ORIGINAL RESEARCH

Comparative Evaluation of the Phytochemical Analysis and Efficacy of Four Plant-derived Extracts against *Streptococcus mutans*: An *In Vitro* Study

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Abstract

Introduction: Oral health is a loyal part of general health. As per the World Health Organization (WHO), dental caries is the most widespread noncommunicable disease and is a significant universal public health concern. The main causative organism associated with this disease is *Streptococcus mutans (S. mutans)*. Various synthetic agents like chlorhexidine and fluorides are commercially available to prevent dental caries. However, these have side effects. Presently, research is engaged in the use of plant extracts to develop an effective and biocompatible material that may be used safely in the oral cavity.

Methodology: Four plants were collected—*Emblica officinalis*, *Vitis vinifera* seeds, *Psidium guajava* (*P. guajava)* Linn leaves, and *Acacia nilotica (A. nilotica*), and extracts were prepared individually. These extracts were subjected to phytochemical analysis, and bacterial growth and fermentation were assessed.

Result: The level of significance was set at $p < 0.05$. All the plant extracts inhibited *S. mutans* growth at all serial dilutions except *P. guajava* Linn and *A. nilotica* showing 55 CFU/mL and 5 CFU/mL, respectively in 1/1000 dilution only. The phytochemical analysis confirmed that all four plant extracts had alkaloids, carbohydrates, tannins, and flavonoids. Steroids and proteins are present in *P. guajava* Linn. Proanthocyanidins were present in *Vitis vinifera*. Saponins and Anthraquinones were present in *A. nilotica* exclusively.

Conclusion: All the extracts were effective against *S. mutans*. These could be tried as herbal alternatives to conventional adjuncts. However, these results must be additionally evaluated for toxicity in animal models, and effectiveness must be assessed using *in vivo* studies on human subjects.

Keywords: *Acacia nilotica*, Dental caries, Dental plaque, *Emblica officinalis*, Phytomedicine, *Psidium guajava* Linn, *Streptococcus mutans*, *Vitis vinifera*.

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Introduction

Oral dental health is an integral segment of overall health, sharing common causal pathways and impacting each other in a symbiotic manner.[1] Oral diseases are one of the most exorbitant conditions to manage in certain nations.[2,3] Research has proven the deadly association between heightened proportions of *Streptococcus mutans* (*S. mutans*) and caries.[4] The appropriate of fluorides, antimicrobials, and chlorhexidine in different forms is recognized in oral hygiene maintenance and prevention of dental caries.[3] Nevertheless, several limitations and problems need to be addressed which include fluoride toxicity, staining of teeth, and taste alteration besides the occurrence of resistant microbes which restrict its long-term application. Plants are revolutionizing a renaissance worldwide with phytomedicines symbolizing safety and feasibility.[5] Hence the hunt for alternative products persists, and long-established traditional phytomedicine is considered a feasible substitute for mainstream medicine. Ayurveda, a holistic practice of medicine that developed in the Indian subcontinent 3,000–5,000 years ago, is now practiced worldwide as complementary alternative medicine.[6,7] Fortifying research on the pharmacological activities of plant-based medicines is salient to validate their dental applications.[8] The first step toward this goal is the *in vitro* evaluation of the phytochemical properties and antibacterial assay.[9] Therefore, this study aimed to compare and evaluate four different natural plant-derived extracts.

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Among the therapeutic plants established hitherto, *Acacia nilotica (A. nilotica*) possesses incredible potential against multiple ailments. *A. nilotica* (Babul), is used as an oral hygiene adjuvant for generations.[10] *A. nilotica* is a rich source of polyphenols, mainly phlobatannin, tannin, gallic acid, ellagic acid, and catechins. It has anti-inflammatory, antimicrobial and antioxidant properties.[11]

Amla (*Emblica officinalis*) possesses antioxidant, antimicrobial, and anti-inflammatory properties due to the existence of polyphenols and tannins. In dentistry, it is used as an intracanal medicament, root canal irrigant, gutta-percha disinfectant smear layer removal, etc.[12] Amla supports the growth of connective tissue when taken systemically benefitting the gums.[6,13] For the enduring advantage of the dental structures, 1–2 gm/day can be taken orally.[14]

Another phytotherapeutic plant that is an abundant source of vitamins, tannins, carotenoids, phenols, saponins, triterpenes, lectins, flavonoids, essential oils and fatty acids is *P. guajava*.[15–17] The guava leaves contain active components like guaijaveric acid, guaiadial, guavanoic acid, and guaijaverin that have remarkable antibacterial, antiplaque, antioxidant, and anti-inflammatory characteristics. Guaijaverin acquired from guava leaves has potential antibacterial activity against *S. mutans*.[18]

Grape seeds are a noteworthy source of polyphenol compounds including catechin, epicatechin, procyanidins, and gallic acid.[19] Grape (*Vitis vinifera*) and its seed extracts (GSE) have anti-cariogenic potential due to the presence of proanthocyanidins which acts by inhibiting glucosyltransferase activity and F-ATPase. Various studies have proved the potential of GSE for remineralization by promoting the deposition of mineral content on the superficial layer of the lesion.[13,20]

In this context, the ongoing experimental research was executed to evaluate the antimicrobial potency of *A. nilotica*, *Emblica officinalis*, *Vitis vinifera*, and *Psidium guajava* (*P. guajava*) extracts on *S. mutans* and to assess their individual phytochemical composition.

Methodology

This *in vitro* study was conducted over a 3-month duration from July to December 2016 at AJ Institute of Dental Sciences, Mangaluru, Karnataka, India.

Sample Collection and Preparation

Emblica officinalis and *P. guajava* Linn were procured from Kannur, Kerala. *A. nilotica* and *Vitis vinifera* were collected from Mumbai, Maharashtra. All solvents/chemicals used were obtained from E-Merck, Mumbai, India. The collected materials were thoroughly double-washed under running water and sterilized after which they were shade dried for 5 days. The dried leaves were then finely powdered individually.

Extract Preparation

Each powdered plant substance was separately extracted in a Soxhlet extractor using petroleum ether (60–80°C for 6 hours). 100 gm of the powder was loaded into the Soxhlet main jar. The solvent was poured into the bottom flask and the extract was condensed under preset temperature (60–80°C) reduced pressure and boiled. The vapor of the solvent was condensed which drew back on the loaded plant material in the main jar prior to collection in the jar. The extraction and collection of material took place at the same time in the main jar. Thus, the extract of the plant material was obtained.

Test for assessing bacterial fermentation and growth—*S. mutans* strain MTCC 497 purchased from "Institute of Microbial Technology," Chandigarh, India was dispersed in Todd Hewitt Broth to assess optimal growth. After which, this was cultured onto mitis salivarius bacitracin agar (MSBA). Petite blackish colonies of frosted glass appearance were detected indicating the presence of *S. mutans*. MSBA was prepared and autoclaved. To this, potassium tellurite and bacitracin were further added to obtain the absolute concentrations of 0.1 units/mL and 0.02 mg/mL, respectively. Biochemical and morphological assessments were performed to estimate the viability of the *S. mutans*. The morphological investigation comprised smear preparation, gram staining, and observation under the light microscope under oil immersion (100 × magnification). Finally, the extract was subjected to serial dilutions of 1/10, 1/100 and 1/1000. 0.01 mL of the aforementioned was streaked on the MSB agar, using a measured loop, and incubated at 37°C for 2 days in plastic bags aerated with exhaled air to enhance CO_2. Following incubation, the number of colonies raised was summed up by employing a Quebec colony counter.

Table 1 shows the list of tests done to analyze the phytochemical constituents of the extracts prepared.

Results

Data entry, database management, and analysis were done using Statistical Package for the Social Sciences version 20.0. Bacterial fermentation and growth were analyzed by a conventional method.

Table 2 shows the growth of *S. mutans* in the presence of the extract at different serial dilutions. *Emblica officinalis* and *Vitis vinifera* showed no growth in any serial dilution. *P. guajava* Linn and *A. nilotica* show 55 CFU/mL and 5 CFU/mL, respectively in 1/1000 dilution only. The percentage of optimal growth was 0.183% for *P. guajava* Linn and 0.1% for *A. nilotica*.

The outcome of the phytochemical assay of the four plant extracts derived is summarized in (Table 3). It displayed that alkaloids, carbohydrates, tannins, and flavonoids are present in all the extracts. Steroids and proteins are present in *P. guajava* Linn. Proanthocyanidins were present in *Vitis vinifera*. Saponins and anthraquinones were present in *A. nilotica* exclusively.

Discussion

One of the most serious plights in public health is an oral illness, which is on the surge in emerging nations.[14] Caries can appropriately be entitled as a misfortune of present-time society and no faction has skipped the repercussions of this ailment.[22] As early as 1924, the contributory relationship between *S. mutans* with plaque and dental caries has been discovered.[23] The World Health Organization (WHO) prophesies that by 2050, plant-derived drug merchandise will be worth US$ 5 trillion signifying an upcoming huge demand for medicinal plants.[15] When a plant is 'medicinal', it is presumed as a drug or therapeutic agent. There are certain noteworthy bioactive compounds present in plants like phenolic compounds, flavonoids, and alkaloids. Roughly 120 distinct chemical substances are derived from diverse plants with medicinal properties and are consequently used as drugs. Apart from conventional treatment protocols, phytomedicine has gained access to oral hygiene aids in the past.[24]

Vitis vinifera seeds are a treasury of polyphenols. GSE constitutes an abundance of proanthocyanidins aiding in remineralization of artificial root caries in vitro, conveying its potential for non-invasive root caries management.[22] Hassan et al. stated that the acetone extract of grape seed possesses exceptional antioxidant

Table 1: Tests used to assess phytochemicals in the four extracts

Phytoconstituent	*Test*	*Observation*	*Inference*
Alkaloids	Mayer's test—extract + 4–5 mL of dilute hydrochloric acid (HCl) shake well + Mayer's reagent[20,21]	White or creamy precipitate	Alkaloids present
	Dragendorff's test—extract + 4–5 mL of dilute HCl + Dragendorff's reagent[21]	Orange-red precipitate	
	Wagner's test—extract + 4–5 mL of dilute HCl and shake well + a few drops of I_2 in KI[21]	Brown precipitate	
Carbohydrates	Molish's test—extract + Molisch's reagent and shake well. Add 2 mL of concentrated H_2SO_4 along the walls of the test tube. Let it stand for 2 minutes	The reddish–violet color at the junction of two solutions	Carbohydrates present
	Anthrone test—extract + 1 mL of water, shake, and warm contents gently	Green or blue color	
Steroids	Salkowski's test—extract (dissolve in chloroform) + concentrated H_2SO_4[21]	Red color	Steroids present
	Liebermann–Burchard's test—extract (dissolved in chloroform) + concentrated H_2SO_4 + acetic acid[21]	Green color	
Glycosides	H_2SO_4 test—extract + concentrated H_2SO_4 shake and allow the content to stand for a few minutes	Reddish color	Glycosides present
	Keller Kiliani test—extract + glacial acetic acid, cool + two drops of $FeCl_3$ transfer the contents to a test tube having 2 mL of concentrated H_2SO_4[20]	Reddish color was observed in junction liquids	
Amino acids and proteins	Ninhydrin test—extract + ninhydrin reagent[20]	Blue/purple color	Amino acids and proteins present
	Millon's test—extract + Millon's reagent	White precipitate which turns to red on heating	
Saponins	Foam test—extract + water, shake well[20]	Formation of foam	Saponins present
Flavones	Aqueous test—extract + concentrated H_2SO_4	Yellow color	Flavones present
	H_2SO_4 test—extract + concentrated H_2SO_4 shake well and allow the contents to stand.	Yellow color	
Phenols	Ferric chloride test—extract + 1 mL 5% $FeCl_3$ solution[21]	Intense color	Phenols present
	Ellagic acid test—extract + three drops of 5% $NaNH_2$ solution	Muddy/Niger brown precipitate	
Tannins	Ferric chloride test—extract + 1% Ferric solution[20]	Blue-green or brownish-green color	Tannins present
	Gelatin test—extract + three drops of 1% solution containing 10% NaCl[21]	White precipitate	
Oils and fats	Spot test—a small quantity of extract is pressed with filter paper[20]	Oil stains on the paper	Oils and fats present
	Saponification test—extract + three drops 0. 1 N KOH + one drop phenolphthalein and heat on a water bath for 30 minutes	Formation of soaps	

characteristics, phenolic compounds, and flavonoid content which is in agreement with our study.[25]

The antimicrobial potency of *A. nilotica* is assumed due to be presence of tannins, flavonoids, and phenolic compounds.[26,27] In a study by Chandra Shekar et al., *A. nilotica* demonstrated the presence of glycosides, flavonoids, anthraquinones, and tannins, and *P. guajava* displayed the presence of glycosides, tannins, anthraquinones, and flavonoids; both of which are in accordance with our study.[3] The antibacterial effect of *A. nilotica* is chiefly contributed by these constituents. Deshpande et al. reported the antimicrobial efficacy of *A. nilotica* on *S. mutans*.[20]

Streptococcus mutans (*S. mutans)* is the most prevalent cariogenic bacteria linked with dental caries.[3] This bacterium can metabolize sucrose and synthesize glucan by cell surface and extracellular glucosyltransferase.[27] In this study, there is no bacterial fermentation in *Emblica officinalis*, *Vitis vinifera*, and minimum amount of growth in *P. guajava* Linn, *A. nilotica*. It can be interpreted that these extracts can be incorporated into oral care products. All the individual plant extracts have been proven to be effective against *S. mutans*. The results of the current study demonstrate that any of these plant extracts could be tried as viable substitutes for chlorhexidine. In our study, all extracts have the presence of phytochemicals like alkaloids, carbohydrates,

Table 2: Growth of *S. mutans* in the presence of the extracts

	S. mutans growth (in CFU/mL)		
	Serial dilutions		
Test samples	*1/1000*	*1/100*	*1/10*
Emblica officinalis (amla)	Nil	Nil	Nil
P. guajava Linn (guava leaves)	55	Nil	Nil
A. nilotica (babul)	5	Nil	Nil
Vitis vinifera (grape seeds)	Nil	Nil	Nil

Table 3: Phytochemical analysis

Component	*Emblica officinalis (amla)*	*P. guajava Linn (guava leaves)*	*Vitis vinifera (grape seeds)*	*A. nilotica (babul)*
Alkaloids	+++	+++	++	++
Carbohydrates	+	++	++	++
Tannins	++	+++	++	++
Flavonoids	+++	+++	++	++
Steroids	–	++	–	–
Proteins	–	++	–	–
Anthraquinones	–	–	–	+
Proanthocyanidins	–	–	++	–
Saponins	–	–	–	+

+++, extensively present; ++, moderately present; +, present; –, absent

tannins, and flavonoids, along with Steroids, proteins present in *P. guajava* Linn, proanthocyanidins present in *Vitis vinifera*, saponins, and anthraquinones present in *A. nilotica*. Thus, confirming that all the extracts are rich in phytochemicals. A marked antibacterial activity can be expected from these extracts of the phytochemicals present in them.

The outcome of this research is only preliminary and further studies evaluating the efficacy of these extracts on plaque colonizers would warrant us to unfold a contemporary approach that can synchronously inhibit plaque microbes and dental caries. Another fact to be kept in mind is that the phytochemical constituents in the extracts vary with seasons.[3] Standardization of these products remains a challenge. Well-structured and clinical trials carried out across various centers are mandatory for precise comparison is the need of the hour.[28] These outcomes must be further estimated for any toxicity in animal models before their effectiveness is assessed on humans. Finally, the efficacy of these extracts as mouthwash/ chewable tablets under in vivo settings is obligatory to substantiate the results of this research.

Conclusion

Even before the epoch of contemporary oral hygiene adjuncts, herbal assets were used by our forefathers for safeguarding oral health. The usage of natural substances is a safe and economically viable remedy to overcome the drawbacks of the currently available synthetic medicaments. Recent research appraising the effectiveness of these products has portrayed revolutionary results. This study opens new avenues for plant extracts to be developed into oral care products that could be used daily without any side effects. The future horizon of our study is to extend its relevance as a sustainable natural and nontoxic substitute. This involves performing many more in vitro and in vivo studies to assess the efficacy of these extracts. The ultimate decision must be made only after long-term randomized clinical trials. Researchers may possibly be guided to extract the vital components from these plant extracts and blend them into oral care products. In this context, the perfectly elucidated statement by Hippocrates stating "nature itself is the best physician" is rationalized to the significance of the plant dynasty against oral dental infections.

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