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Synergistic Activity of Moringa Oleifera and Thyme Vulgaris Essential Oil Against Streptococcus Mutans: An Observational Study

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ABSTRACT

Background: Dental caries still remain a highly prevalent disease among all age groups especially children. It is caused due to repetitive and/or prolonged demineralization of tooth enamel caused by exposure to low pH from organic acids produced by acidogenic oral bacteria during fermentation of dietary carbohydrates. Streptococcus mutans is a facultative anaerobic , gram positive cocci commonly found in the human oral cavity which plays an important role in causing dental caries. Moringa oleifera Lam. (drumstick tree) is a Moringaceae family member which possesses a variety of advantageous biological properties, such as immunomodulatory, anticancer, antiulcer and antimicrobial properties. Thymus vulgaris is a pleasant smelling perennial shrub which has a direct influence on many bacteria species. The use of combinations of these two EOs and their components are thus a new approach to increase the antimicrobial efficacy of EOs , taking advantage of their synergistic and additive effects.

Materials and methods: The broth microdilution method was used to determine the minimum inhibitory concentration(MIC) of the essential oils. The MIC of Moringa, Thyme and combination of the both essential oils against S.mutans which were used in the assay were assessed at a different concentration level.

Results: The antimicrobial activity of M.oleifera, T.vulgaris and combination of both the essential oils was proven with the MIC value of 2.5%v/v, 0.03906% and 0.03906% respectively.

Conclusion: The combination of Moringa oleifera and Thymus vulgaris essential oil can be used as a good antimicrobial agent to treat various Streptococci borne oral infections. Further incorporation of this oil into the drug formulations is also recommended to treat S.mutans borne oral as well as systemic infections.

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INTRODUCTION

Dental caries still remain a highly prevalent disease among all age groups especially children. It is caused due to repetitive and/or prolonged demineralization of tooth enamel caused by exposure to low pH from organic acids produced by acidogenic oral bacteria during fermentation of dietary carbohydrates(1). Dental caries is a biofilm-induced disease which is highly influenced by the patient's dietary habits and frequent sweet food intake. All these factors, together with time, promote the microbial residence in the accumulated dental plaque which eventually leads to dental caries infection (2).

KEYWORDS:
Streptococcus mutans,
Moringa oleifera,
Thymus vulgaris,
essential oils,
antimicrobial activity,
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Streptococcus mutans is a facultative anaerobic , gram positive cocci commonly found in the human oral cavity which plays an important role in forming multidimensional and complex structures (extracellular polysaccharide from sucrose) on tooth enamel and oral mucosa(3). It has the potential to colonise the enamel surface, break down sugar for energy, decrease the pH, make the surrounding acidic and this causes demineralization of tooth enamel and dentine. It is highly aciduric compared to other Streptococci species. Streptococcus mutans are also known to cause bacteremia and infective endocarditis(4).

Moringa oleifera Lam. (drumstick tree) is a Moringaceae family member found from India to Africa, as well as many other tropical and arid countries, and is widely used as food and medicine (5). It has a variety of properties, including nutritional value, amino acid content, and flavonol content, making it an excellent food supplement and cosmetic ingredient (6). In addition to being a significant nutritional component, M. oleifera also possesses a variety of advantageous biological properties, such as immunomodulatory, radioprotective, antiulcer, and tissue protective (liver, kidneys, heart, and lungs) qualities (7).

Thymus vulgaris is a pleasant smelling perennial shrub which is most widespread in Italy. Its chemical composition such as terpenes and aliphatic hydrocarbons (alcohols,aldehydes and ketones) has a direct influence on many bacteria species(8). It is also proven to be antioxidant due to the presence of oxygenated monoterpenes(9).

Because of the high cost of synthetic medicine and the emergence of antimicrobial resistance, the majority of the world's population has turned to traditional medicines for their primary health care needs. Plants' medicinal value is found in chemical substances that have a specific physiologic action on the human body. Alkaloids, flavonoids, tannins, and phenolic compounds are the most important bioactive compounds found in plants. Different activities against microbes have been reported for these compounds.Our team has extensive knowledge and research experience that has translate into high quality publications. (10–19)

Phytochemical research based on ethnopharmacological data is widely regarded as an effective method for discovering new antimicrobial agents from higher plants. Despite this array of uses to which parts of Moringa and thymus plants are put to, scanty literature is available on the uses of Moringa oleifera and Thymus vulgaris plants as sanitizers or preservatives in foods. However, a very important step in the screening of a plant material for sanitising / preservative activity was to evaluate its phytochemical and antibacterial activity. The aim of the present study was to evaluate the synergistic antibacterial activity of Moringa oleifera and Thymus vulgaris essential oil on Streptococcus mutans.

MATERIALS AND METHODS

Strain and Culture Conditions

Streptococcus mutans isolates were routinely grown in sterile brain heart infusion broth (HiMedia, India) and the tube was incubated at 37°C with a shaker incubator (120 rpm) for 24 h.

Evaluation of the Minimum Inhibitory Concentration of the Essential oils

Minimum inhibitory concentration assay was described by Ganesh and Rai, 2015 Briefly , serial two fold dilutions (0.03906-20%v/v) of the test oils were prepared. A sterile Eppendorf tube was set up with S.mutans strain.The test bacteria were as follows: Eppendorf tubes 1-10 contain the serial two fold dilution of the oils (M.oleifera , T.vulgaris and combination of both the oils) mixed with BHI broth and the test organism. Eppendorf tube 11 contained BHI broth (Sterility control), Eppendorf tube 12 contained control strain. All the tubes were vortexed and the tubes were incubated at 37°C for 24h. After 24 h of incubation, the growth of the bacteria was visualized by the addition of 10µl of TTC (1 mg/ml) solution.The lowest concentration with no visible growth was recorded as MIC.

RESULTS

The MIC of the selected essential oils were found for the strain S.mutans using microdilution method at the concentrations ranging from 0.0390-2.5%v/v. M.oleifera essential oil exhibited antimicrobial activity against S.mutans at the concentration level of 2.5% v/v (Fig.1). Whereas, a potent antimicrobial activity was exhibited by T.vulgaris essential oil with the MIC level of 0.0306%v/v(Fig.2). The synergistic activity of both oils was proved against the test organism with the MIC of 0.03906%v/v(Fig. 3).

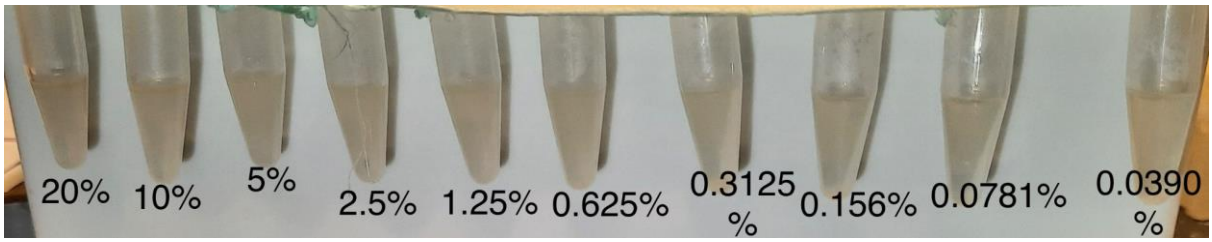

Fig 1: MIC of M.oleifera essential oil for inhibition of S.mutans growth.The MIC value is 2.5% v/v.

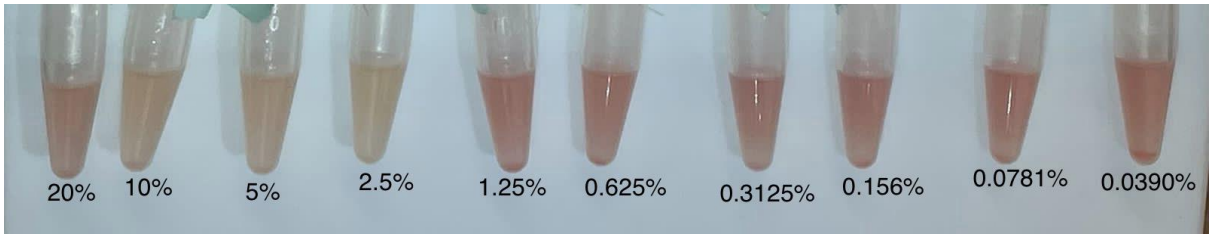

Fig2: MIC of T.vulgaris oil for inhibition of S.mutans. The MIC value is 0.03906%v/v.

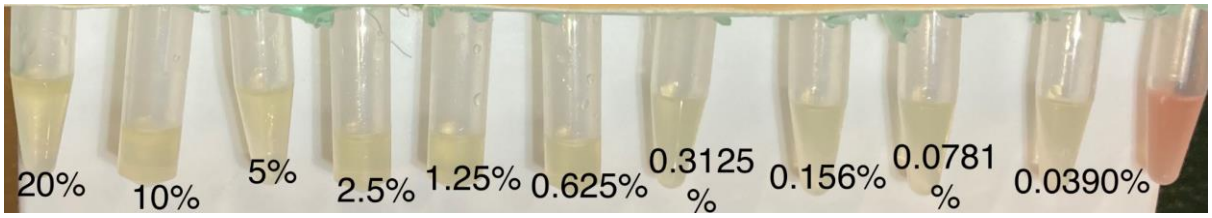

Fig 3: MIC of combination of M.oleifera and T.vulgaris oil(in equal proportion) for inhibition of S.mutans. The MIC value is 0.03906% v/v.

DISCUSSION

Essential oils(EOs) are among the natural plant products that deserve special consideration due to their widespread use in traditional healing practises around the globe. Furthermore, distillation of essential oils from plant organs is now a highly reliable and cost-effective process. EOs have been tested for antimicrobial activity in vivo and in vitro, and some have shown potential antimicrobial activity. Their mechanism of action appears to be primarily on the cell membrane by disrupting its structure, resulting in cell leakage and cell death, with secondary actions including membrane synthesis inhibition and inhibition of cellular respiration. Because of the essential oils' high volatility and lipophilicity, they easily penetrate the cell membrane and exert their biological effect(20). In the present study, the antimicrobial activity of M.oleifera essential oil against S.mutans was illustrated with the MIC value of 2.5%v/v. M.oleifera is high in phytochemicals such as alkaloids, flavonoids, glycosides, tannins, and saponins (21). The antibacterial activity of Moringa oleifera against various test organisms such as Escherichia coli, Staphylococcus aureus,Staphylococcus epidermidis ,Pseudomonas aeruginosa,Proteus vulgaris, Streptococcus mutans and Bacillus subtilis was clearly demonstrated in the study conducted by G.Amabye et,al (22).In the study conducted by,Ehab Ali Fouad et al,M. oleifera is regarded as one of the new infection-fighting methods for preventing camel abscesses caused by pyogenic bacteria, such as C. pseudotuberculosis, S. aureus, and E. coli (23). Antifungal activity against Mucor sp. was also demonstrated by the aqueous Moringa seed extract in the study done by O Donli et al(23) . Moreover, anticancer, anti inflammatory , antispasmodic and diuretic activity was illustrated in many studies (24,25).

The antimicrobial activity of T.vulgaris essential oil is determined by its chemical constituents. The presence of phenolic compounds (thymol) and terpene hydrocarbons (-terpinene) appears to be related to its antimicrobial activity (26). In the present study, the antimicrobial activity of T.vulgaris essential oil against S.mutans was determined with the MIC value of 0.03906%v/v. In the study conducted by J.Antih et al, antibacterial activity of T.vulgaris essential oil was evaluated against respiratory pathogens, namely, H. influenzae,S. pyogenes and S. aureus (27). Strong antimicrobial and antifungal potential was exhibited by T.vulgaris essential oil against food borne pathogens and fungi which included Staphylococcus aureus , Pseudomonas aeruginosa , Salmonella typhimurium , Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis and Candida albicans in the study conducted by O.Boruga et, al (28). T.vulgaris essential oil has exhibited a broad spectrum antifungal activity (Aspergillus,Penicillium,Ulocladium,Mucor, Trichoderma and Rhizopus) (29).T. vulgaris L. demonstrated

significant anticancer potential against experimental mammary carcinoma (30).

Essential oils are natural plant products containing a complex mixture of components and thus having multiple antimicrobial properties. Most of the antimicrobial activity in EOs appears to derive from oxygenated terpenoids, particularly phenolic terpenes, phenylpropanoids and alcohols. Other constituents (e.g., hydrocarbons) can be used in combinations to increase their bioactivities. Interactions between these components may lead to antagonistic, additive or synergistic effects.The use of combinations of EOs and their isolated components are thus new approaches to increase the antimicrobial efficacy of EOs , taking advantage of their synergistic and additive effects (31,32). In the present study ,the combination of M.oleifera and T.vulgaris EOs has exhibited excellent antimicrobial activity against S.mutans with a MIC value of 0.03906%v/v. In the study conducted by F.Hossain et al, a synergistic effect was produced when oregano and thyme were combined, increasing their effectiveness against A. flavus, A. parasiticus, and P. chrysogenum (33).N.Gavaric et al discovered that both combinations (thyme/oregano essential oils and thymol/carvacrol) had an additive effect on gram-positive (S.aureus and B.cereus) and gram-negative bacteria (S.Infantis, E.coli)(34).

CONCLUSION

The combination of Moringa oleifera and Thymus vulgaris essential oil can be used as a good antimicrobial agent to treat various Streptococci borne oral infections. Because they are efficient, selective, biodegradable, and less toxic to the environment, these essential oils have the potential to be further researched as antimicrobial agents to control and treat pathogenic infection in order to lessen the negative effects of synthetic agents.

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