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*INVITRO*ACTIVITIES OF *MYRISTICA FRAGRANS LEAVES* OIL AND ITS INHIBITS CARIOGENIC BIOFILM COLONIZER BACTERIA-NATURE'S GIFT IN ORAL HEALTHCARE

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ABSTRACT

Dental caries and periodontal disease were associated with oral pathogens. Several plant derivatives have been evaluated with concerning their antiplaque effect against such pathogenic microorganisms. In this study, the pharmacological activity of *myristica fragrans* leaf oil and its effects on *invitro* dental Plaque biofilm studies. The volatile oil was isolated from the *myristica fragrans* leaf by using the steam distillation method (Clevenger apparatus). Methods used for evaluation of plaque were the Artificial dental biofilm model, Saliva conditioned flow cell model, Quartz crystal micro balance, and Zurich biofilm model have been reviewed. The VOMFL was evaluated for the inhibitory activity of the major cariogenic bacteria Streptococcus mutans, which has been generally regarded as the primary etiology agent of dental caries in humans, using an artificial dental biofilm model. The inhibitory actions were performed at a concentration of 1µl/ml and exposure of time to the test, standard, and were evaluated. The essential oil of leaves of *myristicafragrans* could be utilized for Pharmaceutical applications.

KEYWORDS: Volatile Oil, Myristica fragrans leaf, Anti-Plaque, Biofilm, Nichrome wire.

INTRODUCTION

Herbal medicines are older than any other type of health care. Every culture has taken advantage of herbs and their benefits. Man's knowledge of herbs and their medicinal uses advanced over time. Arecent review discussed possible etiological associations between periodontitis (the progressive destruction of the supporting structures of the teeth which is triggered by bacterial plaque) and cardiovascular disease. Ancient peoples all had acquired some knowledge of medicinal plants. *Myristica Fragrans* belonging to the family Myristicaceae really do not have any match as a cheap natural and easily available plant. Its leaves traditionally known to be useful for the treatment of wide panel of diseases like epileptic seizure, antihypertensive, antioxidant, anti-platelet aggregation, cardiovascular, antimicrobial, sedative, antidepressant digestive, hemostatics, diuretics, analgesic anti-inflammatory and prostate disorder, wound infection, cough, and sore throat, fever, wounds, tooth decay, gastric ulceration, and reproductive problems and arterial hypertension, recuperation and rejuvenation during pregnancy, lactation and convalescence. *Myristica Fragrans* leaves contain rich source of essential vital minerals, calcium(2.57%), magnesium(0.09%), chlorine(0.60%), silica(4.18%), & amp; potassium(1.79%), vitamins B6,B12, & amp; C, protein 11.38. The several activities reported were anti-epileptic, prostate disorder, larvicidal, antioxidant, antimicrobial, analgesic anti- inflammatory, hepatoprotective, anti-ulcer, and anti-fungal.

MATERIALS AND METHODS

Plant Collection and Authentification

Leaves of the plant *Myristica Fragrans* selected for our study was collected from heliburya estate, idukki District, kerala, India during the month of may 2023 and was authenticated by Dr.Stephen, Department of Botany, American college, Madurai.

MACROSCOPIC ANALYSIS

Macroscopic analysis of the plant was done. The shape, size, surface characters, texture, colour, odour and taste etc were noted.

ISOLATION OF VOLATILE OIL FROM THE LEAVES

The leaves were dried at room temperature under shade, powdered, sieved (60mesh) and stored in a well closed container. From the dried plant VO is isolated (VOMFL) by Clevenger apparatus with steam distillation.

Fig. 1: Myrstica fragransleaf.

Fig. 2: VOMFL.

Caries and periodontal diseases are two of the most common chronic diseases affecting mankind. These are associated with accumulation of bacterial plaque formed as a biofilm on tooth surfaces. Dental plaque is a complex multi-species biofilm community and is a direct precursor of caries and periodontal diseases. Dental caries can lead to tooth dysfunction and loss. Live and dead stains have suggested that bacterial vitality may vary throughout the biofilm, with the most viable bacteria present in the central part of the plaque, and lining the voids and channels. Dental plaque accumulates preferentially at stagnant sites that afford protection from the vigorous removal forces that apply in the mouth. Methods for evaluation of plaque using artificial dental biofilm model, saliva conditioned flow cell model, evaluation using quartz crystal microbalance, Zurich biofilm model has been reviewed. But these methods are expensive cum complicated, so we designed a method for screening plant material for this activity which is adaptable to natural and synthetic materials, simple, rapid, and reliable technique.

ANTIPLAQUE EFFICIENCY OF THE EXTRACT AND QUANTIFICATION

The wire adherent plaques were removed and dipped in the test and standard solutions for 5 and 10 minutes. Then the wire adherent plaques were then rinsed twice in 15 ml of distilled water. The plaque was removed from the wires, suspended in 5ml of sterile 0.85% NaCl and subjected to five 15- second bursts of ultrasonic oscillation. Aliquots of the dispersed plaque suspension were diluted in sterile 0.85% NaCl and spread over petri dishes containing brain heart infusion agar. The plates were incubated aerobically at 370c for 24hrs.colonies were counted and compared with that of standard. The results were tabulated and photographs were taken. Deposition of plaque formed by the S.mutans over nichrome wires in fluid

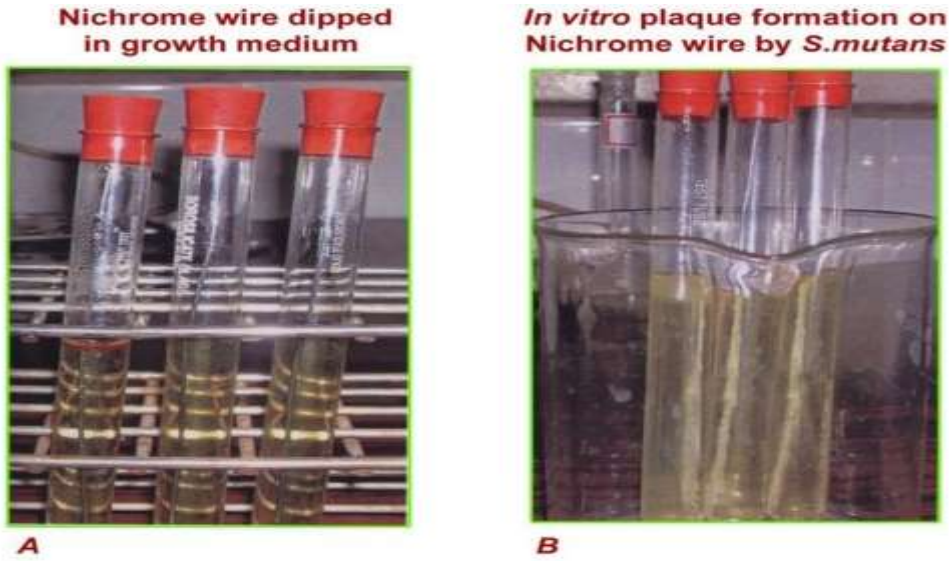
thioglycolate medium supplemented with sucrose and sodium carbonate, was uniform and without any contaminants.

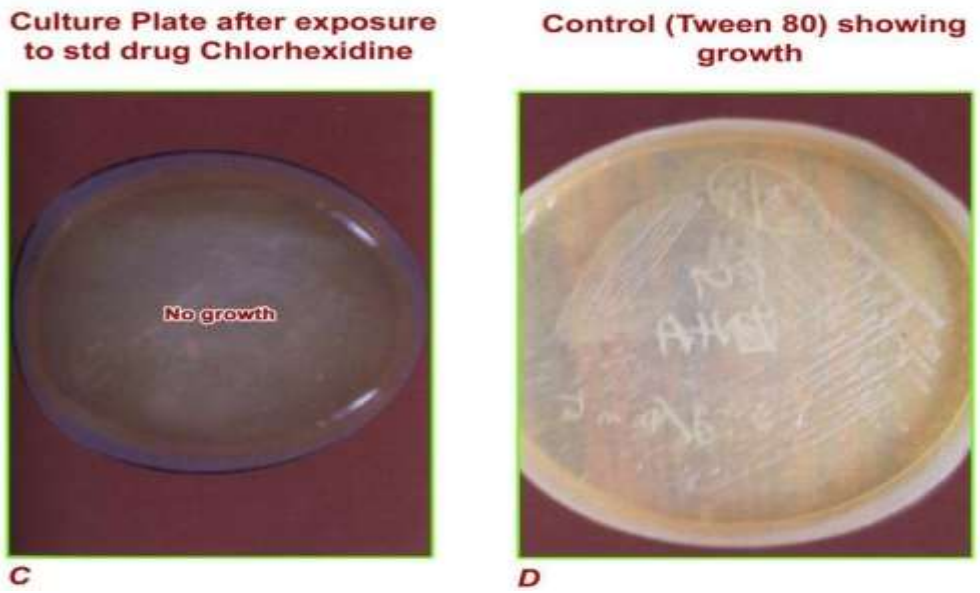
The number of colonies formed after 5, 10 mts exposure to the standard, vehicle control and testVOMFL oil were decreased dose dependently on BHI agar medium petridishes. Exposure to 1µl/ml/ml of to VOMFL 5, 10 mts showed complete inhibition of growth.

RESULTS WITH DISCUSSION

Volatile oils (V.O) are valuable natural products find applications in much area, including pharmaceuticals, cosmetics, perfumes, aromatherapy, phytotheraphy, spices etc. many scientists wasattracted towards the screening of plants to study the biological activities oftheir oils from phytochemical and pharmacological to therapeutic aspects. This may hopefully lead to new directions on plant applications and new perspectives on the potential therapeutic use of these naturalproducts. V.O' are complex mixtures comprising many single compounds. The knowledge of its composition permits for a better and specially directed application. Essentials oils consist of monoterpenes and sesquiterpenes which are the lipophilic secondary metabolites of plants. The VOMFL were evaluated for the inhibitory activity of the major cariogenic bacteria *Streptococcus mutans*, which have been generally regarded as the primary etiologic agents of dental caries in humans, using artificial dental biofilm model. Inhibition of the growth of *S.mutans*was dose dependent. The standard drug chlorhexidine showed no growth**.** Control showed growth. The VOMFL showed significant inhibition at 1µl/ml concentration.

IN VITRO DENTAL PLAQUE BIOFILM INHIBITORY ACTIVITY OF VOMFL OIL





CONCLUSION

Although the antibacterial activities of the crude extracts of each part of *Myristica fragrans* were considerably low, the significant effect of antibacterial activities of each part of *Myristica fragran*s indicated its medicinal potential that could be used against oral pathogens.possible etiological associations between periodontitis and various diseases suggested a potentially valuable role of *myrstica fragrans* phytotherapy in assisting with the management of dental plaque. We studied the effect of *myrstica fragrans*, since it contains tannins and polyphenols which were shown to possess anticariogenic potential and it reduces the caries formation as well as inhibit the glycosyl transferase activity and adsorption of primary etiologic agent *Streptococcus mutans* on the enamel surface. Ethnomedical claim of its use as antiseptic, provide us a clue to study its effect on oral biofilm. The volatile oil showed significant inhibition and it stands as a optimistic choice for the development of safe antiplaque chemotherapeutic agents of great therapeutic potential to control plaque associated infections in human. Therefore, research on development of products from the leaves of *myrstica fragrans*. and its essential oil is required to be inititated urgently for exploring the unique therapeuticpotential of this crop which would also minimize the menacing wastage of this leaves. Moreover, this clearly indicates foreign exchange earning as well as employment generation potentiality of thiscrop, which is required to be strengthened in the interest of our nation. Therefore a well coordinated effort is required to boost up the national economy as well as the employment generation through the proper exploitation of this KING OF SPICES.

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