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Research Article

ANTIBACTERIAL EFFICACY OF E. *JAMBOLANA* (*SYZYGIUM CUMINI*) SEEDS EXTRACTS AGAINST PERIODONTAL PATHOGENS: AN IN-VITRO STUDY

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ABSTRACT

Background: Periodontal disease is progressive, inflammation of dento-gingival complex caused by specific periodontal pathogens. Objective**:** To evaluate the minimum inhibitory concentration and zone of inhibition of *E. Jambolana (S.cumini)* seed extract (aqueous, ethanol and acetone extracts) against *P.Gingivalis*, *F.Nucleatum* and A. *Actinomycetemcomitans* by tube dilution and disc diffusion tests. Methods**:** Aqueous, ethanol and acetone extract of *E. Jambolana* seeds were prepared. Blood agar media with Hemin, Vitamin K and Kanamycin was used to culture P *gingivalis.* Crystal violet Erythromycin Blood Agar and Thioglycolate broth with 1% horse serum were used to culture F. nucleatum and A. *actinomycetemcomitans* respectively. The antibacterial activity of extracts was evaluated by using the disc diffusion method and the Minimum Inhibitory Concentration (MIC) of the test solutions was determined by Tube dilution method. Results**:** *E. Jambolana* seed extracts exhibited antibacterial effect against all three tested periodontal pathogens. Acetone extract of E. *Jambolana* exhibited more potent activity than the aqueous and ethanol extracts. Minimum Inhibitory concentration of *E. Jambolana* seed extract against P. *gingivalis*, was found to be 6.25µl/ml (acetone), against F*. nucleatum* it was 25µl/ml (acetone) and against A. *actinomycetemcomitans* it was 12.5µl/ml (acetone). Conclusion**:** *Syzygium cumini* seeds extracts exhibited antibacterial property against *P. gingivalis, A. actinomycetemcomitans* and *F. nucleatum.*

INTRODUCTION

Periodontal diseases have been considered as one of the major health problems affecting humans.[1] Periodontal diseases are caused by specific pathogens. Although it is generally considered that the disease has multifactorial etiology, some specific gram-negative microorganisms in the plaque biofilm cause initiation and progression of periodontitis and other microorganisms have been implicated as predominant species in the disease process.

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Porphyromonas gingivalis, is a red complex organism which is gram-negative, obligate anaerobe linked to periodontal damage. It is strongly associated with severe chronic periodontitis. Other microorganisms that have been implicated as predominant species in the periodontal disease process are *Aggregatibacter actinomycetemcomitans* a non-motile gram-negative rod which is strongly associated with destructive periodontal lesions and *Fusobacterium nucleatum* a gram- negative bacilli, obligate anaerobe associated commonly with refractory periodontitis.[2]

The main periodontal disease treatment target remains the effective reduction of the supra- and sub-gingival pathogenic flora, mostly by mechanical means and by variety of chemical means, like antibiotics and antiseptics as an adjunct to mechanical therapy but, due to their inappropriate or widespread overuse they can lead to antimicrobial resistance.[3] This has spurred

scientists on the research for plant based antimicrobial agents.

Around 75–80% of the world population, in the developing countries depends on herbal medicine for primary health care because of better cultural acceptability, better compatibility and few side effects. India has well recorded and well-practiced knowledge of traditional herbal medicine. In spite of tremendous development of allopathic medicine, they are found to have some side effects. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programs because the plant-based therapeutics are natural products, non-narcotic, easily bio-degradable, pose minimum environmental hazards, have less adverse effects which are easily available and affordable.[4] *Eugenia jambolana* (*Syn. Syzygium cumini; Family: Myrtaceae*) known as '*Jamun*' is a native medicinal plant of India. It grows naturally in tropical as well as subtropical zones. Rural people of India use the seed, fruit, leaf, bark of this plant as folk medicine to combat different types of diseases and disorders since antiquity. Scientific studies have shown that extracts of different parts of *E. jambolana* possess a range of pharmacological properties such as antioxidant, anti-inflammatory and antimicrobial activities.[5] Seeds are known to be rich in flavonoids known for anti-oxidant properties and phenolics known to exhibit antimicrobial properties.[6] Few studies have shown antimicrobial properties of *E. Jambolana* leaf extract against dental caries producing organisms,[7] but literature search revealed no study testing antibacterial property of *E. Jambolana* seed extract against periodontal pathogens. Hence, an in-vitro study was done to evaluate the antibacterial efficacy of *E. Jambolana (Syzygium Cumini) seeds* extract against *P gingivalis, F nucleatum* and *A actinomycetemcomitans.*

MATERIAL AND METHODS

An in-vitro study design was adopted. The methodology for preparation of the extract and stock solution was based on study done by Anwesa Bag et al.[5]

Collection, identification and processing of *E. Jambolana* (*S. Cumini*) seeds

E.Jambolana seed powder was purchased online for the preparation of extracts with different solvents. The seed powder was ordered from the brand JAIN under the seller Herbocart from Flipkart, with AYUSH license number T-2176/Ayur, ISO certified. Date of production 25th August 2019.

Extract preparation

Aqueous, ethanol and acetone extract of *E. jambolana* seed powder was prepared by immersing 100gm seed powder in each of the three conical flasks stopper with rubber cork containing 600 ml water, 70% aqueous ethanol and acetone respectively with occasional shaking at room temperature for 24h. The mixtures were kept for 24h in case of aqueous and acetone extracts and consecutive three days for ethanol extract and filtered. The process was repeated twice using remaining residues. The pooled filtrates were centrifuged at 3000 rpm for 15 min and concentrated under reduced pressure using a rotary evaporator (yield: 17.26% aqueous extract; 33.4% ethanol extract and 27.2% acetone extract).

Preparation of Stock Test Solutions[5]

The extracts were maintained at 4 ◦C in air-tight jars. Aqueous extract was reconstituted in distilled water and ethanol and acetone extracts in 5% dimethylsulphoxide (DMSO) to obtain a final concentration of 100 mg/ml.

Cultivation of Microorganisms

Blood agar media with hemin, vitamin K and Kanamycin was used to culture *P. gingivalis* and crystal violet erythromycin blood agar was used to culture *F nucleatum* and thioglycolate broth with 1% horse serum was used to culture *A. actinomycetemcomitans*.

Assessment of Antibacterial Activity[7]

The antibacterial activity of extracts was evaluated by using the Agar disc diffusion method and the Minimum Inhibitory Concentration (MIC) of the test solutions was determined by the Tube dilution method. Double dilution was made from a higher dilution to a lower dilution in a series of test tubes. The MIC values of the test extracts was compared against the Minimum Inhibitory Concentration of 0.2% chlorhexidine solution (positive control) against P. gingivalis, F. nucleatum and A. actinomycetemcomitans respectively.

RESULTS

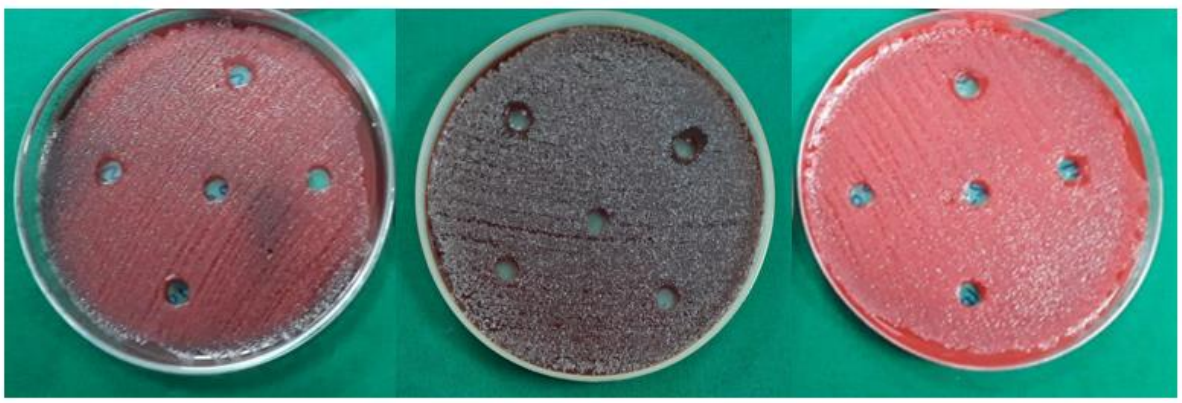
E. Jambolana seed extracts exhibited antibacterial effect against all three tested periodontal pathogens (*P.gingivalis, F.nucleatum and A.actinomycetemcomitans*.) However, acetone extract exhibited more potent activity than the aqueous and ethanol extracts. (Table 1) Minimum Inhibitory concentration of *E. Jambolana* seed extract against *P. gingivalis*, was found to be 6.25µl/ml (acetone), against *F. nucleatum* it was 25µl/ml (acetone) and against A *actinomycetemcomitans* it was 12.5µl/ml (acetone). The minimum inhibitory concentration values were in the range of 6.25µl/ml to 100µl/ml. (Table 1). Results of the Disc Diffusion Test, indicated that the zone of inhibition exhibited at 50µl/ml concentration of *Jamun* seed extract against *P. gingivalis* and *A. actinomycetemcomitans* was 10mm and at 75µl/ml concentration the zone of inhibition against *P.* gingivalis (14-18mm) was more compared to *F. nucleatum* and *A. actinomycetemcomitans*. (Table 2) (Figure 1)

Table 1: Results of minimum inhibitory concentration test											
S.n	**Sample**	**Concentrations of dilutions of *E Jambolana* seed extract in µl/ml**									
		100	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.6**	**0.8**	**0.4**	**0.2**
	P Gingivalis										
1	Acetone extract	S	S	S	S	S	R	R	R	R	R
2	Ethanol extract	S	S	S	R	R	R	R	R	R	R
3	Aqueous extract	S	S	S	S	R	R	R	R	R	R
	F Nucleatum										
1	Acetone extract	S	S	S	R	R	R	R	R	R	R
2	Ethanol extract	S	R	R	R	R	R	R	R	R	R
3	Aqueous extract	S	S	R	R	R	R	R	R	R	R
	A.Actinomycetemcomitans										
1	Acetone extract	S	S	S	S	R	R	R	R	R	R
2	Ethanol extract	S	S	S	R	R	R	R	R	R	R
3	Aqueous extract	S	S	R	R	R	R	R	R	R	R
		S-Sensitive, R-Resistant									

Table 2: Results of Disc diffusion test

Concentration of extracts	75µl/ml	50µl/ml	25µl/ml	10µl/ml	5µl/ml
Zone of Inhibition in millimetres					
P.Gingivalis					
Acetone	18mm	10mm	R	R	R
Ethanol	13mm	R	R	R	R
Aqueous	14mm	R	R	R	R
F.Nucleatum					
Acetone	12mm	R	R	R	R
Ethanol	12mm	R	R	R	R
Aqueous	10mm	R	R	R	R
A. actinomycetemcomitans					
Acetone	10mm	10mm	R	R	R
Ethanol	10mm	R	R	R	R
Aqueous	12mm	10mm	R	R	R
	S-Sensitive, R-Resistant				

Figure 1:Disc Diffusion Test



P gingivalis A actinomycetemcomitans F nucleatum

DISCUSSION

Periodontal diseases lead to damage of the supporting tissues of the teeth (Bone and connective tissue) and affect the oral health related quality of life. It is well known fact that periodontal diseases are infections caused by bacteria that colonize the tooth surface, gingival margin and sub-gingival environment. The "red complex" organism Porphyromonas gingivalis, is related to the initiation and progression of the periodontal destruction.[1] It predominantly consists of *P. gingivalis, B. forsythus,* and *T. denticola* organisms. These organisms are associated with bleeding on probing, which is an important clinical parameter of destructive periodontal diseases.[1] P. gingivalis is a periodontal microorganism that is strongly associated with chronic and aggressive forms of periodontal disease in humans.[1] Gingipains are the group of protease enzymes produced by P gingivalis. They are not only responsible for destruction of collagen, but also linked to attachment properties of organism, which is also an important component of pathogenesis. Another important pathologic mechanism of P.gingivalis is that it induces a decrease of both Interleukin -8 (IL-8) secretion and intercellular adhesion molecule-1 (ICAM-1) expression in oral epithelial cells, which are important for the recruitment of neutrophils, and hence host defenses are subverted.[1] Other microorganisms that have been implicated as predominant species in the periodontal disease process are A actinomycetemcomitans and F nucleatum.[2] Hence, these organisms were considered in the study.

With rising prevalence of antibiotic resistance, and high cost of production of conventional synthetic antimicrobials, there is a need for searching alternative antimicrobial products from natural sources. In this regard, herbal medicine could serve as a safe and economic alternative to combat periodontal diseases. is one such herb that has long been used in Ayurveda for general health and well-being. *Eugenia jambolana* (*Syn. Syzygium cumini; Family: Myrtaceae*) commonly known as '*Jamun*' is a medicinal plant native to India and commonly consumed by people across the nation. An in- vitro study demonstrated that the leaves of S. cumini showed, antibacterial activity against dental caries causing microorganisms.[7,9] Phytochemical analysis of *E. jambolana* seeds showed the presence of high concentration of flavonoids and phenolics with alkaloids and saponins and low concentration of terpenoids which may lead to the antibacterial potential against multidrug resistant human bacterial pathogens like S.aureus [5]. The synthetic products that are currently in use for treatment of periodontitis do suffer from certain disadvantages. Advantages of herbal medicines are: it's safe, easily available, culturally acceptable and economical.[10]

Based on the results of the present study all the extracts (Acetone, ethanol and aqueous) of E. *Jambolana* seed showed antibacterial property against *Porphyromonas gingivalis, Fusobacterium nucleatum* and *A actinomycetemcomitans*. The acetone extract of *E. Jambolana* seed showed most potent antibacterial activity even at lower concentration against *P. gingivalis* and *A. actinomycetemcomitans.* Comparison could not be drawn with other studies as authors could find no study done to test the antibacterial efficacy of E. *Jambolana* seed extract against periodontal pathogens. The antimicrobial property of E. *Jambolana* seeds can be attributed to the presence of high concentration of phenolics, flavonoids, alkaloids, saponins and terpenoids present in the seeds.[11] S Cumini seed is known to be very rich in gallic and ellagic acid polyphenol derivatives which exhibit antioxidant and antimicrobial properties.[12] *E. Jambolana* seed extracts were prepared by cold maceration technique in the present study so that the decomposition of heat sensitive active constituents could be avoided. Review of literature revealed that aqueous and ethanolic extract of *E. Jambolana* seeds was effective in inhibiting the growth of microorganisms.[11] Agar diffusion method is considered to be a standard method of assessing the antimicrobial property of a drug. The agar diffusion method is based on the ability of the test agent to diffuse through the solid agar medium and inhibit the microorganism.[13] In order to substantiate the results of serial dilution method, Minimal Inhibitory Concentration of *E. Jambolana* extracts against periodontal pathogens was also assessed in the present study. Minimal Inhibitory Concentration is considered to be more meaningful in assessing the antimicrobial property of a test agent.[14] Testing antibacterial potential of *Jamun* seed extracts against the tested microorganisms in a biofilm model and in-vivo studies would provide more meaningful insights. The utility of the study is that fresh *E. Jambolana* seed extract can be incorporated into toothpaste or in mouth rinse and prescribed to individuals to reduce the periodontal microbial load in the oral cavity thus serving as both preventive and therapeutic agent against periodontitis. *E. Jambolana* seed extract is generally well tolerated by individuals without any toxic or adverse effects. Hence, the professional supervision required when *E. Jambolana seed* extract is used as a mouth rinse is minimal.

CONCLUSION

Extracts of *Syzygium cumini (Jambolana)* seeds showed antibacterial property against *P. gingivalis, F. nucleatum* and *A. actinomycetemcomitans* in-vitro.

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