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Short Communication

Dentistry Section

Efficacy of Specific Plant Products on Microorganisms Causing Dental Caries

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ABSTRACT

Introduction: Dental caries and periodontal diseases are the most common oral diseases seen globally, both in developed and developing countries. Oral microorganisms that is gram positive and gram negative bacteria are known to be involved in causation of these diseases. Nowadays commercially available dentrifices and mouth rinses are known to contain ingredients that can alter the oral microbial flora and have undesirable side effects such as vomiting, diarrhoea, disarrangement of oral, intestinal flora and tooth staining. Naturally available plant products are known to be less harmful with fewer side effects and also economical for the patient.

Aim: The aim of this study was to determine the antimicrobial properties of 10 naturally available plant products against oral microorganisms causing caries and to check the efficacy of these products in-vitro and to use these in mouth washes and dentrifices.

Materials and Methods: Sample of caries material was scrapped out from the extracted teeth and transferred to liquid broth, streaked over the agar media to allow for the growth of microorganisms. Plant products like clove oil, neem, ginger-garlic paste, tea tree oil, ginger, garlic, cinnamon oil, green tea, eucalyptus oil and turmeric were used. Antimicrobial efficacy of these products, was estimated by measuring zones of inhibition in the nutrient agar media.

Results: Clove oil was the most effective of all products against microorganisms causing caries with zone of inhibition - 30mm followed by ginger-garlic paste - 25mm, Neem - 15mm, tea tree oil - 15mm.

Conclusion: Based on the above results, it can be inferred that these natural products have the maximum efficacy against microorganisms and can be recommended in dentifrices, mouth rinses, topical gels, etc.

Keywords: Antimicrobial activity, Medicinal plants, Phytochemical activity, *Streptococcus mutans*

INTRODUCTION

Medicinal plants have been a great source of novel drug compounds since ages. Plant derived products have made large contributions to the well being of human health. A lot of research has been done for the development of new drugs with novel mechanism of action. Scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very meeker ocean of this tremendous potential drug repertoire has been scientifically screened. In recent years studies have been done to identify the active constituents of medicinal plants [1-3]. Research has also been done to find out the action of various plant extracts on dental caries causing microorganisms [2].

Dental caries is a chemico-microbial process that consists of two stages including decalcification of enamel followed by dentin. The *streptococcus* species are highly cariogenic and are significant to the growth of pathogenic plaque. A vast numbers of *Streptococcus* and *Lactobacilli* play role in dental caries as well as periodontal diseases [3,4].

Several antimicrobial agents such as chlorexidine, amoxicillin and antiseptics have been found to be effective in preventing dental caries; however, these materials have various adverse effects such as tooth staining, disarrangement of oral, intestinal flora and diarrhoea have been associated with use of these drugs [5]. Natural phytochemicals would offer an effective alternative to antibiotics and drugs; hence, represent a promising approach in prevention and therapeutic strategies for prevention of dental caries and other oral infections. The most representative human cariogenic microbes include *Streptococcus mitis, Streptococcus mutans, Staphylococcus aureus, Lactobacillus* species, etc. It has been proven that dental caries is caused by mixture of aerobic and facultative anaerobes. Literature search showed that numerous studies were done to assess antimicrobial activity in an anaerobic culture media [2,3] but few studies were done in aerobic culture media.

In this present study 10 medicinal plant products were selected to assess the antimicrobial activity.

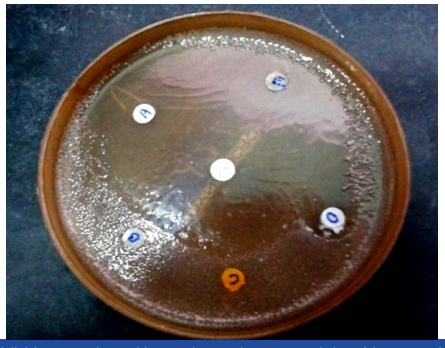
MATERIALS AND METHODS

This in-vitro study was carried out in Oral Pathology and Microbiology laboratory of G. Pulla Reddy Dental College, Kurnool, Andhra Pradesh, India for three months i.e., from January 2016 to March 2016. Ethical clearance was not needed for the study.

Sample Collection: Recently extracted teeth were collected from the Oral Surgery Department [Table/Fig-1], caries material was scrapped with a spoon excavator and transferred to the Hartley's broth (transport media) mixed gently for 1-2 minutes and liquid was streaked over nutrient agar with inoculation loop and incubated for 24 hours at 37°C in the incubator to allow the growth of micro-organisms [Table/Fig-2]. Then, the organisms were identified under microscope using gram stain.

Materials Used: Ten naturally available products and essential oils such as clove oil, turmeric, ginger-garlic paste, neem, tea tree

[Table/Fig-1]: Recently extracted carious teeth. **[Table/Fig-2]:** Carious material scrapped from the extracted tooth.



[Table/Fig-3]: Plant extract material. **[Table/Fig-4]:** Zones of inhibition produced by various plant materials with control disc in center. (a) clove oil; (b) eucalyptus oil; (c) turmeric paste; (d) neem; (e) ginger garlic paste. **[Table/Fig-5]:** Zones of inhibition produced by plant materials; (f) tea tree oil; (g) garlic; (h) ginger; (i) green tea; (J) Cinnamon Oil.

oil, cinnamon oil, green tea, eucalyptus oil, ginger, garlic, were employed in the study.

Preparation of Extracts: Materials were collected from the local market, dried and were ground into fine powder by using mortar and pestle and decoctions were prepared by mixing 10gm with 100ml distilled water and boiled for 15 to 20 minutes, filtered and collected in sterile sample containers and allowed to cool. Essential oils were used directly. All the extracts were stored separately in sterile containers and labeled accordingly [Table/Fig-3].

Preparation of Discs: Circular discs of diameter 6mm were prepared from Whatman filter paper and were placed in the decoctions and essential oils allowing them to be completely infused with the material.

Method: All the 10 materials used were tested by disc method. Aerobic culture method was employed, taking control disc as ciprofloacxin. Swab method was used to transfer the colonies on to the nutrient agar plates. A 500 micro liter of the suspension was spread over the plates containing nutrient agar using a spreader in order to get a uniform microbial growth on test plates. The plates were allowed to dry under aseptic conditions. Turbidity was adjusted visually with the broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. The discs were placed in the culture plate with the control disc in the center with inhibition area 35mm and allowed to be incubated for 24 hours at 37°C in the incubator to check the sensitivity of the materials used.

RESULTS

Antimicrobial efficacy of all the extracts was determined by measuring the zones of inhibition around each disc. Vernier's calliper was used to measure the zone of inhibition. The level of antimicrobial activity was determined by the size of inhibition zone shown by the plant product. A larger inhibition zone usually means that the antimicrobial agent is more potent. In the present study maximum zone of inhibition was seen around clove oil (30mm) followed by ginger-garlic paste (25mm), least zone of inhibition was seen when eucalyptus oil was used among all plant extracts [Table/Fig-4,5].

DISCUSSION

Plant extracts have been used since ancient days as traditional medicines; nowadays these plant extracts have received special attention as they are non-chemical and non-synthetic in nature. These may prove to be better and safer alternatives if they are supported by scientific-based evidence [6]. In the present study maximum antibacterial efficacy was shown by clove oil (30mm), ginger-garlic paste (25mm), neem (15mm), tea tree oil (15mm), minimal inhibition was shown by ginger (5mm), garlic (5mm), green tea (2mm), cinnamon oil (2mm), eucalyptus oil and turmeric showed no zone of inhibition [Table/Fig-6].

Clove oil is one of the essential oil commonly used in seasoning of food. Antimicrobial activity is mainly attributable to eugenol, oleic acid and lipids present in it. Nzeako BC et al., carried out a similar study using oil extracts on dental caries causing microorganisms

CC	BCC subtype
Clove oil	30mm
Ginger and garlic paste	25mm
Tea tree oil	15mm
Neem	15mm
Ginger	5mm
Garlic	5mm
Cinnamon oil	2mm
Green tea	2mm
Turmeric	Nil
Eucalyptus oil	Nil

[Table/Fig-6]: Zones of inhibition when used with different plant materials.

and found the zones of inhibition produced by these oil extracts ranged from of 14.7mm to 33.7mm which were comparable to the present study [7]. Gingerol and Allicin, the chemical compounds present in ginger and garlic respectively are responsible for flavours in them [8]. There are no studies in literature that have used this material against oral microorganisms and dental caries causing microorganisms. Both products when used together surprisingly produced greater antimicrobial action. The primary use of tea tree oil has been historically emphasized as antiseptic and anti-inflammatory agent. Its anti-inflammatory activity can be attributed to its components i.e., hydrocarbon structure and lipophilicity. Similar studies showed use of 2% tea tree oil was effective against, the main causative agents of dental caries i.e., *Streptococcus mutans* and *Lactobacillus*. Mechanisms of antimicrobial and anti-inflammatory action of tea tree oil can be attributed to its components i.e., hydrocarbon structure and lipophilicity [9]. Neem was used in ancient days as a traditional medicine and for cleaning of teeth. According to Chava VR et al., teeth brushing with neem sticks and chewing of leaves helps in reducing the plaque and act as good oral hygiene tool [10]. Green tea has numerous health benefits and it is attributable to the polyphenols present in it like catechins, epigallocatechines and epigallates. Its antibacterial activity is due to the oxidation of polyphenols catalyzed by polyphenol oxidase enzyme [11]. Eucalyptus oil and cinnamon oil both showed stronger inhibitory activity as measured by mean inhibitory concentration determination [12]. Numerous studies on Curcumin showed a good zone of inhibition against *Streptococcus mutans* and *Streptococcus pyogenes* ranging from 9.7mm-10.2mm [13]. Few hurdles must be crossed like testing in large sample and in-vivo testing in oral cavity to adapt this study to new diagnostic procedures.

LIMITATION

There are few limitations in the study such as increasing the sample size and by using other natural available products.

CONCLUSION

Though many studies have been done to evaluate the efficacy of various plant products by anaerobic method on specific

microorganisms, the present study has been done in an aerobic culture method as the initiation of caries is from aerobic environment. According to results of the study it can be concluded that plant extracts and essential oils have the potential to be used in oral dentifrices, mouthwashes, topical gels etc. However, future research efforts are needed for the evaluation of quality and efficacy of the plant products for their regular use in the oral hygiene products. It must be converted to viable form for daily use in tooth pastes and mouth rinses. Further studies on the safety and efficacy of such products must be carried out to establish whether they offer therapeutic benefits, either alone or in combination with conventional therapies that can help to reduce the overall burden of oral diseases worldwide.

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