



## Antimicrobial and anti-inflammatory activity of aqueous extract of *Carica papaya*

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### ABSTRACT

**Introduction:** Over centuries, *Carica papaya* (papaya) has been used as a nutritious and medicinal plant. In view of its medicinal uses, our group focused on aqueous leaves extract of papaya and tried to determine its antimicrobial (using bacterial strains i.e. gram positive and gram negative ones) and anti-inflammatory (virally infected human whole blood) activities. The protein content of the plant and its secondary metabolites were also analysed qualitatively.

**Methods:** The protein content from aqueous leaves extract of *C. papaya* was quantified using Nanodrop method. ELISA studies were conducted in order to determine the antibody production against ovalbumin (OVA) using papaya aqueous leaves extract. Also, its antigen specific proliferation in virally infected human whole blood samples was determined through MTT assay. In addition, antimicrobial studies (using gram positive and gram negative strains) and cytotoxicity were also studied.

**Results:** The aqueous leaves extract of papaya showed enhancement in protein content and also raised antibody production against OVA. In addition, aqueous extract also showed decline in bacterial population and proliferation rate at higher doses as compared to control group. In contrast, aqueous extract showed anti-inflammatory and antimicrobial activities at higher doses.

**Conclusion:** The aqueous leaves extract of papaya plant showed anti-inflammatory and antimicrobial activities. Hence, it might be used for preparation of a new drug for these purposes.

### Implication for health policy/practice/research/medical education:

Aqueous leaves extracts of papaya plant showed some inhibition in bacterial population along with proliferation rate in virally infected human whole blood samples. So, this aqueous extract might be used as anti-inflammatory and antimicrobial agent.

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### Introduction

Since time immemorial, the bulk of the people particularly villagers have been totally relied upon medicinal plants as phyto-therapeutic agents in order to prevent/heal several ailments (1). Recently, pharmaceutical industries have adopted modern as well as scientific techniques and novel biotechnological methods including bioinformatics tools to develop life-saving drugs (2). In this regard, scientists have focused on medicinal plant parts, like leaves, stems and roots to prepare new drugs with minimal side effects (3). This research studied medicinal properties of *Carica papaya* leaves and tried to determine its

immunopharmacological and antimicrobial activities.

*Carica papaya* (papaya; family *Caricaceae*) is one of the laticiferous plants which grows best in a well aerated and rich organic matter soil (pH 5.5–6.7). The plant is considered as one of the most valuable medicinal plants with respect to human health (4,5). Generally, leaves, fruits and seeds of this plant are used as ethno-medicine. The leaves of papaya have been shown to contain alkaloids (i.e. carpaine and pseudocarpine) with some positive specific effects on heart and respiratory rates. In addition, aqueous extract of papaya leaves exhibits anti-tumoral activity. Most biologically active components are abundantly

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found in papaya leaves such as alkaloids (carpaine, pseudocarpaine, dehydrocarpaine); phenolic compounds (caffeic acid, chlorogenic acid, quercetin, kaempferol) and minerals (calcium, potassium, sodium, magnesium, iron, manganese) (4-7). These compounds have been used as antispasmodic, analgesic, antibacterial, antioxidant and as a remedy for digestive disorders.

As per the literature, numerous studies were conducted related to papaya fruits and seeds which were published and mentioning about its macro and micro mineral contents (4-7). In short, papaya is also considered as a good source of carotenoids, vitamin C, thiamine, riboflavin, niacin, vitamin B6 and vitamin K (6,7). Many studies have proved that papaya leaves (in the form of juice or extract) are used as traditional medicine for curing dengue fever without showing any side effects (8,9). In this regard our group focused on aqueous leaves extract and tried to determine its antimicrobial (bacterial population) and anti-inflammatory (virally infected human blood samples) activities.

## Materials and methods

### Plant material

Papaya plant was collected from Nakshatra udyan, Baramati, Maharashtra, India. The papaya leaves were washed thoroughly under tap water and then with distilled water. Thereafter, papaya leaves were shade dried and powdered with a mortar and pestle. The powder was added to phosphate-buffered saline (PBS) to determine its protein content and secondary metabolites qualitatively.

### Estimation of primary and secondary metabolites

Fresh leaves of papaya plant (5 g lot) were macerated in liquid nitrogen and dissolved in phosphate buffered saline (PBS; 50 mL; pH 7.4). Aqueous leaves extract of papaya plant was filtered and the supernatant collected for estimation of primary and secondary metabolites. In order to analyse both primary and secondary metabolites including protein content (Nanodrop method), flavonoids (lead acetate test), saponins (foam test), tannins (ferric chloride test), terpenoids (using chloroform, sulphuric acid) and alkaloids (Mayers test) various assays or tests were accomplished.

### Antimicrobial and anti-inflammatory activity

In this study, we determined antimicrobial activity using various concentrations of aqueous leaves extract of papaya plant against Gram-positive bacteria (*Bacillus subtilis*) and gram-negative bacteria (*Pseudomonas fluorescens*, *Escherichia coli*, *Salmonella typhi*).

For these studies, bacterial samples were collected from soil sample, Vidya Pratishthan except *Salmonella typhi* (ATCC strain). The bacterial samples were identified and screened on the basis of Gram staining method (10). All these antimicrobial studies were conducted under CLSI

guidelines. The bacterial samples (gram positive and gram negative) were normally grown in nutritive rich media containing glucose, yeast extract, sodium chloride, agar and other trace elements with pH 7.4 and maintained in the incubator for 24 hours. After incubation, all bacterial colonies were identified according to Bergey's manual of determinative bacteriology. One colony of each strain of bacteria was diluted in phosphate buffered saline (PBS, pH 7.4) to reach the final concentration of  $10^6$  CFU/mL. Bacterial strains, gram positive and gram negative bacteria ( $10^6$  CFU/mL; 50  $\mu$ L) were taken in 96 well flat bottom tissue culture plates and then exposed to lysed human non-infected whole blood (100  $\mu$ L). Finally they were treated with variable concentrations of aqueous leaves extract of papaya plant (0.625- 2.5 mg/mL, 50  $\mu$ L) for determining the antimicrobial activity (10).

Infected human EDTA blood samples were collected from Mangal Pathology laboratory, Baramati region, District Pune, Maharashtra, India. In this study, we evaluated its antigen specific proliferation in virally infected human whole blood samples using aqueous leaves extract of papaya. The lysed human whole blood ( $2 \times 10^6$  cell/mL, 100  $\mu$ L) was pipetted into 96 well flat bottom tissue culture plates and treated with various concentrations of aqueous leaves extract of papaya (0.625-2.5 mg/mL; 50  $\mu$ L).

Both plates were incubated for 48 hours and then centrifuged to remove the supernatant and added fresh medium containing 10 % FBS. Finally, MTT (5 mg/mL; 10  $\mu$ L) solution was added in 96 well plates and incubated it for another 4 hours. After incubation, formazan crystals were appeared and settled at the bottom. The supernatant was removed after centrifuging and then dissolved in dimethyl sulphoxide (DMSO). The optical density was measured at 570 nm (11,12).

### ELISA

Indirect ELISA was performed using Ovalbumin (OVA, 50  $\mu$ g/well) as coating antigen in high protein binding 96-well plates (Himedia). Variable doses (0.6253-2.5 mg/mL) of aqueous extract of papaya leaves were used with anti-OVA as standard, for the estimation of IgG antibody titre. Horse anti-serum was used as secondary antibody and optical density was measured at 450 nm (13).

### Statistical analysis

Data were reported as mean  $\pm$  standard error (SE). The difference between the control and treated samples of aqueous extract of papaya leaves was determined by one-way analysis of variance (ANOVA) test (Bonferroni multiple comparison test).  $P < 0.05$  was considered as statistically significant.

## Results

### Evaluation of protein content

Estimation of protein content in aqueous extract of papaya

leaves using Nanodrop method is illustrated in Figure 1. The results showed the presence of protein (0.836 mg/mL) in papaya leaves extract.

#### Antimicrobial activity

The effect of aqueous leaves extract of papaya for determining its antimicrobial activity using bacterial strains in lysed non-infected human whole blood is shown in Figure 2. Aqueous leaves extract at higher doses showed a decline in bacterial population as compared to control group. Overall, this study indicated the extract antimicrobial activity.

#### Anti-inflammatory activity

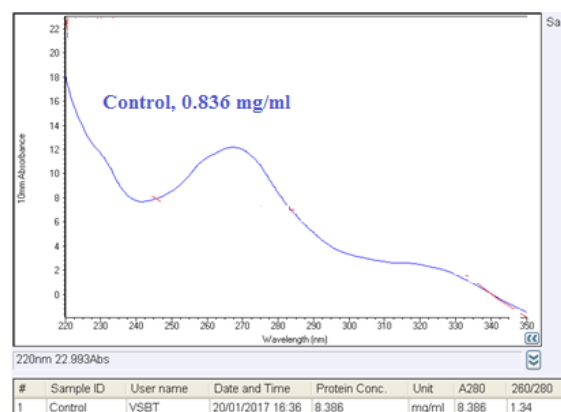
Anti-inflammatory activity was evaluated using various concentrations of papaya leaves extract in lysed infected human whole blood (Figure 3). Aqueous leaves extract showed decline in cell population at higher doses. Overall, this study indicated the plant anti-inflammatory activity.

#### ELISA

The effect of aqueous leaves extract of papaya for determining its antibody production against OVA is shown in Figure 4. Higher doses of aqueous leaves extract enhanced antibody production as compared to control group.

#### Discussion

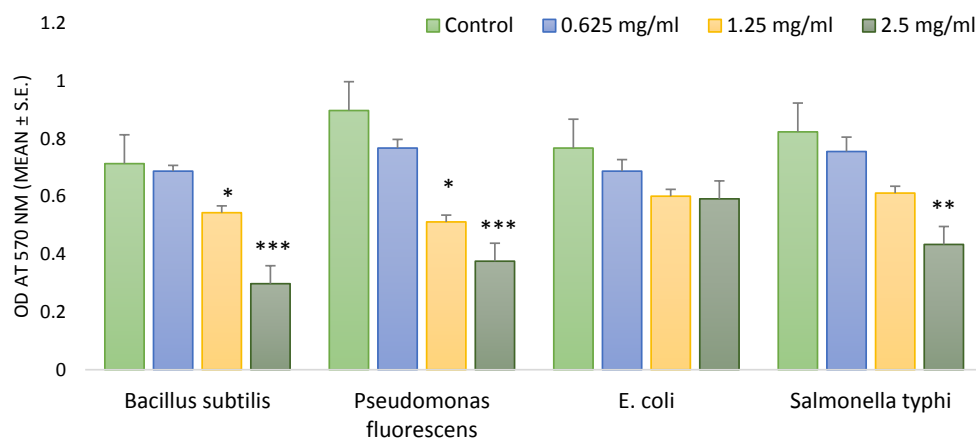
Numerous studies have been conducted extracting secondary metabolites from papaya leaves (14,15). In the present study, we determined the antimicrobial and anti-inflammatory activities of aqueous leaves extract of papaya plant. The use of these medicinal plant extracts with well-known antimicrobial properties is of great significance for the therapeutic treatments. In view of this, the aqueous extract showed considerably inhibition with respect to the



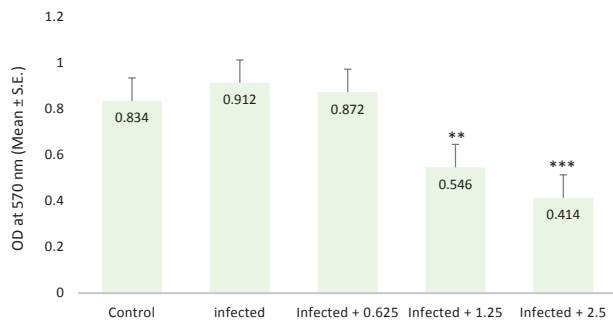
**Figure 1.** Estimation of protein content in aqueous leaves extract of papaya plant using Nanodrop method. In this figure, line indicates the presence of protein content using PBS as blank. This protein is extracted from leaves using Tris HCl and ice cold acetone.

growth of gram positive and gram negative bacteria as compared to control group. Maximum inhibition of these bacterial strains was observed at higher doses and this could be due to the presence of primary and secondary metabolites. In other words, this study may lead to the establishment of some valuable herbal medicines derived from aqueous leaves extract of papaya plant (11). Further antimicrobial studies are needed to identify the most active biological compounds and to evaluate the efficiency of the active constituents against pathogenic microorganisms associated with various human diseases.

In this study, the results also showed that papaya aqueous leaves extract possessed anti-inflammatory properties. This activity could be due to decline in proliferation rate in case of virally infected human whole blood after treating with variable doses of aqueous leaves extract of papaya plant. These studies suggest that aqueous leaves extract has a suppressive effect on lymphocyte activation



**Figure 2.** Antimicrobial activity of *Carica papaya*. To determine the effect of variable doses of papaya leaves extract (0.625–2.5 mg/mL, 50  $\mu$ L) on different strains of bacterial pathogens in lysed human whole blood. Values are expressed as mean  $\pm$  SE. The difference between the controls versus variable doses of terpenoid is determined by one-way ANOVA test (Bonferroni multiple comparison test). \* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Figure 3.** Anti-inflammatory activity of *Carica papaya*. To determine the effect of variable doses of papaya leaves extract (0.625 – 2.5 mg/mL, 50  $\mu$ L) on virally infected lysed human whole blood. Values are expressed as mean  $\pm$  SE. The difference between the controls versus variable doses of terpenoid is determined by one way ANOVA test (Bonferroni multiple comparison test). \* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

in case of virally infected blood samples even at non-toxic concentrations. In other words, lymphocytes activation is usually modulated (stimulatory or suppressive) by expression of co-stimulatory molecules present in the cell membrane and also through the action of several cytokines. So, cytokines depending upon lymphocyte proliferation play a crucial role in controlling the expansion of T cells during immune response to pathogenic antigens (16-18). Similarly, various immunoassay methods have been developed related to medicinal plants on the basis of antibody production against weak antigen i.e. OVA. In this study, there was an enhancement in antibody production with respect to papaya leaves extract against OVA at higher doses. This technique related to medicinal plants can be developed into a novel technique for detecting antibody production (13).

### Conclusion

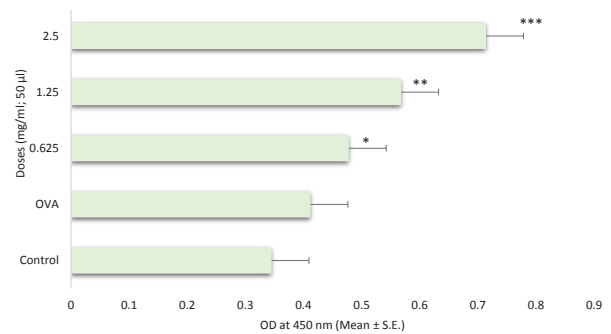
This study suggests anti-microbial and anti-inflammatory activities of papaya aqueous leaves extract. Further investigations on the aqueous leaves extract of papaya plant should be done through in vivo assessment of immune-pharmacological relevance to identify the major active components responsible for anti-microbial and anti-inflammatory activities.

### Authors' contributions

This work was carried out in collaboration of three authors. AG designed the study, wrote the protocol and interpreted the data where SSP anchored the field study, gathered the initial data related to his MSc degree. Preliminary data analysis and microbiology dissertation work were done under AG supervision. NP managed the literature searches whereas AG produced the initial draft. The final manuscript was read and approved by all authors.

### Conflict of interests

The authors declared no competing interests.



**Figure 4.** ELISA assay. To determine its antibody production against OVA using variable doses of papaya leaves extract (0.625–2.5 mg/mL, 50  $\mu$ L). Values are expressed as mean  $\pm$  SE. The difference between the controls versus variable doses of terpenoid is determined by one way ANOVA test (Bonferroni multiple comparison test). \* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

### Ethical considerations

Not applicable.

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