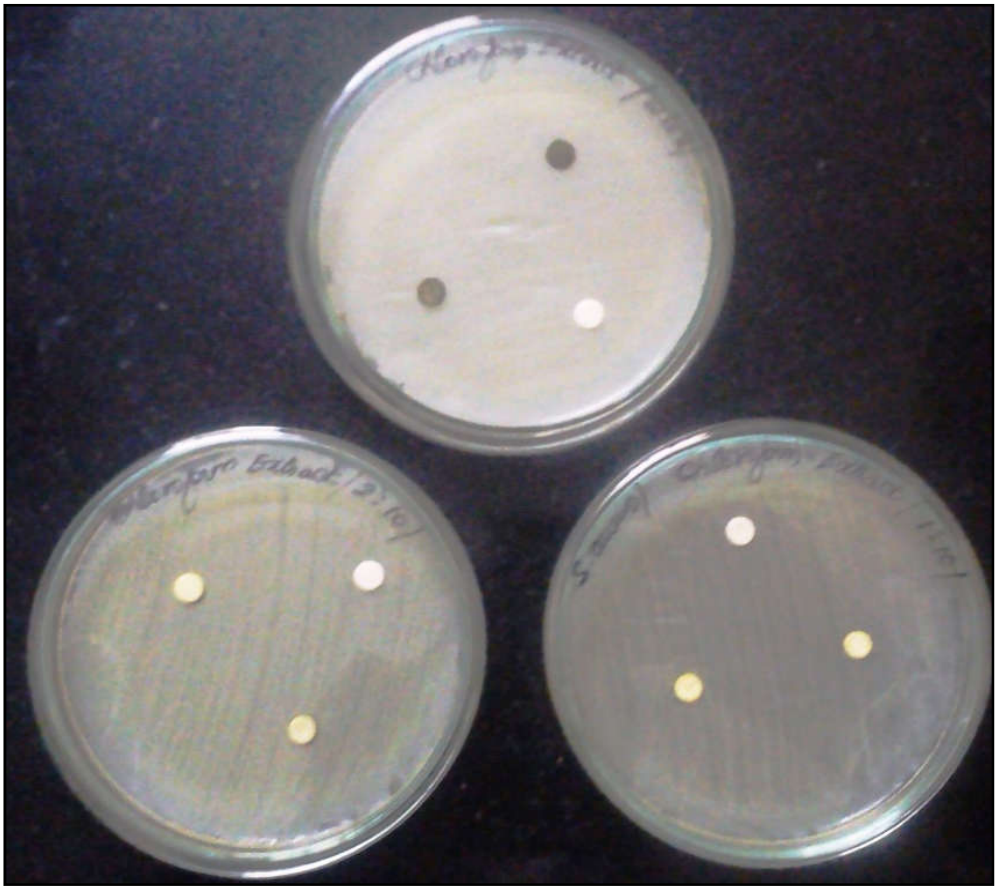

Figure 3. Antibacterial activity –Disc Diffusion method-Chloroform

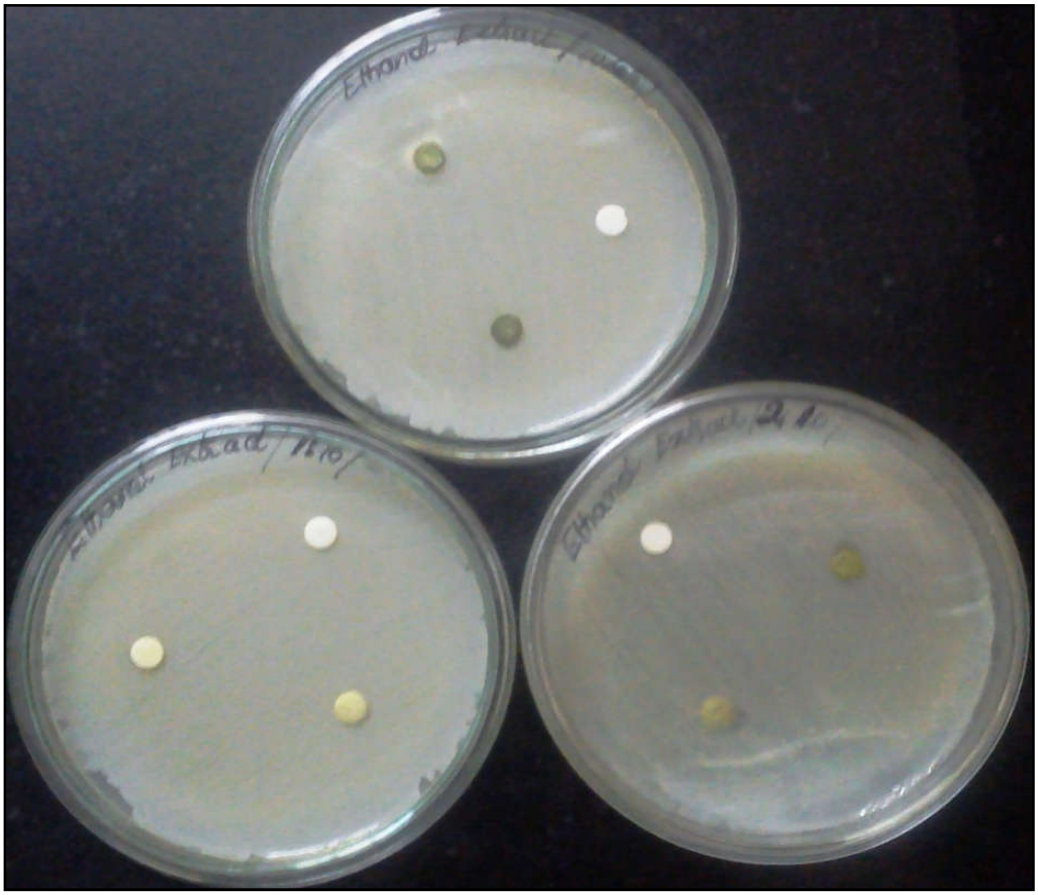

Figure 4. Antibacterial activity –Disc Diffusion – Ethanol

Figure 5. Shows Inoculated culture with the extract – Broth test

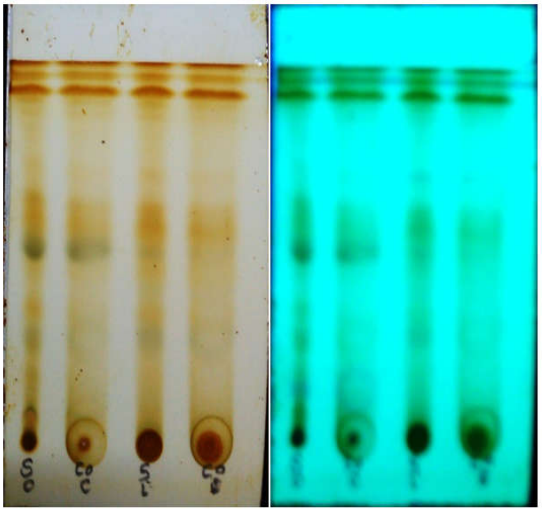

Figure 6. Shows Mobile phase I- Inference on Iodine and UV visuals – Phytochemicals

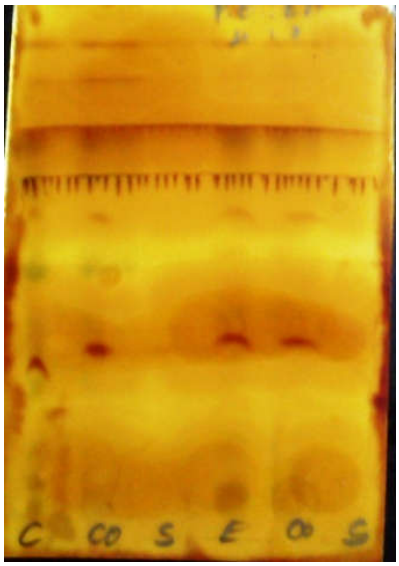

Figure 7. Shows Mobile phase I- Alkaloid localization using Wagner's reagent

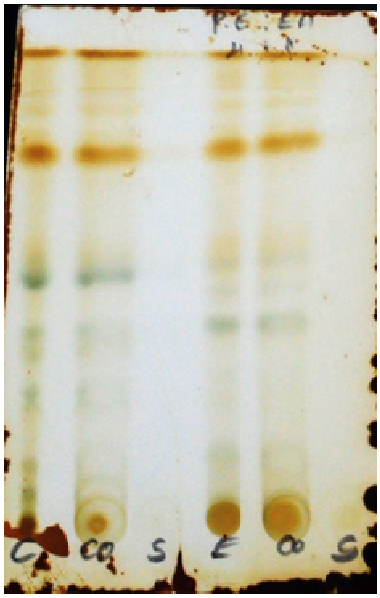

Figure 8. Shows Mobile phase II- Inference on iodine and UV Visuals – Phytochemicals and its activity

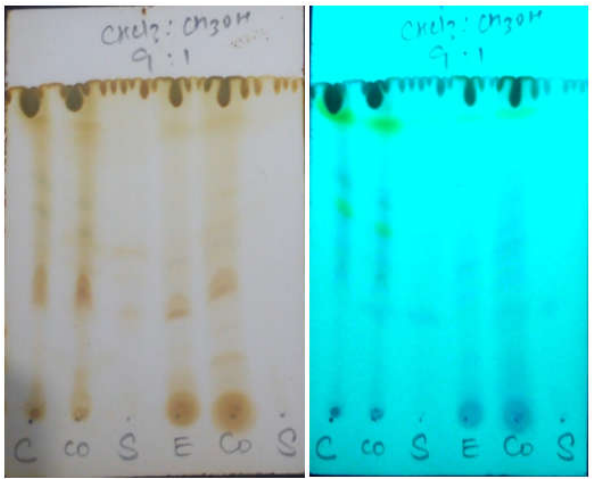

Figure 9. Inference on Iodine and UV visuals – Phytochemicals and its activity with S. aureus

Chemical constitutents and their antibacterial activity against *staphylococcus aureus* measured by TLC method: The chromatograms of chemical constituents studied via TLC plates (silica gel $6OFe_2SO_4$) were measured using two different mobile phases.

Mobile phase – I Petroleum ether: Ethyl acetate (9:1).

Mobile phase –II Petroleum ether: ethylacetate (8:2)

n-Hexane : ethyl acetate : Acetic acid (2:4:0:5).

TLC finger prints showed that there are more separate spots in the *Carica papaya L.*chloroform and ethanol extract. In the comparison with standards in similar conditions, the spots in the chromatogram of *Carica papaya* extract and the standards (Rf values= 0.48,0.60,0.89,0.93,0.95 respectively) Table 3. Rf value of *Carica papaya L.* extract was found (Table 4) Rf value (mobile II) of the extract and the combination is ethanol- 0.13, 0.36, 0.41, 0.46, 0.73 & 0.94 chloroform – 0.24, 0.29,0.54, 0.73 &0.94. The brown spot highlighted was alkaloid on reacting with Wagner's reagent the alkaloid derivatives are prominent to give a visible coloration (Figure 6).

Figure 7, 8, 9 shows the separation technique thin layer chromatography, the separation of different photochemical was observed subsequently with their activity toward *Staphylococcus aureus.* Two different mobile phases was used to separate both the phytochemicals and their respective activity. The separated components identified using iodine vapour illustrated different bioactive with the chloroform and ethanol extracts and there was no bioconversion of these components by *Staphylococcus aureus*. The result was compared with the disc diffusion method which also portrays interpretation which gives the confirmation that the phytochemicals extracted do not have antibacterial activity

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Conclusion

Based on the results of the present study, it was found that fresh extracts of *Carica papaya leaves* showing good antibacterial activity. The test bacteria *Staphylococcus aureus* were more susceptible to the extracts. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents. The zone of inhibition varied suggesting the varying degree of efficiency and different phytoconstituents of herb on the target organisms the antibacterial activity of the plants may be to the presence of various active principles in their leaf.

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