

Assessment of Antimicrobial Potential of Different Concentrations of Ethanolic Papaya Leaf Extract Against *Candida Albicans* and *Enterococcus Faecalis* – An In Vitro Study

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Abstract

Original Research Article

Context: Medicinal plants signify a rich source of antimicrobial agents. Plants are used medicinally in many countries and are sources of various potent and prevailing drugs. *Carica papaya* is a known medicinal plant in that it comprises constituents that can be used for therapeutic purposes. **Aim:** To assess antimicrobial potential of different concentrations of *ethanolic papaya leaf* extract against *Candida albicans* (*C albicans*) and *Enterococcus faecalis* (*E faecalis*). **Settings and Design:** Laboratory setting and Experimental design. **Methods and Material:** In first part of study, 10%, 20% and 30% ethanolic papaya leaf extract was prepared in the laboratory of Pharmacy College. 10%, 20% and 30% ethanolic papaya leaf extract was then subjected to microbiological assay to determine its zone of inhibition using Agar disk diffusion test and Minimum Inhibitory concentration (MIC) using Serial broth dilution method against *C albicans*, and *E faecalis*. **Results:** 10%, 20% and 30% ethanolic papaya leaf extract showed: a) Maximum zone of inhibition of 10 mm, 12 mm and 14 mm respectively against *C albicans* b) Maximum zone of inhibition of 11mm, 12 mm and 15 mm respectively against *E faecalis*. b) MIC of 2.5% and 1.25% against, *Candida albicans* and *Enterococcus faecalis* respectively. **Conclusion:** Different concentrations of ethanolic papaya leaf extract possess antimicrobial potential against both pathogens used in the study.

Keywords: Papaya leaf, *Candida albicans*, *Enterococcus faecalis*, Antimicrobial potential, Ethanolic extract.

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INTRODUCTION

The advent of multidrug resistance among pathogenic bacteria is endangering the worth of antibiotics, which had formerly transformed medical sciences. Antimicrobial resistance poses a severe worldwide threat of budding concern to human, animal and environment health which is due to occurrence, spread and persistence of Multi Drug Resistance (MDR) [1]. Green dentistry is an advanced way of dental practice which is environment friendly and at the same time saves money and time by minimizing waste, conserving energy and reducing pollution with the use of latest techniques and procedures [2]. It is a high tech methodology, which decreases the environmental impact of dental practices in moving forward to an ecologically sustainable health care system [3]. Green

dentistry is a developing approach to decrease the environmental impact from dental practice [4].

Ayurvedic medicine was considered to be one of the world's first medical systems, which was invented in India dating back over thousands of years. There is a long history regarding plants for the enhancement of dental health and oral hygiene to study various plants and their products as effective medicines in management of various illnesses since ancient times [5]. Plants are used medicinally in many countries and are sources of various potent and prevailing drugs [6].

Papaya Plant (*Carica papaya*) is a known medicinal plant in that it comprises constituents that can be used for therapeutic purposes [7]. This plant has been acclaimed as anti-ulcerogenic, anti-amoebic, anti-fungal, anti-microbial, anti-tumor, hypolipidaemic and

employ in wound healing activity, free radical scavenging activity, diuretic activity, uterotonic activity and antifertility activity [7-9].

Candida is the summarized name used to describe a class of fungi that comprises more than 150 species of yeast [10]. In healthy persons, *Candida* exists harmlessly in the mucous membranes such as your ears, eyes, gastrointestinal tract, mouth, nose, reproductive organs, sinuses, skin, stool and vagina etc [10]. It is known as “beneficial flora” and has a advantageous purpose in the body. When an imbalance in the regular flora occurs, it causes an overgrowth of *Candida albicans*. Candidiasis or thrush is a fungal infection of any of the *Candida* species of which *C. albicans* is the most common [10].

E. faecalis, the major human enterococcus, has been also linked to oral diseases, such as caries, endodontic infections, periodontitis, and peri-implantitis [11-13]. It has been often implicated in failure of endodontic treatment, due to high resistance to endodontic drugs, and the ability to form recalcitrant biofilms both in treated and untreated root canals [14, 15].

On review of literature scarce studies are available which has tried to assess the antimicrobial potential of Papaya plant parts against oral micro-organisms. Hence an attempt was made to assess the antimicrobial potential of various concentration of ethanolic papaya leaf extract against *C. albicans* and *E. faecalis*.

AIM OF THE STUDY

- To assess antimicrobial potential of different concentrations of ethanolic papaya leaf extract against *Candida albicans* (*C. albicans*) and *Enterococcus faecalis* (*E. faecalis*).

OBJECTIVES OF THE STUDY

- To assess antimicrobial potential of 10% concentration of ethanolic papaya leaf extract against *Candida albicans* (*C. albicans*) and *Enterococcus faecalis* (*E. faecalis*).
- To assess antimicrobial potential of 20% concentration of ethanolic papaya leaf extract against *Candida albicans* (*C. albicans*) and *Enterococcus faecalis* (*E. faecalis*).
- To assess antimicrobial potential of 30% concentration of ethanolic papaya leaf extract against *Candida albicans* (*C. albicans*) and *Enterococcus faecalis* (*E. faecalis*).

MATERIALS AND METHODS

Taxonomical Classification, Common Names and Parts Used [20]

The papaya belongs to a small family-Caricaceae, having four genera in the world. The genus

Carica Linn. It is represented by four species in India, of which *C. papaya*. Linna. is the most widely cultivated and best-known species. The taxonomical classification includes kingdom (Plantae), order (Brassicales), family (Caricaceae), genus (*Carica*), species (*C. papaya*). Common names include papaya, pawpaw, papaw, papita, arand-kharpuja, papaya, papayabaum, papaia. The parts used contain fruit, leaves and bark.

Chemical Constituents

The different parts of papaya such as fruit, fruit juice, seed, root, leaves, bark, latex contain various chemical constituents, which are shown as follows:

- Fruit-Protein, fat, fiber, carbohydrates, minerals: calcium, iron, vitamin C, thiamine, riboflavin, niacin, and carotene, amino acid, citric acid and malic acids (green fruits), volatile compounds: benzylisothiocyanate, cis and trans 2, 6-dimethyl-3,6 epoxy-7 octen-2-ol, alkaloids, carpaine.
- Juice-N-butyric, n-hexanoic and n-octanoic acids, lipids; myristic acid, palmitic acid, stearic acid, linolenic acid, linoleic acid, oleic acid.
- 3.Seed-Fatty acids, crude protein, crude fibre, papaya oil, carpaine, caricin, glucotropacolin, and an enzyme myrosin.
- Root Carposides and an enzyme myrossin.
- Leaves- Alkaloids carpain, pseudocarpain, dehydrocarpaine I and II, choline, vitamin C and E, carposide.
- Bark-Glucose, fructose, sucrose, xylitol, β -sitosterol.
- Latex-Papain, chemopapain, peptidase A and B, lysozymes.

Phytochemicals Analysis [24]

Phytochemicals were analyzed for the extracts particularly to ascertain the presence of different bioactive components present in 70% ethanolic *Carica papaya* leaf extract. The presence of alkaloids, saponins, tannins, flavonoids, steroidal terpenes and carbohydrates were determined, as described by the method of Harborne [23].

Test for Carbohydrates: Molisch, Fehling's, Benedict's and Barfoed test was performed to determine the presence of Carbohydrates.

Test for Proteins: Biuret, Millon's and Xanthoprotein test was performed to determine the presence of Protein.

Test for Steroids and Triterpanoids: Salkowski and Liebermann-burchard test was performed to determine the presence of Steroids and Triterpanoids.

Test for Amino Acids: Ninhydrin test was performed to determine the presence of Amino Acids.

Test for Flavonoids: Shinoda, Lead acetate and NaOH test was performed to determine the presence of Flavonoids.

Test for Tannin and Phenolic compounds: FeCl₃, Lead acetate, dil. HNO₃, Acetic Acid and Iodine test was performed to determine the presence of tannin and Phenolic compounds.

Test for Alkaloids: Dragendorff's, Mayer's, Wagner's and Hager's test was performed to determine the presence of Alkaloids.

Test for Saponins: Foam test was performed to determine the presence of Saponins.

Test for Fats: To determine fats few drops of sample was taken on blotting paper and checked for oily surface of blotting paper. The oily surface of blotting paper determines the presence of fats.

Materials Used In the Study

- Hot air oven
- Whatmann Filter paper
- Thimble
- Round bottom Flask
- Soxhlet apparatus
- Distillation Unit
- Water bath
- Dimethyl Sulfoxide
- Potato Dextrose Agar
- Luria Bertani Agar media
- Autoclave
- Petriplates
- Well Borer
- Culture plates

Study Design: Experimental design (In-vitro laboratory study).

Duration of the Study: The study was carried out for duration of 2 months (April- May 2019).

Ethical Clearance: After review of the study protocol by Institutional Review Board (Rajarajeswari Dental College and Hospital), Ethical clearance was obtained to conduct the study.

Collection of Papaya leaves: The leaves of *papaya* were collected in and around Bangalore, Karnataka. Botanical identification of papaya leaf was done in Department of Botanical Sciences, Ayurvedic College, Bangalore.

Extraction of papaya leaves extract from Ethanol (70%)

The leaves were washed and dried at 40°C in hot air oven until complete dry and powdered (Fig-1). 30g of powdered sample was filled in a Whatmann

filter paper pouch and kept inside thimble. 200mL of the (70%) Ethanol was added in thimble. The thimble was fit into a round bottom flask containing 700mL of the solvent and run for 6-8 hours at the temperature based on the boiling point of the Ethanol using soxhlet apparatus (Fig 2). Later, the extract was subjected for the distillation for 2 hours (Fig-3). Further, the extract were kept in water bath at 40°C for drying. The dried extract (Fig-4) thus obtained was used for antimicrobial activity.



Fig-1: Drying leaves in hot air oven



Fig-2: Soxhlet Apparatus extraction of the sample



Fig-3: Distillation of the sample



Fig-4: Drying of extract in water bath

Sample Preparation

10 mg of the sample were dissolved in 1mL of DMSO (Dimethyl sulfoxide). Different concentration of the sample containing 100µg, 200µg, 300µg, and 400µg was prepared by pipetting different aliquot 10µL, 20µL, 30µL and 40µL of sample and the final volume of sample was made up to 50µL by adding DMSO.

Media Preparation

- For bacteria (*E.faecalis*- ATCC 35550) Luria Bertani (LB) agar media (tryptone 10g, sodium chloride 10g, yeast extract 6g, agar 15g, distil water 1000mL)
- For fungus (*Candida albicans*- ATCC 2091) Potato Dextrose Agar (PDA: Potato-200g, dextrose-20g, agar-20g, distilled water-1000mL) was prepared by boiling 200g of potato in 500mL distilled water and filtering it. The remaining components was added into the filtrate and the volume was made up to 1000mL with distilled water. The LB agar and PDA media was prepared and autoclaved at 121°C for 15 minutes.

Plate Preparation

Approximately 25mL of the media was poured into the sterilized petriplates and allowed it to solidify, later 24hrs cultured 100µL inoculum of *E.faecalis*, *Pseudomonas aeruginosa* poured into the LB agar plates and 72hrs cultured 100µL of *C.albicans* was poured into the PDA media in another plate respectively and spread throughout the plate using spreader. Five wells were made using well borer and the samples containing 100µg, 200µg, 300µg, and 400µg are loaded into the respective wells and 50µL of DMSO loaded in the centre well as control blank and incubated at 37°C for 24hrs for bacterial plates and 25°C. Zone of inhibition was recorded in mm (millimetre).

Determination of Minimum Inhibitory Concentration Using Ethanolic Ginger Extract Procedure

Revival of organisms – The selected bacterial and candidal strains was revived by plating on blood agar medium. After overnight incubation at 37⁰ C,

isolated colonies were selected and identities of the organisms were confirmed. Isolated colonies were then transferred to sterile BHI broth and Sabouraud dextrose broth for the bacterial and *Candida* strains respectively and once again incubated overnight. The growth concentration was adjusted to 10⁵ organisms/ml by using 0.5 McFarland's turbidity standard. An ethanolic solution of 10% concentration was prepared from the *Carica papaya* extract powder as the stock solution. A volume of 200µl of the BHI broth was added in each of ten MIC tubes per bacterial strain. For the *Candida* strain 200µl of the Sabouraud's broth was added in each of the ten MIC tubes. In the first MIC tube containing 200µl broth, 200µl of stock solution was added. After mixing well, 200 µl was transferred to the second MIC tube. This was continued till the last (10th tube). From the last tube 200µl final solution was discarded. By following this serial dilution, the concentration of papaya leaf extract powder was achieved as following 30%, 20%, 10%, 5%, and 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, 0.07%, 0.03% and 0.01 % respectively. To each of the ten such prepared MIC tubes with various concentrations, 200µl of the earlier prepared strains of *Enterococcus faecalis* was added such that the final volume per tube was 400µl. The procedure was repeated for the *Candida albicans* strain. The tube was then incubated for 24 hours at 35°C. After the incubation, the MIC values were determined by visual inspection of tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the MIC tube indicated growth of bacterial / *Candida* strain implying that the organisms were resistant to ethanolic papaya leaf extract.

RESULTS

Table-1 shows, For 10% ethanolic papaya leaf extract the zone of inhibition was found to be 12 mm and 11 mm in case of *E.faecalis* and *C.albicans* respectively (Fig 5 and 6). For 20% ethanolic papaya leaf extract it was observed that the zone of inhibition of 13 mm and 12 mm was observed for *E.faecalis* and *C.albicans* respectively and it was observed that the inhibition zone for the following organisms increased with the concentration. For 30% ethanolic papaya leaf extract, zone of inhibition increased to 15 mm and 13 mm in relation to *E. faecalis* and *C albicans* respectively. In relation to control (0.2% Chlorhexidine), it was observed that the zone of inhibition was 10 mm for *E.faecalis* and control (Flucanazole) 11 mm for *C.albicans*. The results of the study thus showed that different concentrations of papaya extract had better antimicrobial potential when compared to standard control used.

Table-2 shows shows Minimum inhibitory concentration of different concentrations ethanolic papaya leaf extract against *E.faecalis*, and *C.albicans* respectively. Minimum inhibitory concentration of

different concentrations of extract for *E. faecalis* was

established at 1.25% and *C. albicans* at 2.5%.

Table-1: Disc Diffusion Test [Zone Of Inhibition] for 10%, 20% and 30% Ethanolic Papaya leaf extract

<i>Carica papaya</i> extract in Percentage	MICROBIAL ZONE OF INHIBITION IN mm	
	<i>Enterococcus faecalis</i>	<i>Candida albicans</i>
10%	12 mm	11 mm
20%	13 mm	12 mm
30%	15 mm	13 mm
Control	10 mm (0.2% Chlorhexidine solution)	11 mm (Flucanazole)

Table-2: Minimum Inhibitory Concentration Different concentrations of Ethanolic Papaya leaf Extract by Serial Broth Dilution Method

Test organisms	30%	20%	10%	5%	2.5%	1.25%
<i>C. albicans</i>	S	S	S	S	R	R
<i>E. faecalis</i>	S	S	S	S	S	R

Legends

R- Resistant

S- Sensitive



Fig-5: Zone of inhibition for *E. faecalis*

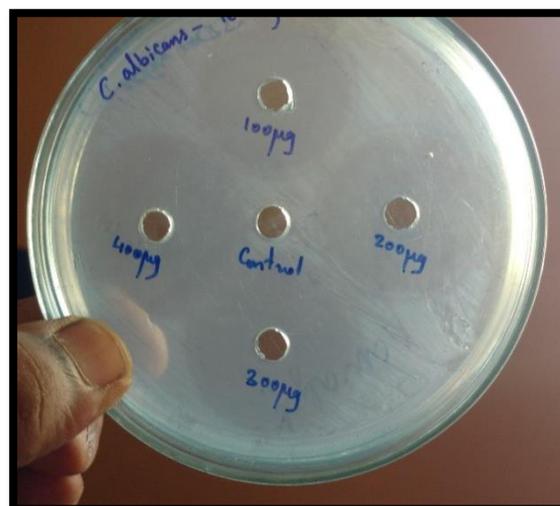


Fig-6: Zone of inhibition for *C. albicans*

DISCUSSION

The Therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane [25]. Flavonoids are a major group of phenolic compounds reported for their antiviral [26], antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties [27]. The presence of saponins supports the fact that papaya leaf has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic [28]. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties [29].

In the present study, 10%, 20% and 30% ethanolic papaya leaf extract showed zone of inhibition of 12mm, 13mm and 15mm respectively against *E. faecalis*. Comparison couldnot be done with any previous studies since this was first study which tried to assess the antimicrobial potential of ethanolic papaya extract of different concentration against *E. faecalis*. The following result can be attributed to the presence of phytochemical constituents like flavinoids, saponins, alkaloids and tannins which are mainly responsible for antimicrobial properties.

In the present study, 10%, 20% and 30% ethanolic papaya leaf extract showed zone of inhibition of 11mm, 12mm and 13mm respectively against *C. albicans*. Similar results were seen in studies conducted by Subramanian *et al.*, [15], De Boer HJ *et al.*, [30], Srinivasan D *et al.*, [31] and Bhaskaran *et al.*, [18]. The antifungal potential of the ethanolic papaya leaf extract can be attributed to the presence of presence of

carbohydrates, flavonoids, Terpenoids, saponins and tannins. This study was first of its kind where different concentration of ethanolic papaya leaf extract along with positive control was used against two test microorganisms in order to compare the efficacy of ethanolic papaya leaf extract.

In the present study, 0.2 % chlorhexidine and flucanazole was used as a control to test its antimicrobial potential against *E faecalis* and *C albicans*. It exhibited zone of inhibition of 10 mm and 11mm against *E faecalis* and *C albicans* respectively. It was observed that different concentration ethanolic papaya leaf extract exhibited maximum zone of inhibition against the two test organism when compared to the control, which had maximum zone of inhibition of 10 mm and 11mm respectively against *E faecalis* and *C albicans*. This is indicative that test extract used in our study has shown more antimicrobial potential than control used in the study.

Further research in future is still needed to isolate and characterize the bioactive principles of papaya leaf to develop new antimicrobial drugs.

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