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Papaya extract as new endodontic irrigant

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Abstract: ***Introduction:*** *The purpose of the study was to compare the antimicrobial efficacy of crude papaya extract with 5.25% Sodium hypochlorite(NaOCl) and 2% Chlorhexidine(CHX) against Enterococcus faecalis and Candida albicans at 1 min, 5 min and 15 min time intervals.* ***Methods****: #72 absorbent paper points were immersed in experimental suspensions (E. faecalis and C. albicans) for 5 mins and placed on test tubes to cover with irrigants (NaOCl, CHX, papaya extract, and distilled water. Distilled water was used as negative control. At 1 min, 5 mins and 15 mins intervals, 18 absorbent points for each irrigants were removed from contact with irrigants, individually transported to immerse in Letheen broth and incubated at 37ºC for 48 hr. Microbial growth was analyzed by turbidity of culture medium and measured by spectrophotometer at 560 nm. Inoculum obtained from Letheen broth was serially diluted for spread plate. Colony Count was done for viable cell count.* ***Result:*** *The result of turbidimetric measurement showed crude papaya extract to be effective against E. faecalis after 5 mins whereas it was effective against Candida albicans at all time intervals. The result of viable cell count of crude papaya extract shows gradual decrease of colony count from 1 min to 15 min against E. faecalis and Candida albicans.* ***Conclusion:*** *Papaya extract demonstrated potent antimicrobial activity against E. faecalis and Candida albicans comparable to 5.25% NaOCl and 2% CHX at 5 min and 15 min time intervals.*

Keywords - *Antimicrobial, C.albicans, E.faecalis, papaya extract*

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I. Introduction

Success of root canal treatment depends upon complexity of the pulp canal space. Its proper debridement and disinfection is core requisite for the endodontic success. Hence irrigation of root canals with antibacterial solutions is considered as an integral part [1]. Researchers have shown that even after meticulous and advanced instrumentation substantial part of root canal wall is left un-instrumented [2, 3]. Hence, irrigation may be considered as the primary method to clean and disinfect these parts of the root canal system [4, 5].

Root canal irrigants should have antibacterial and antifungal properties, in addition it must not be toxic to periapical tissues while in contact with them. To combat the toxicity of synthetic irrigants, use of natural plant extracts as endodontic irrigants is the growing trend of research in dentistry [6, 7]. One of potent natural plant extract with well known antibacterial and antifungal property is *Carica papaya* [8]. Its leaves extract contains folic acid, vitamins B12, A and C, alkaloids, saponins, glycosides, tannins, and flavonoids [8,9]. These secondary metabolites have bactericidal, bacteriostatic and anti-inflammatory characteristics [10]. Flavonoids provide potential protection against oxidative and free radical damage. They are called as "biological response modifiers" as they modify the body's reactions to allergens, carcinogens and viruses. Hence they have been described as having anti-inflammatory, anti-allergic, anticarcinogenic, antioxidant and antiviral properties [11, 12].

In root canal, *Enterococcus faecalis* and *Candida albicans* dominate primary lesion as well as these are recalcitrant and are persistent in periradicular lesions even after root canal treatment. This is because *E. faecalis* has ability to survive extreme environment, resists different antimicrobial even high pH of calcium hydroxide and can bind to dentin [13, 14]. *C. albicans* is also versatile microbe, it can adapt to range of pH, changes gene expression according to environmental conditions, adheres to variety of surfaces, produces degradative enzymes and changes morphologic forms to evade the immune system[15]. In addition to these virulence factors, complexity of root canals promotes bacterial growth and limits the action of disinfectants adding more

challenges for obtaining a disinfected state of the root canal system[13,16]. Hence, recent studies have focused on evaluating the effectiveness of different root canal irrigants and medicaments against these virulent microbes.

In the verge of the search for potential ideal endodontic irrigants, different synthetic and herbal irrigants are field of research these days. As papaya extract is used as a potent antibacterial and antifungal agent with proven medicinal property [8], but its efficacy against root canal pathogens is still unclear. Thus, the objective of the study was to evaluate the antimicrobial and antifungal potential of papaya extract and compare with the popular endodontic irrigants i.e. sodium hypochlorite and chlorhexidine.

II. Material And Method

2.1 Preparation of papaya extract: Healthy/ disease free, mature, fresh plant leaves of *Carica papaya* were handpicked and authenticated by Institute of Agriculture and Animal Sciences (IAAS), Tribhuvan University, Bhairahawa, Nepal. The leaves were surface sterilized in 0.1% mercuric chloride for 5 min and air dried at room temperature for 24 hrs. Sterilized leaves were grounded fine by electric blender. The crude extract obtained was centrifuged at 4000 rpm for 30 mins. The supernatant layer obtained was filtered through Whatman filter paper no: 1.The extract was then stored at 4ºC.

2.2 Inoculum preparation: Standard strain of *E. faecalis* (ATCC 29212) and *C. albicans* (ATCC 10231) was revived from lyophilized state. The inoculums prepared of test isolates were matched with 0.5 MacFarland to get concentration of $1.5x10^8$ cells/ mL.

2.3 Experiment: 72 sterile paper points #50 (Dentsply, Maillefer) were taken and divided into 2 groups of 36 paper points for 2 test organisms*: E. faecalis* and *C. albicans*. For each irrigants used i.e. 5.25% NaOCl, 2% CHX, crude Papaya extract and Distilled water, 3 paper points were used for specified time interval of 1 min, 5 min and 15 min.

2.4 Direct Contact Test: The paper points were immersed individually into test tubes containing 5 ml of freshly prepared bacterial suspension of *E. faecalis* and *C. albicans* respectively for 5 mins. Paper points were transferred to test tubes containing irrigating solution for different time intervals i.e. 1 min, 5 min and 15 min respectively. After definite contact time, paper points were immediately transferred into test tubes containing 5 ml of Leethen broth (neutralizing solution) and incubated at 37ºC for 48 hrs.

2.5 Interpretation of microbial growth: After incubation, test tubes were observed for the presence of turbidity and optical density was measured using Spectrophotometer (Spectrochem-i, Double Beam 2800CE, New Delhi) at 560 nm wavelength. Gram-staining was done to check unwanted microbial growth. Spread plate method was used for the viable cell count. The tubes were serially diluted upto 10^{-6} and 10µl of solution of was spread on to Nutrient agar and Sabourad's Dextrose agar plate. The plates were incubated at 37ºC for 24-48 hrs. Colony morphology was studied and gram staining was done and biochemical tests were performed to confirm the organism isolated. The colonies grown on the media were counted with the help of Colony counter machine (Optics Technology, New Delhi).

2.6 Data analysis: Experiment was conducted in triplicate form. Data obtained was expressed as mean ± standard deviation. Statistical evaluation of turbidimetric analysis was done using ANOVA test and individual analysis of each group at each time interval was done by Tukey's test. Analysis of viable cell count was done using independent't' test.

III. Result

The result of crude papaya extract against *E. faecalis* showed good antimicrobial property by both colony count and turbidimetric measurement, there is significant decrease in mean CFU count from 112 ± 3 to 1 ± 1 ($p< 0.05$) from 1 min to 15 min time intervals (Fig 1). Similarly, the mean optical density also decreased significantly from 0.693 ± 0.023 to 0.550 ± 0.03 ($p<0.05$) in 1 min to 15 min (Table 1). Comparison within each time interval shows 5.25% NaOCl to be most effective irrigants as its shows significant mean difference of optical density at all-time intervals ($p< 0.05$) whereas 2% CHX and crude papaya extract shows significant mean difference of optical density only after 5 min($p< 0.05$) (Table 2).

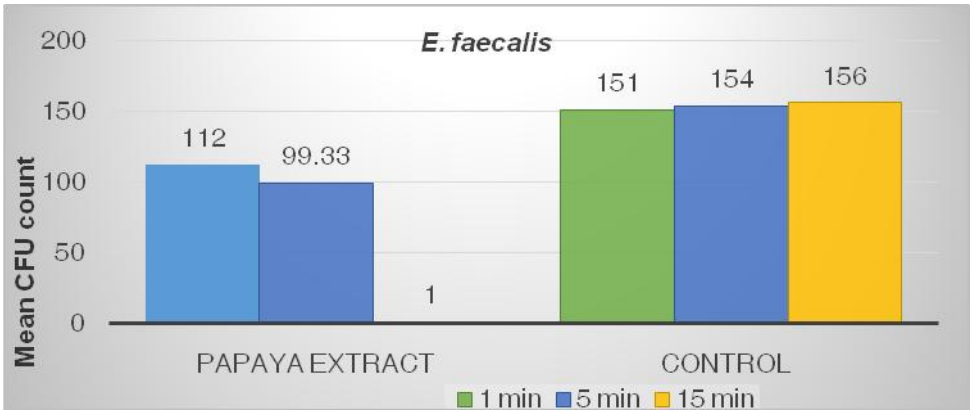

Figure 1: Comparison of mean colony forming unit (CFU) of papaya extract and control against *E. faecalis* at different time intervals.

Table 1: Mean Optical density (OD) (mean ± SD) of different irrigant against *E. faecalis* and *C. albicans*

Groups	Time intervals	*E.faecalis*	*C. albicans*	p-value* of *E. faecalis* group	p-value* of *C. albicans* group
NaOCl	1min	0.403±0.006	0.007±0.001	0.000	0.001
	5min	0.347±0.020	0.005±0.000		
	15min	0.008±0.002	0.002±0.001		
CHX	1min	0.009±0.001	0.008±0.001	0.008	0.001
	5min	0.004±0.000	0.005±0.000		
	15min	0.004±0.002	0.002±0.001		
Papaya	1min	0.720±0.023	0.333±0.041	0.008	0.000
	5min	0.693±0.026	0.217±0.020		
	15min	0.550±0.030	0.000±0.000		
DW	1min	0.623±0.015	0.547±0.025	0.355	0.984
	5min	0.580±0.060	0.550±0.036		
	15min	0.587±0.006	0.550±0.010		

*ANOVA test was performed to identify the significant groups at 5% level

Table 2: Comparison of mean optical density (OD) of different irrigants within the groups against *E. faecalis*

Irrigants	Contact time		Mean Difference in Optical Density between Contact time groups (I-J)	p-value[#]
	(I) groups	(J) groups		
NaOCl	1 min	5 min	0.0566667*	0.004
	5 min	15 min	0.3390000*	0.000
	15 min	1 min	-0.3956667*	0.000
CHX	1 min	5 min	-0.0003333	0.965
	5 min	15 min	-0.0053333*	0.015
	15 min	1 min	0.0056667*	0.012

Papaya	1 min	5 min	-0.0266667	0.483
	5 min	15 min	0.1700000*	0.001
	15 min	1 min	-0.1433333*	0.001
Control	1 min	5 min	0.0433333	0.372
	5 min	15 min	-0.0066667	0.973
	15 min	1 min	-0.0366667	0.478

Tukey HSD test was performed to identify the significant groups at 5% level.*The mean difference is significant at 0.05 level.

For *C. albicans* also the papaya extract showed appreciable antimicrobial property as the mean colony count decreased from 2.67 ± 1.15 to 0.00 ($p<0.05$) in 1 min to 15 min (Fig 2).

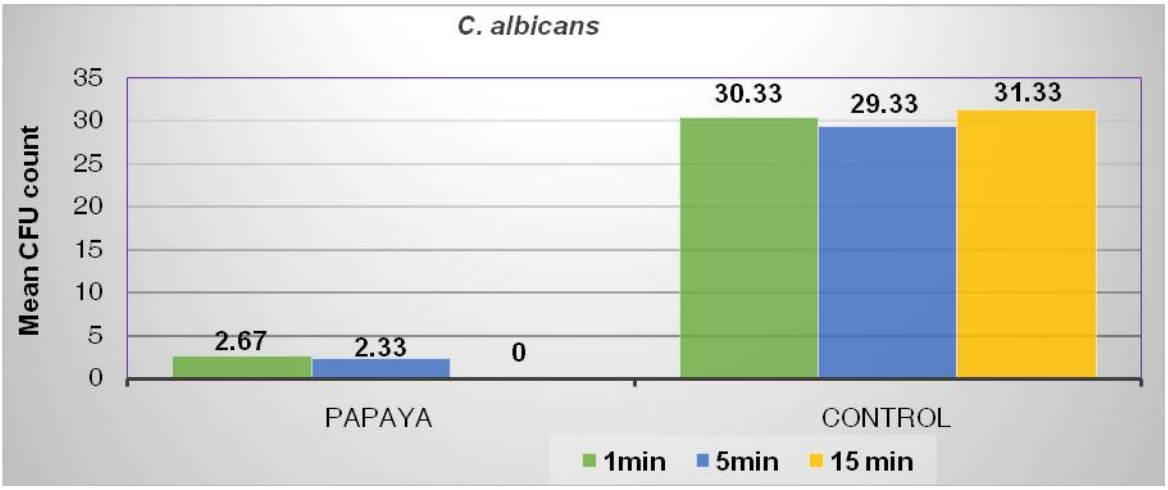

Figure 2: Comparison of mean colony forming unit (CFU) of papaya extract and control against *C. albicans* at different time intervals.

Similarly, mean optical density also decreased significantly from 0.333 ± 0.041 to 0.00 ($p<0.05$) from 1 min to 15 min (Table 1). Comparison within each time interval shows comparable antimicrobial efficacy of 5.25% NaOCl, 2% CHX and papaya extract against *C. albicans* with significant mean difference of optical density at all-time intervals (Table 3).

Table 3: Comparison on mean optical density (OD) of different irrigants within the group against *C. albicans*

Irrigants	Contact time		Mean Difference in optical density between contact time groups (I-J)	p-value#
	(I) group	(J) group		
NaOCl	1 min	5 min	0.0023333*	0.041
	5min	15 min	0.0026667*	0.023
	15 min	1min	-0.0050000*	0.001
CHX	1min	5min	0.0030000*	0.019
	5min	15 min	0.0026667*	0.031
	15min	1min	-0.0056667*	0.001
Papaya	1min	5min	0.11667*	0.004
	5min	15 min	0.21667*	0.000

	15min	1min	-0.33333*	0.000
Control	1min	5min	-0.0033333	0.987
	5min	15 min	0.0000000	1.000
	15min	1min	0.0033333	0.987

Tukey HSD test was performed to identify the significant groups at 5% level.* the mean difference is significant at 0.05 levels.

IV. Discussion

Till date NaOCl and CHX have gained popularity as most effective irrigants in combating persistent endodontic infection because of their high antimicrobial efficacy. However, unpleasant taste and odor, toxicity, allergic potential, resorption, inability to remove smear layer are the main disadvantages of NaOCl [17]. In addition, Grigoratos D et al. [18], reported decrease in flexural and elastic strength of dentin after 2 hr submersion in NaOCl. The toxic effect of 5.25% NaOCl is postulated to be greater than 2% CHX as reported by Oncag O et al. [19]. CHX has a reasonably wide range of activity against aerobic and anaerobic organisms as well as the Candida species. But the presence of inflammatory exudates and killed microorganisms can inhibit the action of chlorhexidine in root canals and in addition, it is incapable to dissolve tissues [4]. These undesirable characteristics of current synthetic irrigants leads to opt for alternatives like use of natural plant extracts as endodontic irrigant.

For the preparation papaya leaf extract manual method was used as suggested by Oyagade et al [20].This technique provides extraction of enzyme or protein in a more delicate or softer method and minimizes the use of chemical compounds as enzymes and proteins are prone to denaturation and are thermolabile. Time intervals of 1 min, 5 min and 15 min were taken, as minimum irrigation time is 1 min and maximum 15 min while performing endodontic treatment. So the aim was to evaluate whether the irrigants have efficient antimicrobial property in the given time intervals.

In present study, direct contact test (DCT) was used to assess the antibacterial activity as it measures the antimicrobial effect regardless of the solubility and the diffusion of the antimicrobial components. The results of DCT are more quantitative and reproducible when compared to those of the Agar Diffusion Test [21]. Spectrophotometric measurement was used to measure the turbidity as it indirectly measures all bacteria (cell biomass), dead and alive. Hence, to evaluate viable cell count left after antimicrobial activity, colony count method was performed in addition to turbidimetric measurement. Moreover, the test micro-organisms i.e E.faecalis and C.albicans in the methodology are used in planktonic form because the test was conducted in an intention of primary screening of antibacterial and antifungal property of papaya extract.

The result of viable cell count showed that 5.25% NaOCl and 2% CHX had no colony forming unit at all-time intervals against *E. faecalis* and *Candida albicans*. This is in agreement to the study done by Silva et al. [22] , C. E. Radcliffe et al [23] and Waltimo et al. [24] whereas, the study conducted by Retamozo et al showed effective irrigation regimen was 5.25% NaOCl at 40 minutes [25].

The antibacterial activity of CHX was delayed due to the adherence of antiseptic particles to the bacterial cell wall which takes longer than 5 min [26]. Hence, the clinical implication of these findings is that 2% CHX and papaya extract should be in direct contact with the infected dentinal surface for a prolonged time (>5 min) in order to achieve their maximum antibacterial effect against *E. faecalis*.

The papaya extract showed appreciable antimicrobial property against both *E. faecalis* and *C. albicans*. These results are in agreement with the study done by Okunola A. Alabi et al [27] in which 100 mg/ml papaya extract showed growth inhibition of *E. faecalis* and *C. albicans.* The antimicrobial activity of papaya extract can be attributed to the presence of different bioactive compounds such as alkaloids, saponin, flavonoids, reducing sugar, tannin and steroid as suggested to the study done by Yusha'u M et al [28].Similarly LSS. Abirami et al [29] also performed phytochemical analysis of papaya leaf which showed the presence of flavonoids, alkaloids and terpene. These compounds are posulated to influence its mycotoxicity by interacting with fungus membrane constituents. 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) to be in the leaf extract [30] of which alkaloids are quite probably an important element in defense against pathogens [31].

V. Conclusion

Within the limitation of the in vitro study, it can be concluded that the antimicrobial efficacy of crude papaya extract was comparable to 5.25% NaOCl and 2% CHX at 15 min time interval for both *E. faecalis* and *C. albicans*. Hence, papaya extract can be hypothesized to be more beneficial as in context of patient. The

magnitude of this effect was influenced by the experimental method, characteristics of the microorganisms and the exposure time. For conclusive result further clinical studies with large sample size should be carried out.

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The authors deny any conflicts of interest related to this study.

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