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IN VITRO ANTIPYRIAL ACTIVITY OF *PSIDIUM GUAJAVA* LEAF EXTRACT

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ABSTRACT

Chemical substances used for prevention of dental caries are known to have many side effects. Thus natural products should be explored for their anti caries action. Dental plaque when allowed to accumulate may lead to caries formation and discomfort due to inflammation of the gingival area. Natural compounds may offer significant advantages over the chemical ones, and formulation of such compounds if easy to use and safe by people may lead to improvement in the general dental health of the population. *Streptococcus mutans* is normally found in oral cavity, which is responsible to cause dental caries and bad breath odor. Hence this study is Performed to Evaluate the effect of *Psidium guajava* leaf extract against *Streptococcus mutans.*

KEY WORDS: *Psidium guajava* Leaf, Antipyrial Activity, *Streptococcus mutans*.

INTRODUCTION

Periodontal disease is characterized by inflammation and destruction of supporting tissue of the teeth. The most effective method of prevention and maintenance of periodontal disease is mechanical as well as chemical plaque control. Several chemical plaque control agents have been evaluated for their effectiveness on supra gingival plaque including, essential oils, enzymes, and even herbal extract. Some of these substances have been associated with various side effects incapacitating their long term use, so new formulation of equal efficacy and fewer side effects are required to be evaluated.

Dental infections such as dental caries are infectious diseases in which the oral bacterium *Streptococcus mutans* has been implicated as a principal etiological agent, although other oral bacterial species probably contribute to disease. These bacteria are known to cause bad breath odor. Among the various oral micro-organisms, *Streptococcus mutans* has been identified as a plaque-forming bacterium capable of producing dental caries.

Streptococcus mutants is the most important oral bacteria which plays a major role in dental caries, bacteremia and consequently bacterial endocarditic. Application of antibiotics for prevention of dental caries is not recommended, since there is risk of development of MDR (multiple drug resistance) strains. The use of plants and their extracts in the treatment of diseases dates back to 460-370 BC when Hippocrates practiced the art of healing by use of plant based drugs. Different plants and their parts have been used for thousands of years. S. mutants were found to form biofilm on the surface of screw cap tube in the presence of 1% dextrose. In this study plant extratcs of *Psidium guvjava* has significant antibacterial activity and thus can be employed as an effective anti plaque agent and can be used in the prevention of dental caries. The plant extract was evaluated for antistreptococcal and antibiofilm activity against *Streptococcus mutans*. *Psidium guajava* was found to be active against *Streptococcus mutans* also has potential anti biofilm activity. Currently, there is an increasing interest to investigate the effect of natural compounds, especially plants extracts, on the residence of the oral cavity. The present study has shown that the extract of *Psidium guajava* exhibited anti-gingivitis activities.

Streptococcus mutans is a gram positive bacteria which is the primary causative agent in the formation of dental cavities in humans. *Streptococcus* is a genus of spherical gram positive bacteria belonging to the phylum *firmicutes* and the lactic acid bacteria group. *Streptococcus mutans*, a member of the human oral flora, is widely recognized as the main etiological agent of dental cavities.

Dental caries is a dental biofilm related oral diseases associated with increased consumption of dietary sugar and fermentable carbohydrates. When dental biofilms remain on tooth surfaces, along with frequent exposure to sugars, acidogenic bacteria will metabolize the sugars to organic acids, susceptibility to diseases varies between

individuals and immunological mechanism have been proposed to confer protection or susceptibility to the disease.

The important constituents of guava are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids. Leaves of *Psidium guvjava* contain phenolic compounds, isoflavonoids, Gallic acid, catechin, epicatechin, rutin, naringenin, kaempferol having hepatoprotective, antioxidant, anti-inflammatory, and antispasmodic, anticancer, antimicrobial, anti-hyperglycemic, analgesic actions. The leaf contain two important flavonoids quercetin known for its spasmolytic, antioxidant, antimicrobial, anti-inflammatory actions and guaijaverin known for its antibacterial action. Pulp contains ascorbic acid, carotenoids (lycopenes, β-carotene) possessing antioxidant, anti-hyperglycemic, antineoplastic. The seed contains glycosides, carotenoids, phenolic compounds having antimicrobial actions.

Guava is proven for its antidiarrheal, antimicrobial[2,5,7,12,13], antiparasitic[18], antitussive[9, 11], hepatoprotective, and antioxidant[8], and antigenotoxic, antimutagenic, antiallergic[10,17], Anthelmintic[19], anticancer, Periodontitis[1,4,6,7], Gingivitis[3] and anti-hyperglycemic effects. Acclaimed as the "poor man's apple of the tropic"; guava has been used for various purposes in different regions of the world. It has been used in the treatment of diarrhea, dysentery, menstrual disorders, vertigo, anorexia, digestive problems, gastric insufficiency, inflamed mucous membrane, laryngitis, skin problems, ulcers, vaginal discharge, cold, cough, cerebral ailments, nephritis, jaundice, diabetes, malaria and rheumatism to mention a few. Leaves are chewed to relieve toothache and to cure bleeding gums and bad breath. Guava leaf decoction is gargled to relieve mouth sores, coughs, throat and chest ailments and inflamed and bleeding gums. The paste of tender leaves of guava is used as toothpaste.

Dental plaque[1] is the principal etiologic factor in periodontal disease. Plaque if allowed to accumulate, with no intervention or oral hygiene methods, leads to gingivitis which further progresses to periodontitis. Effective plaque control strategies to prevent or limit bacterial adhesion and further growth on the tooth surface are essential to prevent and control periodontal disease. The paste of tender leaves of guava has been traditionally used to maintain oral hygiene. Guava has shown antibacterial activity against both Gram-positive and Gram-negative bacteria. The antimicrobial activity of guava is mainly attributed to flavonoids, guaijaverin and quercetin. The bark has exhibited antibacterial properties due to the presence of tannins.

Psidium guajava contains greater percentage of vitamin C than oranges, about four times than oranges. Having a higher concentration of vitamin C, it is useful in the treatment of bleeding and swollen gums. The astringents contents of guava leave juice are a useful remedy for toothache pain, swollen gums and ulcers. The gargling of guava leaves is an important toothache remedy. The properly masticated guava stopped the blood leakage from gums. Guava juice is one of the important home remedies to treat gums problems. Guava is rich in Flavonoids that prevent bad breath which is responsible for gum disease gingivitis.

Guava as an anti-inflammatory agent: Guava has been known for its anti-inflammatory action. The anti-inflammatory action of guava is in its ability to inhibit prostaglandin, kinin and histamine. It has been used in the treatment of diarrhea, dysentery, menstrual disorders, vertigo, anorexia, digestive problems, cold, cough, inflamed mucous membrane.

MATERIALS AND METHODS

Equipment and Materials: The tools used in this study include analytical scales, blenders, sieves, desiccator, petridish, stir bar, beakers, pipettes, Erlenmeyer flask, evaporator, filter paper. Materials used in this study are the guava leaves, distilled water, alcohol.

Sample extraction

The leaf sample- washed, dried, grounded into powder-alcohol (solvent)used for extraction procedure and to make concentration-covered with lid to avoid evaporation-exposure to light at room temperature-after 24hrs of soaking-transferred into tubes for-centrifuged-supernatant was collected-stored until use for extraction.

Plant extract was poured in Petriplates to detect the presence of anti pyrial activity. Prior to streaking the plates with bacteria, all the plates were inoculated with the test bacterium (S*treptococcus mutans*) sterile cotton swab was dipped in the suspension and spread firmly around the plate. The plates are allowed 3 to 5 minutes to dry the excess moisture. Each extract were dispensed into each plate with bacteria and incubated. The diameter of inhibition zone for each concentration was measured and compared with the standard antibiotic (ofloxacin).

Antipyrial activity

Antipyrial susceptibility testing was done using the well diffusion method according to the standard of the national committee for clinical laboratory standards. The plant extract were tested on plates to detect the presences of antipyrial activity. Prior to streaking the plates with bacteria, 5mm diameter wells were punched into the medium using a sterile borer. All plates were inoculated with the test bacteria which has been previously adjusted to the 0.5 standard solution, a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculums. The surface of the agar was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculums with a final swab around the rim. The plates are allowed 3-5mins to dry the excess moisture. Each test

extract was dispensed into each well after the inoculation of the plates with bacteria. The wells were also arranged in the formation 2inches apart. The same extract was used on each plate. The plates were sealed, labeled and placed in an incubator set to 37^{O}C. After 24 hours of incubation, each plate was examined for zone of inhibitions. A ruler was used to measure the inhibition zones in millimeter.

RESULTS AND DISCUSSION

This study was designed to evaluate the antipyrial activity of Alcoholic extract from leaves of *Psidium guajava* and result shows inhibitory activity of the alcohol extract against bacteria, with zone of inhibition 16-26mm.

Table No.1: Antibacterial and Antipyrial activity of *Psidium guajava* leaf extract Zone of inhibition (Diameter in mm)

Micro-organisms	100mg	200mg	300mg	400mg
Streptococcus mutans	19	20	24	26
E Coli	18	19	21	22
Pseudomonas	16	18	19	20
Staphylococcus aureus	16	17	19	20
Ofloxacin (S)	22	24	25	28

Table No.2: Phytochemical Tests For *Psidium Guajava* Leaf Extract

S. No	Test Performed to detect	Observation	Inference
1.	Flavonoids	Orange pink colour	Present
2.	Volatile oil	Colorless solution	Present
3.	Glycosides	Brown ring	Present
4.	Alkaloids	Pale yellow colour	Present
5.	Phenols, Tannins	Blue green or black colour	Present
6.	Terpenoids	Reddish brown interphase	Present

CONCLUSION

From the observations it was concluded that the leaf extract of *Psidium guajava* has inhibitory efficacy against *Streptococcus mutans.* This makes it suitable to apply for the treatment of dental caries and can be used as oral cavity consumer herbal product as it as less side effects. The observed zone of inhibition of bacteria *Streptococcus mutans* suggests that *Psidium guajava* leaf extract possess compounds containing anti-bacterial properties that can effectively suppress the growth when extracted using alcohol as solvent.

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