



TO STUDY ANTIMICROBIAL ACTIVITY OF SYZYGIUM AROMATICUM EXTRACT AGAINST DENTAL PATHOGENS

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ABSTRACT

The present study was conducted to assess the antimicrobial potential of *Syzygium aromaticum* against bacterial strains which causes the dental caries by disc diffusion technique. The organisms reported to cause dental caries such as *Streptococcus mutans*, *Streptococcus salivarius* and *Fusobacterium nucleatum* were used to evaluate the antimicrobial activity of *Syzygium aromaticum* extract, which were isolated from dental caries. Ethanolic extract of *Syzygium aromaticum* at various (100 – 500 µg) concentrations were used and the antimicrobial activity was evaluated by disc diffusion method. Doxycycline (100mg) was used as positive control for comparison. Ethanolic extract of *Syzygium aromaticum* exhibited dose dependent inhibition against *Streptococcus mutans* and *Streptococcus salivarius*. All the concentrations of ethanolic extract of *Syzygium aromaticum* showed strong antibacterial activity against *Fusobacterium nucleatum*. From the result it was concluded that, ethanolic extract of *Syzygium aromaticum* exhibited antimicrobial activity against the organisms which causes dental caries.

Keywords :- *Syzygium aromaticum*, Dental caries, Antimicrobial activity, *Streptococcus mutans*, *Streptococcus salivarius*.

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INTRODUCTION

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years recommends a combination of lifestyle management, and treatment with specific herbs to cure various diseases. There are approximately 1,250 medicinal plants being used in formulating beneficial measures [1]. Herbal medicines have two special characteristics that distinguish them from chemical drugs, use of crude herbs and prolonged usage. Experience has shown that there are real benefits in the long-term use of whole medicinal plants and their extracts, since the constituents in them work in conjunction with each other. Several popular conventional drugs on the market are from various herbs.

Herbal medicines have fewer side effects and are safer to use than conventional medications. It is well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacteria's responsible for dental caries. Phytochemicals for the prevention, treatment and maintenance of periodontal diseases are identified. They may be tannins, terpenoids, flavonoids, alkaloids, etc. Antimicrobial activities of these have been found to be particularly useful for periodontal diseases [2]. Cloves (*Syzygium aromaticum*) are dried aromatic unopened floral buds of an evergreen tree 10-20 m in height, belonging to the family Myrtaceae, indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon [3].

They are esteemed as a flavouring agent and also used as a spice for scenting, chewing tobacco and an ingredient of betel chew. Cloves have many therapeutic uses: they control nausea and vomiting, cough, diarrhoea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves [4]. In addition, the cloves are highly antiseptic, antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic, antifungal, antiviral and antiparasitic [4].

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. The demand of plant based therapeutics is increasing in both developed and developing countries due to the growing recognition that they are natural products, being non narcotic, having no side effects, easily available at affordable prices and sometimes the only source of healthcare available to the poor. In dental complications, Cloves was found to be used in the sites of periodontal surgery, toothpick injuries, gum abscesses, dry socket, and gingival problems associated with AIDS, leukemia, migratory glossitis, geographic tongue and burning mouth syndrome, desquamative [13]. The major challenge and problem is the lack of scientific evidence on clove for its effect on dental caries, so effort was taken to study the antimicrobial activity of ethanolic extract of clove against the bacteria causing dental caries.

MATERIAL AND METHODS

Collection of Clinical Sample, Isolation and Identification of Dental Pathogen

Dental plaque samples were collected from the adult patients in Sri Lakshmi Narayana Institute of Medical Sciences and Pondicherry Institute of Medical Sciences, Pondicherry. The dental plaque sample was inoculated on blood agar plates and incubated for 18-24 hours at 37°C streak plate technique and the pathogens were isolated and identified by Bergey's manual [14].

Cloves Collection & Authentication

Syzygium aromaticum was collected from the medicinal garden of Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry and the plant was authenticated Plant Anatomy Research Centre, Chennai. The voucher specimen was deposited in the herbarium for future reference.

Extraction

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30°C) for two days and pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. Ethanol was used as solvent for extraction. 10 grams of this powder was soaked in 100ml ethanol as solvent, for 24 h. The contents were then filtered through Whatman filter paper no. 1 and the filtrate was evaporated to dryness. The filtered extract was concentrated under vacuum below 40°C using Heidolph. The dried extract thus obtained was exposed to UV rays for 24h and checked for sterility on nutrient agar plates and stored in labeled sterile bottles in a freezer at 4°C until further use. This dried extract was further powdered and stored in refrigerator.

Preparation of Disc

The discs were prepared by sterile filter paper dried in an oven to remove moisture. The extracts were applied on the dried filter paper disc by micropipette to obtain disc containing 100µg, 200µg, 300µg, 400µg and 500µg of extract concentration in each disc.

Antibacterial Assays

Antibacterial activities of ethanolic extracts of *Syzygium aromaticum* were evaluated by disc diffusion method. A 100 µL of diluted bacterial suspension (5×10^6 cfu mL⁻¹) of test bacterial strains was spread on the surface of Muller Hinton agar. Then sterile disc containing 100 µg, 200 µg, 300 µg, 400 µg and 500 µg of *Aloe vera* gel extracts was placed onto the surface of agar plate. For negative control, discs were impregnated with solvent. Plates were incubated at 37°C for 24 h and diameters of inhibition zones (mm) were determined.

RESULTS AND DISCUSSION

Antimicrobial activity of ethanolic leaf extract of *Syzygium aromaticum* (100 µg – 500 µg) was studied against *Streptococcus mutans*, *Streptococcus salivarius* and *Fusobacterium nucleatum* by disc diffusion method and the results were given in table 1. Doxycycline was used as reference control. *Syzygium aromaticum* extract [14] exhibited dose dependent antimicrobial activity against *Streptococcus mutans* and *Streptococcus salivarius*.

The zone of inhibition of *Syzygium aromaticum* extract at 100 µg against *Streptococcus mutans* and *Streptococcus salivarius* was 9.35 ± 0.78 and 5.77 ± 0.29 respectively. The zone of inhibition of *Syzygium aromaticum* extract at 500 µg against *Streptococcus mutans* and *Streptococcus salivarius* was increased to 17.42 ± 0.99 and 16.78 ± 0.84 respectively. There was dose dependent increase in antimicrobial activity of *Syzygium aromaticum* extract

against *Streptococcus mutans* and *Streptococcus salivarius*. The zone of inhibition [13] of *Syzygium aromaticum* extract at 100 µg and 500 µg against *Fusobacterium nucleatum* was 15.37±0.74 and

16.81±0.77 respectively. The antimicrobial activity of *Aloe vera* gel extract was comparable to the effect of Doxycycline.

Table 1: Antimicrobial activities of ethanolic leaf extract of *Syzygium aromaticum* against *Streptococcus mutans*, *Streptococcus salivarius* and *Fusobacterium nucleatum* by disc diffusion method

Extract	Concentrations	Zone of Inhibition (mm)		
		<i>Streptococcus mutans</i>	<i>Streptococcus salivarius</i>	<i>Fusobacterium nucleatum</i>
Doxycycline	100mg	18.35±0.95	17.66±0.42	16.88±0.74
Ethanolic Leaf Extract of <i>Syzygium aromaticum</i>	100 µg	9.35±0.78	5.77±0.29	15.37±0.74
	200 µg	11.40±0.99	9.84±0.47	15.72±0.34
	300 µg	12.82±0.89	12.24±0.89	16.23±0.97
	400 µg	16.31±0.71	14.48±0.57	16.41±0.89
	500 µg	17.42±0.99	16.78±0.84	16.81±0.77

CONCLUSION

The antimicrobial activity of ethanolic leaf extract of *Syzygium aromaticum* extract was studied on dental caries causing dental pathogens. From the result it was concluded that, ethanolic extract of *Syzygium aromaticum* exhibited dose dependent inhibition

against *Streptococcus mutans* and *Streptococcus salivarius*. All the concentrations of ethanolic extract of *Syzygium aromaticum* showed strong antibacterial activity against *Fusobacterium nucleatum*. From the study it was found that, natural products may be the easy way in controlling the various dental disorders with a least side effect.

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