# ANTIMICROBIAL ACTIVITY OF COLD AND HOT EXTRACTS OF *CARICA PAPAYA* L. LEAVES

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## Summary

*Carica papaya* L. (Caricaceae) is extensively used in traditional medicine for several diseases including microbial infections The different concentrations of the cold and hot alcoholic leaf extracts (soxhlet extraction) of *C. papaya* plant were tested for *in-vitro* antimicrobial activity using agar well diffusion technique against five bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*) and five fungi (*Candida albicans, Aspergillus niger, Rhizopus, Penicillium* and *Mucor* species). The results of the present study clearly displayed that, the cold alcoholic extract of papaya showed significant antimicrobial activity against all the test pathogens, except *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* In contrast to this, hot extract of papaya was moderately effective to *Klebsiella pneumoniae.* While, *Escherichia coli, Candida albicans, Aspergillus niger, Rhizopus* and *Penicillium* were also showed sensitivity only at higher doses of the hot extract. Where as, *Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Mucor* species were found to be resistant to the hot alcoholic extract. As a conclusion, Papaya leaves act as a good source of antimicrobial agent. Comparatively hot extract, the cold alcoholic extract of papaya leaves possess even more potency with respect to consistent bacteriocidal and fungicidal properties. The results of the biological evaluations are strongly supports traditional medicinal use of papaya in crude form especially for the conditions such as microbial infections.

**Key words:** Antimicrobial activity, Cold alcoholic leaf extract of papaya, Hot alcoholic leaf extract of papaya

## Introduction

Papaya (*Carica papaya* L.) is one of the important tropical fruit crops. It is a small-unbranched tree, single stem growing up to 5-10 m tall, with the spirally arranged leaves; the lower trunk is conspicuously scarred with the leaf. The leaves are large, 50-70 cm diameter, deeply palmate lobed with 7 lobes. The fruits are rich in vitamin-A (2020IU) and vitamin-C (46g). Latex is extracted from unripe papaya, which has many pharmaceutical and cosmetic applications (1). The fruits, leaves and latex of *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, typhoid fever and wound infections, asthama, rheumatism, fever, diarrhea, boils, hypertension etc.(2-4). Recently, Antifertility (5), Anthelmintic activity (6), Anti-inflammatory activity (7). The latex from the leaves of this plant has been used as anthelmintcs and for the treatment of infections of bacterial origin (8). In spite of the diverse use of *Carica papaya* plant, it was also necessitated to evaluate extracts of this plant to antimicrobial activity in order to provide scientific proof for its wide applications particularly microbial infections in traditional medicine.

## Materials and Methods

**Collection and Identification of Plant materials**: The leaves of *Carica papaya* were collected from the Botanical Garden of Gulbarga University, Gulbarga, Karnataka, India. Identity of this plant was confirmed with the help of standard floras (9-10). Later it was authenticated and the voucher specimen was deposited in Herbarium of Department of Botany, Gulbarga University, Gulbarga, India. Later leaves of this plant were subjected for surface sterilization using 30% alcohol and then shade dried for further analysis.

**Preparation of the hot and cold Extracts:** The 100g leaves of *Carica papaya* were extracted with 95% alcohol at 60 - 80°C in a soxhlet apparatus. The obtained extract was concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) Where as, in case of cold alcoholic extraction, the 100g fresh leaves of papaya were blended into fine pieces and soaked in 150ml of 95% alcohol for 5 days and shaken vigorously to allow for proper extraction. Then obtained extract was filtered using a sterile muslin cloth, later filtrate was concentrated to dryness under room temperature and note down the yield of both the extracts.

**Microorganisms Used:** Clinical laboratory bacterial isolates of *Klebsiella pneumoniae, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Candida albicans, Mucor*, *Rhizopus* and *Penicillium* species were collected from the standard stock cultures of Microbiology Laboratory, Basaveshwara Hospital, Gulbarga, Karnataka, India. The bacterial and fungal cultures were maintained on nutrient agar and Sabouraud Dextrose Agar medium respectively and were stored at 4˚C for determining antimicrobial activity of some medicinal plants.

**Culture Media:** The nutrient agar, nutrient broth and Sabouraud dextrose agar were purchased from HiMedia, Laboratories Limited, Mumbai. Nystatin an antifungal agent, purchased from, Tocelo chemicals, Netherlands, Streptomycin (powdered form) from Nanjing Asian chemicals Co., Ltd. The solvents and other chemicals used were analytical grade.

**Antimicrobial activity:** Here the *in vitro* antibacterial and antifungal activities were assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar plate and Sabouraud dextrose agar plates for bacterial and fungal cultures. The wells were bored with 6mm borer in seeded agar and then the desired concentrations (2mg, 4mg, 6mg, 8mg/0.2ml/well) of the plant extracts and 10µg/0.2ml/well of streptomycin sulphate and Nystatin were added in each separate well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. Later, the zone of inhibition was measured and recorded (11-12)

**Statistical Analysis:** All the data are expressed as mean ± S.E.M. (standard error of the mean). The significance level was determined using the Student't' test. A *p*-value of $<0.05$ was considered statistically significant.

## Results

The present study showed that the cold and hot alcoholic extracts of papaya plant leaves inhibited bacterial and fungal growth but their effectiveness varied (table-1 and table-2). The agar well diffusion method for antimicrobial activity showed significant reduction in bacterial and fungal growth in terms of zone of inhibition around the disc. The zone of inhibition increased on increasing the concentration of extract in wells, this showed the concentration dependent activity. The cold alcoholic extract of papaya leaf showed significant antimicrobial activity against majority of test pathogens, among *Escherichia coli, Proteus vulgaris, Candida albicans, Aspergillus niger,* and Rhizopus were found to be more sensitive when compared with *Staphylococcus aureus, Mucor,* and *Penicillium* species, which showed moderate sensitivity, where as, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed resistance to cold alcoholic leaf extract of papaya leaves. Where as, the hot alcoholic extract was effective against *Klebsiella pneumoniae,* but *Escherichia coli, Candida albicans, Rhizopus, Penicillium* species showed mild to moderate level of sensitivity only at higher doses (6mg and 8mg/well). The hot alcoholic extract of papaya leaves was failed to inhibit certain tested pathogens like *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger* and *Mucor* species, which are proved to be resistant to hot alcoholic extract of papaya leaves.

**Table-1** Antimicrobial activity of cold alcoholic extract of *Carica papaya* Linn leaves

| Microorganisms | Zone of inhibition (cm) | | | | | |
|---|---|---|---|---|---|---|
| | **2 mg/0.2 ml /well** | **4 mg/0.2 ml /well** | **6 mg/0.2 ml /well** | **8 mg/0.2 ml /well** | **Streptomycin (10µg/0.2ml /well)** | **Nystatin (10µg/0.2ml /well)** |
| ***Escherichia coli*** | 0.97 ± 0.05 | 1.01 ± 0.06 | 1.17 ± 0.05 | 1.23 ± 0.04 | 1.90 ± 0.09 | NT |
| ***Klebsiella pneumoniae*** | - | - | 0.60 ± 0.20 | 0.62 ±0.05 | 1.87 ± 0.04 | NT |
| ***Proteus vulgaris*** | 0.95 ±0.05 | 0.97 ± 0.09 | 1.24 ± 0.01 | 1.27 ± 0.00 | 1.92 ± 0.16 | NT |
| ***Pseudomonas aeruginosa*** | - | - | - | - | 1.87 ± 0.04 | NT |
| ***Staphylococcus aureus*** | 0.85 ± 0.07 | 1.07 ± 0.07 | 1.25 ± 0.07 | 1.29 ± 0.09 | 1.62 ± 0.09 | NT |
| ***Candida albicans*** | 1.05 ± 0.19 | 1.21 ± 0.19 | 1.55 ± 0.19 | 1.75 ± 0.20 | NT | 1.92 ± 0.00 |
| ***Aspergillus niger*** | 0.97 ± 0.25 | 1.01 ± 0.04 | 1.13 ± 0.25 | 1.27 ± 0.15 | NT | 1.60 ± 0.40 |
| ***Mucor* spp** | 0.87 ± 0.25 | 1.02 ± 0.25 | 1.05 ± 0.02 | 1.21 ± 0.02 | NT | 1.35 ± 0.02 |
| ***Rhizopus* spp** | 1.02 ± 0.07 | 1.05 ± 0.60 | 1.22 ± 0.70 | 1.35 ± 0.07 | NT | 1.53 ± 0.06 |
| ***Penicillium* spp** | 0.77 ± 0.08 | 0.96 ± 0.08 | 1.07 ± 0.07 | 1.10 ± 0.03 | NT | 1.77 ± 0.09 |

Note: Not Tested; Spp – Species

**Table-2** Antimicrobial activity of hot alcoholic extract of *Carica papaya* Linn leaves

| Microorganisms | Zone of inhibition (cm) | | | | | |
|---|---|---|---|---|---|---|
| | **2 mg/0.2 ml /well** | **4 mg/0.2 ml /well** | **6 mg/0.2 ml /well** | **8 mg/0.2 ml /well** | **Streptomycin (10µg/0.2ml /well)** | **Nystatin (10µg/0.2ml /well)** |
| ***Escherichia coli*** | 0.62 ± 0.080 | 0.74 ± 0.090 | 0.82 ± 0.005 | 0.89 ± 0.060 | 1.90 ± 0.090 | NT |
| ***Klebsiella pneumoniae*** | 0.71 ± 0.011 | 0.72 ± 0.080 | 0.92 ± 0.041 | 0.98 ± 0.040 | 1.87 ± 0.040 | NT |
| ***Proteus vulgaris*** | - | - | 0.47 ± 0.050 | 0.52 ± 0.050 | 1.92 ± 0.166 | NT |
| ***Pseudomonas aeruginosa*** | - | - | - | - | 1.87 ± 0.040 | NT |
| ***Staphylococcus aureus*** | - | - | - | - | 1.62 ± 0.090 | NT |
| ***Candida albicans*** | - | - | 0.67 ± 0.005 | 0.97 ± 0.060 | NT | 1.92 ± 0.00 |
| ***Aspergillus niger*** | - | 0.50 ± 0.040 | 0.50 ± 0.024 | 0.55 ± 0.025 | NT | 1.60 ± 0.40 |
| ***Mucor* spp** | - | - | - | - | NT | 1.35 ± 0.02 |
| ***Rhizopus* spp** | - | - | 0.62 ± 0.070 | 0.95 ± 0.079 | NT | 1.53 ± 0.06 |
| ***Penicillium* spp** | - | - | - | 0.97 ± 0.010 | NT | 1.77 ± 0.09 |

Note: Not Tested; Spp - Species

## Discussion

The results of the present study clearly demonstrated that, the various concentrations of papaya plant leaves especially cold alcoholic extract was found to be more effective against majority of the tested pathogens. The results of the present studies are also strengthened by the several earlier reports. The extracts of ripe and unripe fruits of *Carica papaya* produced very significant antibacterial activity on *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella flexneri*.

The MIC of the substance was small (0.2 - 0.3 mg/ml) for gram-positive bacteria and large (1.5 - 4 mg/ml) for gram-negative bacteria. The substance was bactericidal and showed properties of a protein (13). The methanol and ethanol extracts of *C. papaya* flowers were effective only against *S. aureus* and *S. pneumoniae,* with the latter also effective against *Cornebacterium diptheriae* (3). Similarly, methanolic extract of the *C. papaya* roots and the minimum inhibitory concentration of the root extracts ranged between 50 – 200 mg/ml (4).

## Conclusion

It can be concluded that the cold alcoholic extract of *C. papaya* leaves has the most potential antimicrobial properties. The work carried out was a basic approach to find out the antimicrobial activity in *C. papaya.* Further works on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes. Additional *in-vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this plant as an antibiotic agent in topical or oral applications. The results of biological evaluations are strongly supports traditional medicinal use of papaya in crude form especially for the conditions such as microbial infections.

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