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In-Vitro Antimicrobial Activity of *Costus pictus* "A Multifaceted Wonder Herb" Against Periodontogenic Bacteria

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Abstract

Background: Periodontitis is an inflammatory disease, initiated by colonization of bacteria, which triggers an immunoinflammatory response in the host tissues, resulting in the destruction of the periodontium.

Objectives: The aim of the present study was to assess the antimicrobial activity of pure *Costus pictus* extract (Insulin plant) on periodontal pathogenic microorganisms; *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Fusobacterium nucleatum (Fn).*

Materials and Methods: The minimum inhibitory concentration (MIC), and minimum bactericidal concentrations (MBC) were performed to evaluate the antimicrobial effect of both ethanolic and aqueous extract of pure *Costus pictus* against periodontopathogenic bacteria by serial dilution method and colony forming units respectively.

Conclusion: Both aqueous and alcoholic extract of *Costus pictus* exhibited effective antibacterial activity, however, ethanolic extract exhibited increased antibacterial activity in comparison with aqueous extract.

Keywords: Antibacterial Activity; Costus Pictus; Minimum Bactericidal Concentration; Minimum Inhibitory Concentration

Introduction

Periodontitis results from a complex interaction between subgingival biofilm and host immune inflammatory events that develop in periodontal tissue in response to the challenge promoted by bacteria. Thus, periodontitis is an inflammatory disease initiated by a bacterial pathogen. However, host immunological response through the bacterial challenge can cause more severe destruction than the pathogenic bacteria and its by-products itself [1].

Mechanical treatment can remove the local factors and disrupts the subgingival biofilm but they cannot act on the bacteria in tissue which can be reduced either by administration of systemic antibiotics or locally delivered antibiotics into the periodontal pocket. This modifies the host's immune response to the bacteria and reduces the self-destructive immunologic response to the bacterial pathogen [1]. However indiscriminate use of systemic antimicrobials as an adjunct to the conventional periodontal treatment was well established due to the tissue penetrable nature of pathogenic microbes [2]. In this regard, various herbal products have been tried and tested as a local drug agent, due to their antimicrobial properties and minimal side effects as compared to the systemic antimicrobials which are known to develop multi-resistant microorganisms [3].

Costus pictus (C. pictus) is one of the herbal product with an antibacterial property. It is commonly known as 'spiral ginger', 'step ladder' or 'insulin plant' originated in Mexico [4]. It has been reported that Costus pictus is known to possess therapeutic effects like antibacterial, antioxidant, antitumour and antidiabetic action. The leaves and rhizomes of *C. pictus* contain appreciable amounts of the elements potassium (K), calcium (Ca), chromium (Cr), manganese (Mn), copper (Cu) and zinc (Zn), which may be responsible for potentiating insulin action.

The different parts of the insulin plant (leaf, flower, stem and root) exhibited pronounced antibacterial activity against microorganisms such as *Shigella flexneri, Klebsiella pneumonia, Bacillus subtilis and Escherichia coli*, and it was observed that the activity was quite comparable with the standard antibiotics screened under similar conditions [5].

To the best of our knowledge, till date, no study has been done to assess the antimicrobial effect of Costus pictus on periodontal

pathogens. Hence, the aim of the present study was to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of both aqueous and ethanolic pure Costus pictus extract that can be safely and effectively administered as local drug delivery system on specific periodontal pathogens like *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Fusobacterium nucleatum* (Fn) *and Aggregatibacter actinomycetemcomitans* (Aa).

Materials and Methods

Costus pictus Extract

About 100% pure *Costus pictus* (powder form) was obtained from Adhiti agro farm, Pammal Chennai. It was certified to be free from any form of bacteria, yeast, or mould by the manufacturer after microbial analysis.

Bacterial Strains

Bacterial strains used in this study were American type culture collection, Manassas, VA, USA. The tested bacterial strains in this study were Pg, AT CC 33277, Pi AT CC 25611, Fn, AT CC 25586 and Aa, AT CC 29523.

Minimum Inhibitory Concentrations (Serial dilution method)

To determine the antibacterial activities, 9 serial dilutions of *Costus pictus* extract were prepared in thioglycollate (TG) broth medium by means of standard protocols given by Schwalbe *et al. Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Fusobacterium nucleatum* (Fn) *and Aggregatibacter actinomycetemcomitans* (Aa) were suspended in TG broth medium. An about 200 µl concentration of *Costus pictus* extract was diluted in serial dilution manner [6]. 10 µl of each stock culture was added to the test tube containing antimicrobial agents, respectively. The test tubes were shaken well and incubated at 37 °C for 24 hours in an anaerobic jar/chamber and observed for turbidity. The minimum concentration of the drug in the tube which does not show any turbidity is considered as the minimum inhibitory concentration of the drug.

Minimum Bactericidal Concentration

After the MIC procedure, four dilution tubes which were showing sensitivity to the antibacterial agent at lower concentrations were taken and inoculated into the respective culture medium to check the growth of microorganisms. Formerly plates were incubated in anaerobic jar/chamber for ≥48hrs and then colonies were counted.

µg/ ml	100	50	25	12.5	6.25	3.12
Fn						
Aqueous extract	S	S	S	S	S	S
Ethanol extract	S	S	S	S	S	S
Aa						
Aqueous extract	S	S	S	S	S	S
Ethanol extract	S	S	S	S	S	S
Pi						
Aqueous extract	S	R	R	R	R	R
Ethanol extract	S	R	R	R	R	R
Fn						
Aqueous extract	S	R	R	R	R	R
Ethanol extract	S	R	R	R	R	R

S- Sensitive, R- Resistant

Table 1: MIC values of *Costus pictus* extract against *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Fusobacterium nucleatum (Fn)*

µg/ ml	100	50	25	12.5	6.25	3.12
Fn						
Aqueous extract	59	61	66	69	73	79
Ethanol extract	13	18	22	34	61	63
Pg						
Aqueous extract	81	96	99	108	116	142
Ethanol extract	69	71	82	84	98	106
Pi						
Aqueous extract	74	78	84	96	108	116
Ethanol extract	62	75	79	91	95	99

µg/ ml	100	50	25	12.5	6.25	3.12
Aa						
Aqueous extract	62	68	78	88	96	103
Ethanol extract	NG	NG	84	95	222	224

NG- No Growth

Table 2: MBC values of *Costus pictus* extract against *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Fusobacterium nucleatum (Fn)*

Results

All the microorganisms were sensitive at the highest concentration (100 µg/ml), whereas Aa and Fn were sensitive to the least concentration also (3.12 µg/ml). Pg and Pi were sensitive only at the highest tested concentration (100 µg/ml). However, the number of colony forming units reduced as the concentration of extract increased and also the ethanolic extract showed few colony forming units as compared to the aqueous extract. Only Aa showed no growth at 50 and 100 µg/ml for ethanolic extract (Table1 and 2).

Discussion

Periodontal disease is a chronic inflammatory condition initiated by microbial infections that lead to a host response resulting in an inflammatory breakdown of tooth-supporting soft and hard tissues [6]. The emerging use of antimicrobials as well as host-modulating agents, as adjuncts in the management of the periodontal diseases, is a common practice. Inspite the availability of wide variety of allopathic medicines herbal products have been chosen to overcome adverse effects caused by them. The use of natural plant extracts with medicinal properties emerged as a new horizon in the treatment of different diseases associated with microbial etiology. Various natural products were under practice as antimicrobial agents, despite the absence of scientific basis [7]. Natural herbs used either exclusively or in combination is proven to be safe and effective in the management of many oral health problems such as halitosis, bleeding gums, mouth ulcers and dental caries etc [5].

Costus pictus is one such medicinally important herb, which is commonly called as 'Insulin plant', as its leaves are proved to produce antidiabetic effects. Whereas certain phytochemical constituents such as tannins, flavonoids, alkaloids, saponins and several other aromatic compounds are secondary metabolites that serve as a defence mechanism against many microorganisms [8]. A part from antibacterial and antiviral functions, tannins are also found to have antiulcer property [9].

There is limited literature supporting the antimicrobial property of Costus pictus. A study was done by Reddy, *et al.,* on *Costus pictus* showed its significant activity against both gram-positive and gram-negative microorganisms [4]. Similar results were obtained from another study done by Majumdar, *et al.,* who compared different parts of the *Costus pictus* (leaf, flower, stem and root) against gram-positive as well as gram-negative microorganisms. It was found that the results were quite comparable with the standard antibiotics screened under similar conditions [5].

After extensive literature search and to the best of our knowledge, this is the first study that determines the MIC and MBC of pure *Costus pictus* extract against four gram-negative periodontal pathogenic bacteriae, such as *P. gingivalis, P. intermedia, F. nucleatum and A.actinomycetemcomitans.* The MIC and MBC are measures to identify the potency of an antimicrobial drug and are considered as the important entity in diagnostic laboratories to confirm the resistance of antimicrobial agent. Sensitive strains will have relatively low values, whereas resistant strains have relatively high values [10]. As a general rule of thumb, the concentration of antimicrobial drug in the blood should exceed the MIC by a factor of 2-8 times to offset the tissue barriers that restrict the access to the infected site [11].

The microorganisms were tested for MIC and MBC values for both aqueous and ethanolic extracts at the concentration ranging from 100 µg/ml to 3.12 µg/ml. The results of MIC were expressed whether the tested microorganisms were sensitive or resistant whereas MBC values were expressed in terms of the number of colony forming units. Microorganisms showed colony forming units at all the tested concentrations. The ethanolic extract of *Costus pictus* is more effective for *Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans* than aqueous extract for the same, but for *Porphyromonas gingivalis and Prevotella intermedia*, the aqueous, as well as the ethanolic extract, is least effective. Also in MBC, the aqueous extract is showing more colony count than ethanolic extract.

Hence it can be inferred that *Costus pictus* extract exhibited both bacteriostatic and bactericidal activity. Probably higher concentration of the drug is required to exhibit a better bactericidal action. Thereby this study helps us to focus on an intervention approach to design and conduct a clinical trial to detect the beneficial effect of pure *Costus pictus* extract on patients at the risk of periodontitis. But *in vitro* values of MIC & MBC may not hold good for in vivo studies due to their inherent limitations. The growth of micro-organisms *in vitro* is exponential whereas the growth *in vivo* can be very slow to none.

Conclusion

From the present study, it can be concluded that the lowest concentration of ethanolic extract of pure *Costus pictus* extract inhibited

bacterial growth than aqueous extract. However, since periodontitis is a polymicrobial disease, the susceptibility of various other periodontal pathogens to this extract must be evaluated. Further studies are recommended to assess the *in vivo* and *in vitro* determination of their concentration in GCF and serum samples to evaluate and ensure effective therapeutic dosage required for antimicrobial and regenerative activity of pure *Costus pictus* extract to treat periodontitis.

Disclosures

The authors of this manuscript encompass no disagreement of interest to report.

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Conflict of Interest

We authors of this manuscript have no conflict of interest to report.

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