Preparation of dual nutmeg and tulsi gel along with the evaluation of antimicrobial property and cytotoxic potential.

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ABSTRACT:

AIM/ OBJECTIVE: The objective of the study is to prepare the dual gel using nutmeg and tulsi and then the evaluation of antimicrobial property and the cytotoxic potential.

MATERIALS AND METHODS : The gel preparation has been done by mixing the powder of nutmeg and tulsi.Then to this mixture 5ml of the concentrate is added and mixed thoroughly until the proper gel formation is done. The antimicrobial property is checked in the Porphyromonas gingivalis organism. The cytotoxic potential is checked in the Braine variety of the shrimp.

Results : the results stated that the nutmeg and tulsi gel is having high antimicrobial potential in case of Porphyromonas gingivalis.

CONCLUSION : The antimicrobial property of Nutmeg tulsi gel has been demonstrated to be effective against P.gingivalis. This suggests that it could be used as an affordable and effective "adjunct" alongside standard care for managing periodontal conditions.

INTRODUCTION:

Throughout the course of their lives, all living organisms - including human beings are consistently subjected to potential harmful pathogens. This persistent exposure encompasses a vast array of diseases that profoundly impact human health. Despite possessing evolved defense mechanisms to combat these challenges the initial line of defense often proves inadequate. Consequently relying on chemotherapeutic agents becomes necessary in tackling pathogen induced infections(1). Historical precedent demonstrates the discovery or synthesis of numerous chemical agents designed for treating and curing such infections. Unfortunately the widespread and indiscriminate application of these medications has engendered the development of drug resistant strains which is a significant quandary with global ramifications as existing drugs lose efficacy against them even now. It is imperative that we urgently explore alternative avenues in lieu of synthetic antibiotics and adjunctive drugs(2).

Spices and aromatic herbs offer a promising solution to the aforementioned problems due to their recognized antioxidant and antimicrobial qualities. Furthermore these natural remedies have long played an essential role in traditional medicine across various cultures. An example of such herbal

resources is nutmeg – obtained by drying the kernel of an ovoid seed called Myristica fragrans (Family: Myristicaceae). Nutmeg holds multiple uses; it serves as a popular spice while also being employed in alternative medicine for its reputed aphrodisiac effects [31]. Memory enhancing properties, ability to alleviate diarrhea, anti inflammatory attributes, and even potential in fighting cancer [23].

Ocimum tenuiflorum, commonly known as Ocimum sanctum, Tulsi, or Holy Basil, is indeed a highly revered plant in traditional medicine, particularly in India. It belongs to the family Lamiaceae, which includes many aromatic herbs. Tulsi has been referred to as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities[26].

Tulsi has a long history of use in traditional medicine, and almost every part of the plant has been found to possess therapeutic properties. The leaves, in particular, are commonly used in different forms. Aqueous extracts can be obtained from fresh or dried leaves, which are then used in herbal teas. The leaves can also be ground into a powder, which is used either alone or mixed with other herbs or honey to enhance its medicinal value.The medicinal properties attributed to Tulsi are diverse. It is known for its antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects. Tulsi is considered beneficial for various ailments, including respiratory disorders, digestive issues, skin conditions, and cardiovascular problems. It is also used for its potential anti-cancer properties and for promoting longevity[32].

Tulsi (Ocimum tenuiflorum) has been traditionally used to treat a wide range of ailments, as you have mentioned. The extracts of Tulsi have been used in the treatment of various conditions, including Poisoning, Stomach Ache,Common colds as it is often used to alleviate symptoms of the common cold, such as cough, congestion, and sore throat. Its antimicrobial and expectorant properties may help in providing relief, Headaches, Malaria, Inflammation,Heart disease- Its hypotensive, hypolipidemic, and antioxidant properties may contribute to its potential cardiovascular benefits.

In addition to the aqueous extracts, oils derived from the leaves and inflorescence of Tulsi are also believed to possess various useful properties, including Expectorant, Analgesic, Antiemetic, Antipyretic,Anti-asthmatic, Hypoglycemic, Hepatoprotective, Immune modulators[36].

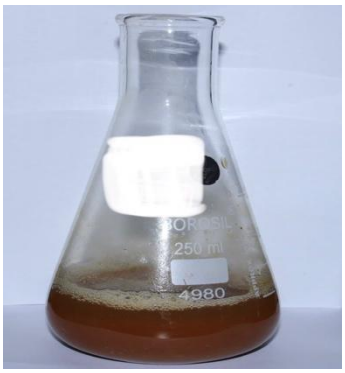
The combination of nutmeg and Tulsi in traditional medicine is additive, as both herbs have been recognized for their potential health benefits. Nutmeg (Myristica fragrans) is a spice commonly used in culinary applications, and Tulsi

(Ocimum tenuiflorum) has a long history of traditional medicinal use, as we discussed earlier.

However the additive potential of both nutmeg and Tulsi in terms of their antioxidant and antimicrobial properties has not been specifically assessed. But, both herbs are known to contain a variety of active phytochemicals, including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, and phenolics.These phytochemicals are known for their diverse biological activities and may contribute to the observed health benefits of nutmeg and Tulsi. Specifically, the compounds found in these herbs can act as antioxidants through mechanisms such as radical scavenging, metal chelation, inhibition of lipid peroxidation, and quenching of singlet oxygen. Antioxidants help protect the body against oxidative stress, which is associated with various chronic diseases[36].Furthermore, both nutmeg and Tulsi have antimicrobial properties, which may be attributed to their bioactive compounds. Antimicrobial activity can help inhibit the growth of microorganisms, including bacteria, fungi, and viruses[38].

Materials and methods:

Preparation of the gel: The nutmeg and tulsi gel has been prepared by taking equal quantity of commercially available products of nutmeg and tulsi. 5 grams of nutmeg powder is mixed with 5 grams of tulsi powder and 100 ml of distilled water is added and the solution has been heated for 65 degrees centigrade for 15 minutes for obtaining a thick extract form.



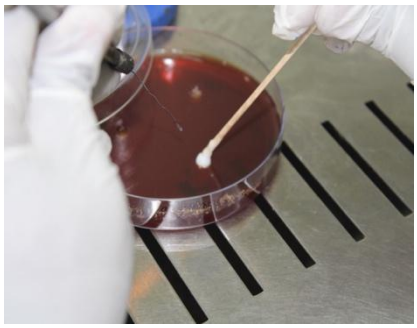
Then 1.5 grams of carboxymethyl cellulose and 1.5 grams of carbopol is prepared into a thick mixture using 20 ml of distilled water. 5ml of the concentrated extract of nutmeg and tulsi has been added to the above-mentioned mixture to obtain a gel form of the nutmeg and tulsi.

Cytotoxic potential: The cytotoxic potential of the nutmeg and tulsi gel has been checked in the BRINE variety of the shrimp.The BRINE shrimp were seeded on a 6-well plate with 10 shrimp per well in a 100 microlit of the growth medium and incubated for 24 hr at 37 degree centigrade. The gel is added in different concentrations such as 5 microliter, 10 microliter, 20 microliter, 40 microliter and 80 microliter by diluting gel with distilled water along with the control group and shrimp were incubated for 12 hrs at 37

degree.

Antimicrobial activity:

A gram of this gel was dissolved in 10 ml of dimethyl formamide to obtain a 10% concentration of nutmeg and tulsi gel.Similarly, concentrations of 0.5%, , 1%,5%, and 10% of nutmeg tulsi gel extracts were obtained. These gel extracts were collected in sterile containers, transported for microbiological assays.In this study, nutmeg-tulsi gel has been used as an experiment and dimethyl



formamide is used as a control. The culture of the plates has been done in the anaerobic jar to check its antimicrobial property against gram- negative bacteria.

Results:

Cytotoxic potential: The effect of the nutmeg and tulsi gel on the brine shrimp was determined by using presto- blue test. The shrimp were treated with different concentrations of the gel ranging from 5 microlitre to 80 microlitre, and the results demonstrate that the cytotoxicity of the gel decreased in relation to the concentration. The shrimp viability is decreased by less than 50% when the concentration is 80 microlitre. Both the nutmeg and tulsi concentrates in the gel of 80mg/ml were within the range as these concentrations led to decrease in the shrimp viability by less than 50% and therefore are not considered as cytotoxic.

Antimicrobial activity: The microbiological investigation focuses on evaluating the antimicrobial activity of Tulsi extract against two specific bacteria: Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. The agar well diffusion method was used to determine the concentration of Tulsi gel extract that produced an inhibition zone similar to that of doxycycline, which is a commonly used antibiotic.The data obtained from the study were analyzed using one-way analysis of variance (ANOVA), which is a statistical test used to compare the means of multiple groups. In this case, it was likely used to compare the antimicrobial activity of different concentrations of Tulsi extract and the control (doxycycline). The Tukey post-hoc test, which is a pairwise comparison test, was then used to assess the differences between the groups.

The results of the study indicated that at concentrations of 5% and 10%, Tulsi extract demonstrated antimicrobial activity against Aggregatibacter actinomycetemcomitans, similar to the activity observed with doxycycline. This means that the inhibition zones produced by Tulsi extract at these concentrations were comparable to those produced by doxycycline, and the difference between them was not statistically significant ($P > 0.05$).However, when it came to Porphyromonas gingivalis,these bacteria showed resistance to Tulsi extract. The inhibition zones produced by Tulsi extract against these bacteria were significantly smaller compared to those observed with doxycycline ($P < 0.05$). This suggests that Tulsi extract was less effective in inhibiting the growth of Porphyromonas gingivalis when compared to doxycycline.

DISCUSSION:

The effect of nutmeg and Tulsi extract on cell viability was assessed using the Presto-Blue test, a commonly used method to evaluate cell metabolic activity and viability. The cells were exposed to different concentrations of the extract, ranging from 2.5 to 20 mg/ml.The results of the study indicated that the cytotoxicity of the extracts decreased with decreasing concentration. In other words, as the concentration of the extract decreased, the negative impact on cell viability also decreased. This suggests that the extracts of nutmeg and tulsi were less harmful to the cells at lower concentrations.Specifically, the study found that even at the highest tested concentration of 20 mg/ml, the cell viability decreased by less than 20%. This means that the cells retain at least 80% of their metabolic activity and viability when treated with this concentration of the extract. As a result, the concentration of 20 mg/ml was considered non-cytotoxic in this context.

Both the essential oil and concentrated leaf extract, when used at a concentration of 20 mg/ml, were found to be within the expected range of cytotoxicity. This suggests that these concentrations did not significantly decrease cell viability, as the decrease observed was less than 20%.It is important to note that the specific variety of shrimp and experimental conditions used in the study may influence the cytotoxic effects of nutmeg and Tulsi extract.

The present study it has been observed that there is a significant antimicrobial activity of nutmeg extracts against the tested microorganisms. Specifically, the acetone extract of nutmeg exhibited the strongest antimicrobial activity compared to other extracts used in the study.This finding states that the nutmeg seeds possess potent antimicrobial properties against important pathogenic bacteria and fungi. The antimicrobial activity of the different nutmeg extracts can be attributed to the presence and concentration of various chemical substances present in each extract.

Nutmeg seeds contain a variety of bioactive compounds that have been isolated and found to have antimicrobial importance. These compounds likely contribute to the observed antimicrobial activity.Further research is needed to identify and characterize the specific antimicrobial compounds present in nutmeg seeds. Understanding the chemical composition and mechanisms of action of these compounds can provide valuable insights into their potential applications in antimicrobial therapies.

The additional information regarding the antimicrobial compounds found in nutmeg include the key findings mentioned Trimyristin and myristic acid: Narasimhan and Dhake reported the compounds such as Trimyristin and myristic acid as the chief antibacterial principles isolated from nutmeg. These compounds are believed to contribute to the antimicrobial activity of nutmeg against bacteria[20]. Three lignans, namely erythro-austrobailignan-6, meso-dihydroguaiaretic acid, and nectandrin-B, were isolated from the methanolic extract of nutmeg. These lignans were found to possess antifungal activity, indicating their potential role in combating fungal infections.

Takikawa et al. reported the antimicrobial activity of nutmeg against entero-hemorrhagic Escherichia coli O157. They found that E. coli O157 was highly sensitive to b-pinene, a compound present in nutmeg. This suggests that b-pinene contributes to the antimicrobial effects against this specific strain of bacteria[32]. Many plant phenolic compounds have been reported to possess antimicrobial activity. While the specific phenolic compounds present in nutmeg were not mentioned in the provided information, it suggests that these compounds may also contribute to the overall antimicrobial properties of nutmeg.These findings highlight the presence of various bioactive compounds in nutmeg, such as trimyristin, myristic acid, lignans, b-pinene, and plant phenolics, which likely play a role in its antimicrobial activity against bacteria and fungi[24,4].

The a-pinene and b-pinene are pinene-type monoterpene hydrocarbons present in nutmeg. Both compounds have been reported to possess antimicrobial activity. They are believed to contribute to the antimicrobial effects by potentially disrupting cell membranes through their lipophilic properties[11]. The compound named p-cymene is another antimicrobial compound found in nutmeg. It is known for its potential antimicrobial properties, although the specific mechanisms of action were not mentioned.

The b-caryophyllene compound is present in nutmeg and is a sesquiterpene. While its antimicrobial activity was not specified in the provided information, b-caryophyllene is known to have various biological activities, including anti-inflammatory and antioxidant properties. Carvacrol is an important component of nutmeg and has been reported to exhibit antimicrobial activities. It is known to have the ability to cross cell membranes and penetrate inside the cell, where it interacts with intracellular sites critical for its antimicrobial effects[8,33].

These compounds, including a-pinene, b-pinene, p-cymene, b-caryophyllene, and carvacrol, contribute to the antimicrobial properties of nutmeg[12,17]. They are believed to act through various mechanisms, such as membrane disruption and interactions with intracellular targets.

While p-cymene may exhibit weak antibacterial activity on its own, it plays a crucial role as a precursor to carvacrol, another antimicrobial compound found in nutmeg. It has been reported that p-cymene works synergistically with carvacrol. Together, they have the ability to expand the bacterial membrane, leading to membrane destabilization and ultimately causing antimicrobial effects. In addition to its potential antimicrobial activity, b-caryophyllene has been reported to have anti-inflammatory and anti-fungal activities[34]. This compound contributes to the overall beneficial properties of nutmeg, including its potential to combat inflammation and fungal infections.These findings emphasize the importance of considering the synergistic effects and interactions between different compounds present in nutmeg. The combination of p-cymene and carvacrol, as well as the presence of b-caryophyllene, contribute to the overall antimicrobial and health-promoting properties of nutmeg[25].

The agar diffusion method and the dilution method are two commonly used techniques for examining the antimicrobial activity of essential oils.

The agar diffusion method involves creating wells or placing paper disks soaked with the essential oil onto an agar plate inoculated with microorganisms. Antimicrobial activity is determined by measuring the zone of inhibition, which is the area around the well or disk where the growth of microorganisms is inhibited.While the agar diffusion method has been widely used in earlier studies due to its simplicity and ease of implementation, there are certain limitations to its application, particularly when examining essential oils. One of the main concerns is the volatility of the compounds present in essential oils. Essential oils consist of volatile compounds that have a tendency to evaporate from the disks during the incubation period. This evaporation can lead to a decrease in the concentration of essential oil reaching the microorganisms, affecting the accuracy and reliability of the results.

To overcome this issue, the dilution method is often preferred for assessing the antimicrobial activity of essential oils. In the dilution method, the essential

oil is diluted in a liquid broth or incorporated into agar at various concentrations. The microorganisms are then exposed to these dilutions, and the minimum inhibitory concentration (MIC) can be determined, which is the lowest concentration of the essential oil that inhibits the growth of microorganisms.The dilution method provides more precise and reproducible results compared to the agar diffusion method when testing essential oils. It allows for a more accurate determination of the concentration of essential oil required to inhibit the growth of microorganisms.

The low solubility of essential oils in agar can hinder the diffusion of volatile compounds, potentially affecting the accuracy of results when using the agar diffusion method. As a result, the broth dilution method using 96 well flat-bottom microtitre plates has gained popularity as the method of choice for determining the antimicrobial activity of essential oils.In the broth dilution method, the essential oil is diluted in a liquid broth and serial dilutions are prepared in the microtitre plate wells. Each well contains a different concentration of the essential oil. Microorganisms are then inoculated into each well, and the plates are incubated. The antimicrobial activity is assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil.

The MIC is the lowest concentration of the essential oil that inhibits the visible growth of microorganisms, while the MBC is the lowest concentration that results in the complete killing of the microorganisms. These values provide valuable information about the potency of the essential oil against specific microorganisms.To facilitate comparisons between different studies, Kalemba and Kunicka (2003) recommended publishing only the MIC and MBC values. This standardization helps researchers and readers better understand and compare the antimicrobial activity of essential oils across different studies.

The broth dilution method using microtiter plates allows for more accurate determination of MIC and MBC values and has become the preferred method for assessing the antimicrobial activity of essential oils. It provides quantitative data that can be used to evaluate the efficacy of essential oils and facilitates comparisons between different studies, enhancing the overall understanding of their antimicrobial properties.

Based on your results, the nutmeg-Tulsi gel demonstrated bacteriostatic activity at levels of 2.25-2.5 µg/ml against P. gingivalis and Aggregatibacter actinomycetemcomitans. However, it showed less activity against P. intermedia. These findings are consistent with previous studies that utilized

disk diffusion or optical density reduction methods.Mahmood et al. (2008) reported similar results using a disk diffusion assay, where zones of inhibition were observed around both Gram-positive and Gram-negative species. However, the activity was found to be more effective against Staphylococcus aureus (a Gram-positive bacterium) compared to Gram-negative species.

The differences in the activity of nutmeg-Tulsi gel toward Gram-positive and Gram-negative bacteria have been observed in various studies. These differences may be attributed to variations in the bacterial strains used, the concentrations and formulations of the nutmeg-Tulsi gel, as well as the specific antimicrobial mechanisms involved.Further research is necessary to elucidate the underlying mechanisms of action and to evaluate the efficacy of nutmeg-Tulsi gel against a broader range of bacterial strains. Additionally, it is important to consider the potential synergistic effects of nutmeg and Tulsi in the gel formulation, as both herbs have their own antimicrobial properties.The findings suggest that nutmeg-Tulsi gel exhibits bacteriostatic activity against certain periodontal pathogens, such as P. gingivalis and A. actinomycetemcomitans.

The study done by Mishra and Mishra (2011) reported good inhibition of both Gram-positive and Gram-negative species using optical density reduction as the measure of activity. Interestingly, P. intermedia exhibited slightly better activity compared to S. aureus under their test conditions. This indicates that the efficacy of nutmeg-Tulsi gel against specific bacterial strains can vary depending on the experimental conditions and methodologies employed.

Additionally, Helen et al. (2011) found that Tulsi essential oil from one subspecies of O. tenuiflorum was more effective against P. vulgaris, S. aureus, P. aeruginosa, and E. coli compared to extracts from two other subspecies. These differences in the antimicrobial activity of Tulsi oil could be attributed to variations in the composition of volatile compounds present in the oil, which can be influenced by factors such as the geographical source of the plants or specific cultivars used.The discrepancies in reported results regarding the antimicrobial activity of Tulsi oil could also arise from differences in the methodologies used to determine antimicrobial activity. Varied experimental conditions, concentrations, and exposure times can contribute to differing outcomes.

Furthermore, it is important to note that P. aeruginosa is known for its intrinsic and acquired resistance to many classes of antimicrobial agents. This resistance may explain the variability in the effectiveness of Tulsi oil against P.

aeruginosa compared to other bacterial strains[poole 2011].Overall, the observed variations in the antimicrobial activity of Tulsi oil and nutmeg-Tulsi gel is influenced by multiple factors such as the composition of volatile compounds, geographical source, specific cultivars, and the inherent resistance of certain bacterial strains.

Conclusion: The present study has successfully demonstrated the antioxidant and antimicrobial activity of different extracts of nutmeg. Specifically, the acetone extract exhibited the highest antioxidant and antimicrobial properties among the extracts.The observed high antioxidant and antimicrobial activity in nutmeg extracts can be attributed to the presence of various bioactive compounds, including a-pinene, b-pinene, myrcene, 1,8-cineole, carvacrol, terpinen-4-ol, eugenol, and iso eugenol. These compounds are known for their antioxidant and antimicrobial properties, and their presence in nutmeg likely contributes to its beneficial effects.

The findings of this study provide support for the ethno-pharmacological importance of nutmeg. Traditional uses of nutmeg in folk medicine may be justified by its antioxidant and antimicrobial activities. The antioxidant properties of nutmeg can help prevent or slow the progression of oxidative stress-related diseases, which are often associated with the accumulation of reactive oxygen species in the body. The antimicrobial activity of nutmeg can be valuable in preventing or combating infections caused by opportunistic pathogenic microorganisms. The presence of antimicrobial compounds in nutmeg may help inhibit the growth and survival of these microorganisms, potentially offering therapeutic benefits. Thus, the synergistic combination of nutmeg and tulsi gel helps in preventing or treating oxidative stress-related diseases and microbial infections such as periodontitis.

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