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Research Article

Phytochemical Analysis of Papaya Leaf Extract: Screening Test

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Abstract

Introduction: Medicinal plant extracts are endowed with several biologically active compounds which possess potent antimicrobial activity and could be used to effectively replace synthetic chemicals. To assess the clinical utility, the present study was undertaken to screen the phytochemical compounds of papaya leaf extract.

Material and Methods: Plant leaves of *Carica papaya* were handpicked at University of Agriculture and Animal Science. The preparation of papaya extract and its phytochemical analysis was done for detection of alkaloids, flavonoids, glycosides, reducing sugar, saponin, steroids and tannins. Antimicrobial activity was assessed against two resistant endodontic pathogens i.e. *Enterococcus faecalis* and *Candida albicans*.

Results: The test showed presence of all the bioactive compounds such as saponin, flavonoids, reducing sugars, steroid and tannin except glycosides. Colony count of *E. faecalis* and *Candida albicans* decreased from 1 minute to 15 minutes contact time of papaya extract.

Conclusion: The phytochemicals of *Carica papaya* can be observed as a potential source of antimicrobials and can be made available commercially for its medicinal value.

Keywords: *Antimicrobial; Papaya Leaf Extract; Phytochemical Analysis*

Introduction

Antibiotics are considered both as a boon and a bane to medical field these days due to emergence of resistant strains of pathogens. The development of resistant strains has heralded search for recent, effective, economical and easily available sources such as medicinal herbs. Medicinal herbs are age old sources of drugs [1]. Various active compounds mainly secondary metabolites are synthesized by medicinal herbs. These secondary metabolites are mainly responsible for antimicrobial property. These pharmacologically active substances singly or in combination with other inactive substances act as reservoir of antimicrobial agent [2,3]. These antimicrobial agent are not only potent against infectious diseases, but also mitigates the adverse affect of synthetic antimicrobial agents [4,5]. The antimicrobial active compounds can be obtained from the roots, barks, stems, leaves and seeds extracts of medicinal plants.

Carica papaya is a herbaceous plant whose fruits, leaves, seeds and latex are used medicinally. The fruit has a juicy taste rich in antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids, folate, pantothenic acids and minerals such as potassium and magnesium. The biologically active constituents of papaya include chymopapain and papain which are used in the treatment of arthritis and digestive disorders. Extracts of the ripe fruits are used for a variety of medicinal purposes ranging from treatment of ringworm, malaria and hyper-

tension [6]. The extract is also known to have antioxidant properties. Despite the wide and historical use of *Carica papaya* in the traditional management of many diseases, the scientific validation of its use as antioxidant is lacking. While extracts of unripe fruit have been used in treatment of diabetes [7]. Traditionally, the leaf extract was used as a tonic for the heart, analgesia and treatment for stomach ache [8].

Carica papaya leaves and seeds are known to contain [9,10]:

- Proteolytic enzymes such as papain, chymopapain
- Alkaloids such as carpain, carpasemine
- Sulfurous compounds such as benzyl isothiocyanate
- Flavonoids
- Triterpenes
- Organic acids and
- Oils.

Whole fruit extract contains ferulic, p-coumaric acid, caffeic acid, carotenoids, and vitamin C. Leaves extract contains folic acid, vitamins B12, A, and C, alkaloids, saponins, glycosides, tannins, and flavonoids. Seeds extract contains different phenolic compounds, vanillic acid, and vitamin C [11].

Hence, the purpose of this study was to screen the presence of bioactive substances in papaya leaf extract for its futuristic use as irrigant in dentistry against root canal pathogens.

Materials and Method

Collection and identification of plant material: Fresh healthy/disease free, mature, plant leaves of *Carica papaya* were handpicked at University of Agriculture and Animal Science. The plant was identified and authenticated by Department of Agriculture Research. The leaves were washed and surface sterilized in 0.1% mercuric chloride for 5 minutes. It was washed thrice with sterile distilled water for 9 minutes. The sterilized leaves were air dried at room temperature for 24 hrs.

Preparation of papaya extract: 200 grams of sterilized leaves were grounded fine by electric blender. The extract was squeezed out through sterile gauze pieces. About 16 ml of crude extract was obtained followed by centrifugation at 4000 rpm for 30 minutes. The supernatant layer obtained was filtered through Whatman's filter paper number 1. The crude extract was stored at 4ºC.

Phytochemical analysis of papaya extract

Test for alkaloids

2-3 drops of Dragendoff's reagent was reacted in the test tube containing 0.1 ml of crude papaya extract. The presence of alkaloids was indicated by appearance of an orange red precipitate with turbidity [12].

Test for flavonoids

4 mg/ml of papaya extract was taken in a test tube in which a piece magnesium ribbon was added. Then gradual drop of concentrated HCl was added to the test tube leaded to change in color from orange to red and then red to crimson. A colour change from orange to red indicated the presence of flavones; red to crimson indicated the presence of flavonoids [13].

Color observed	Sugar concentration
(+) Green precipitate	< 0.5g%
(++) Yellow precipitate	0.5 - 1g%
(+++) Orange precipitate	1 - 1.5g%
(++++) Red precipitate	> 2.0g%

Table 1: *Benedict's test for reducing sugar showing particular color at different concentration.*

Test for glycosides

1 ml of papaya extract filtrate was taken in which 10 ml of 50% H_2SO_4 was added and the mixture was heated for 15 minutes. 10 ml of Fehling's solution was added and boiled which showed brick red precipitate indicating presence of glycosides [13].

Test for reducing sugars

- To 1 ml of extract, 2 ml of distilled water was added followed by addition of Fehling's solution (A + B) and the mixture was warmed at 40ºC in serological water bath. At the bottom of test tube brick red precipitate appeared indicating the presence of reducing sugar [14].
- **Benedict's test**: To 5ml of Benedict's reagent. 0.5 ml of extract was added and homogenously mixed. The solution was heated for 2 minutes and final colour was observed after cooling under running tap water. The colour observed denoted sugar concentration as follows.

Test for saponins

0.5 gram of the papaya leaf powder was taken in a test-tube and 5.0 ml of distilled water was added and shaken vigorously. A persistent froth that lasted for about 15 minutes indicated the presence of saponins [15].

Test for steroids

2 ml of the extracts was evaporated to dryness and the residue was dissolved in acetic anhydride followed by addition of chloroform. Concentrated H_2SO_4 was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet colour in the supernatant layer denoted the presence of steroids [12].

Test for tannins

Two milliliters of the extract was diluted with 3 ml distilled water, 2 - 3 drop of 5% Ferric Chloride ($FeCl_3$) solution was added. A green-black or blue colouration indicated presence of tannin [12].

Antimicrobial efficacy: To evaluate the antimicrobial activity of the papaya extract, two endodontic microbes (*E. faecalis* and *C. albicans*) were tested by direct contact test against the papaya leaf extract at 1 minute, 5 minutes and 15 minutes time interval. After the respective time of contact of papaya leaf extract with suspension of *E. faecalis* and *C. albicans*, spread plate technique was performed on to nutrient agar and Sabouraud Dextrose Agar (SDA) plate with the help of L- shaped rod. The plates were incubated at 37ºC for 24 - 48 hrs. Next day, colony morphology was studied and gram stain and biochemical tests were performed to confirm the organism isolated.

Results

The test showed presence of all the bioactive compounds such as saponin, flavonoids, reducing sugars, steroid and tannin except glycosides. Fehling's test has confirmed the presence of reducing sugar which is further quantified as presence of less than 0.5 mg of reducing sugar by Benedict's test. Presence of these bioactive compounds infers antimicrobial potential of papaya extract. On test with *E. faecalis* and *C. albicans* also showed gradual decrease in colony count in 1 minute, 5 minutes and 15 minutes of contact with papaya leaf extract. There were uncountable small colonies of *E. faecalis* in 1 minute and 5 minutes time intervals whereas prominent countable colonies can be observed at 15 minutes as shown in figure 1. Similarly, the decrease in colonies of *C. albicans* can be observed from 1 minute to 15 minutes time interval as shown in figure 2.

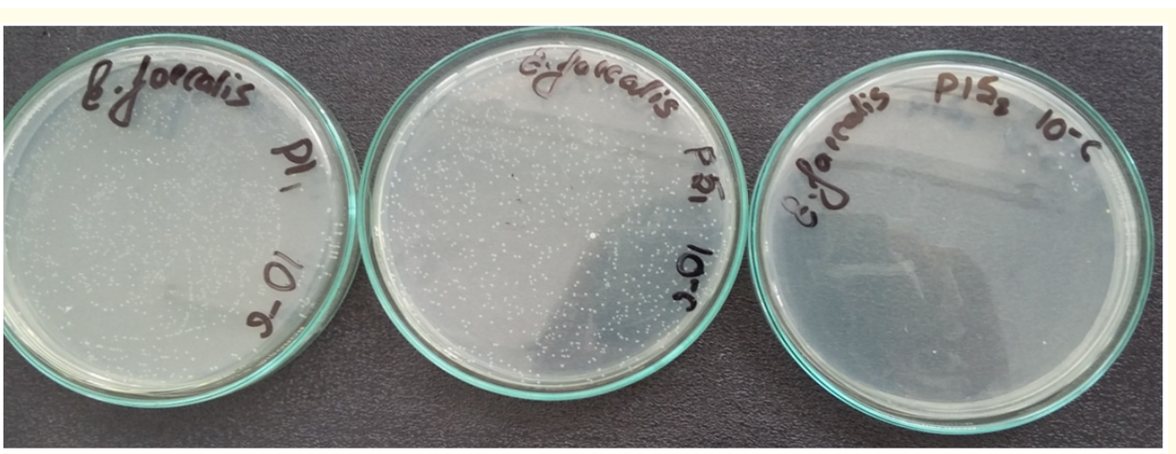

Figure 1: *Gradual decrease of E. faecalis colonies from 1 minute, 5 minutes to 15 minutes time interval.*

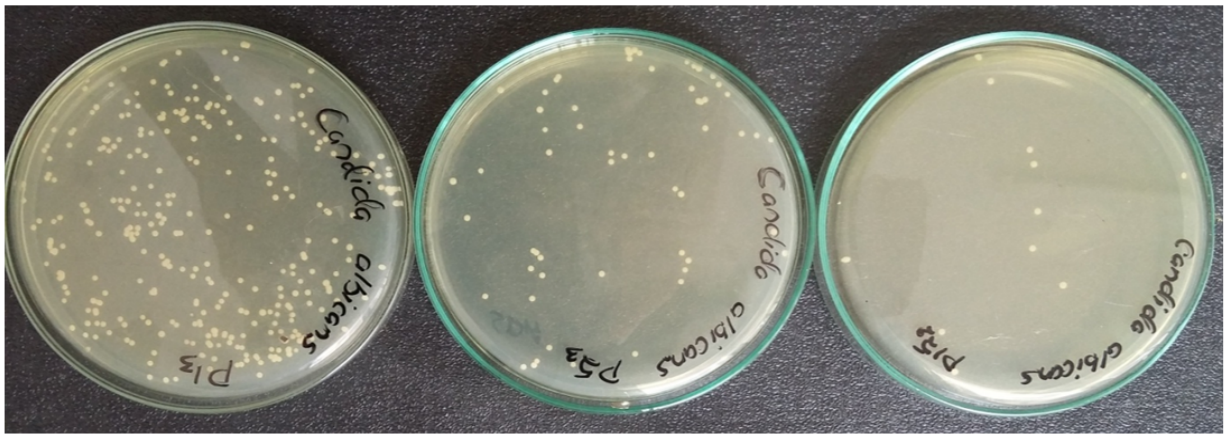

Figure 2: *Gradual decrease of Candida albicans colonies from 1 minute to 15 minutes time interval.*

Discussion

Among various herbal plants, *Carica papaya* is also well known for its medicinal properties in traditional system of medicine. It is effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects often associated with synthetic antimicrobial agents.

The antimicrobial activity of papaya extract can be attributed to the presence of different bioactive compounds which was confirmed by the phytochemical analysis as shown by table 2 showing the presence of alkaloids, saponin, flavonoids, reducing sugar, tannin and steroid but absence of glycosides which is similar to the study done by Yusha'u M., *et al* [16]. Similarly, LSS Abirami., *et al.* [17] also performed phytochemical analysis of papaya leaf which showed the presence of flavonoids, alkaloids and terpene. These compounds are postulated to influence its mycotoxicity by interacting with fungus membrane constituents.

Phytochemicals	Observation	Inference
Saponin	Frothing of extract which persisted for 15 minutes	+
Flavoids		
Flavones	Color change from Orange to pale Red	+
Flavonoids	Color change from pale Red to Crimson	+
Glycosides	Absence of Brick Red precipitate	-
Reducing sugars:		
Fehling's test	Presence of Brick Red precipitate	+
Benedict's test	Presence of Green color	+ (< 0.5 g %)
Steroids	Beautiful Brown color ring at interface	+
Tannin	Black Green	+

Table 2: *Phytochemical constituents of Carica papaya leaf extract.*

Key: -: Absence; +: Presence.

The phytochemical tests performed by Pedro., *et al.* showed the highest presence of compounds with antibacterial and antifungal activity (e.g. alkaloids, triterpenes, flavonoids, and saponins) in the leaf extract [18]. Of these compounds, alkaloids are quite probably the most important elements in defense against pathogens [19].

The presence of bioactive substance has implied its potential role for antibacterial and antifungal agent against root canal pathogens. There were uncountable small colonies in 1 minute and 5 minutes time intervals whereas prominent countable colonies can be observed at 15 minutes for growth of *E. faecalis* as shown in figure 1. Moreover, the decrease in colonies of *C. albicans* can be accentuated from 1 minute to 15 minutes time interval as shown in figure 2. Its action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract [20]. While phytochemical compounds such as tannin coagulate the wall proteins, saponins facilitated the entry of toxic material or leakage of vital constituents from the cell [21]. Flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity or microbial membranes at low concentrations [22].

Conclusion

The phytochemicals of *Carica papaya* can be observed as a potential source of antimicrobials and can be made available commercially for its medicinal value.

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