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COMPARATIVE EVALUATION OF ANTIMICROBIAL PROPERTIES OF AQUEOUS & ALCOHOLIC GLYCARRHIZA GLABRA ROOT EXTRACT AND CHLORHEXIDINE AGAINST STREPTOCOCCUS MUTANS AND ENTEROCOCCUS FAECALIS –AN IN VITRO STUDY

Dental Science

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ABSTRACT

Introduction- Glycarrhiza Glabra commonly known as licorice is used since years to relieve coughs sore throats & gastric inflammation. Thus considering antimicrobial properties of licorice, present study is conducted for benefits of oral diseases including dental caries. Streptococcus Mutans is highly associated with dental caries as an important etiological agent in initiation of caries and Enterococcus Faecalis is prevalent in endodontic infections. Hence, to evaluate cariostatic properties of licorice the present study is carried out.

Aim-To evaluate antibacterial activity of Glycarrhiza Glabra on selected oral microbes

Method-The antibacterial activity of Glycarrhiza Glabra against oral pathogens is evaluated by disk diffusion methods

Results -The results depict that licorice both extract have marked activity against tested samples in which alcoholic showing better zones of growth inhibition

Conclusion- Hence, due to cariostatic properties of licorice, it can be involved into caries preventive regimen by selectively inhibiting cariogenic bacteria basically targeted for use in pediatric population

KEYWORDS

Introduction:

Plants have been one of the important sources of medicines since the beginning of human cultivation. Nature is an inexhaustible storehouse of riches.

One of these is the therapeutic effect of various medicinal plants that have been described in traditional medicinal practice.

Since ancient times, several societies have resorted to nature, mainly to plants as medical and health sources. A large no. of the world population, particular in developing countries are seen using plants for facing primary needs of medical assistance It has been estimated that such use of medicinal plants possibly go back in time to around 3000 years.

Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure several human diseases. Consumption of herbal medicines is widespread and increasing in recent years and approximately 80% of the people in developing countries depend on traditional medicines for primary health care. Hence there is increased awareness for the use of medicinal plants in society due to its excellent healing properties and minimal or no side effects.

However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and 15% have been investigated phytochemically. This shows a need for planned activity guided phyto-pharmacological evaluation of herbal drugs.

Glycyrrhiza glabra Linn is one of the most widely used herb from the ancient medical history of Ayurveda, both as a medicine and also as a flavoring herb. It is found mainly in Mediterranean and certain areas of Asia.1

The earliest record of its use in medicine is found incode Hammurabi (2100 BC). It was also one of the important plants mentioned in Assyrian herbal (2000BC). Hippocrates (400BC) mentioned its use as a remedy of ulcers and quenching of thirds. The drug was also mentioned by Theophrastus and Dioscorides. In traditional Siddha system of medicine, Liquorice is used as a demulcent, expectorant, anti-tussive, laxative and sweetener.[2]

Origin

The roots are unearthed in the autumn of the fourth season. It is grown in India, Spain, Iran, Russia,China & Italy.[3]

Dental caries has been one of the most commonly occurring diseases in children. According to the National Oral Health Survey in 2004 caries prevalence in India was 51.9%,53.8%,63.1 % at ages 5,12, 15 years respectively4 It's very high morbidity potential has brought this disease into the main focus of the dental health profession.

StreptococcusMutans is an important etiological agent of dental caries in humans that attaches to the tooth surface and forms a biofilm. Considerable evidence exist implicating S. Mutans as an important etiological agent in initiation of enamel caries, both in laboratory animals and humans. E. Faecalis is often found in caries and lesions associated with periodontal diseases. Faecalis is also particularly prevalent in endodontic infections, in which the pulp may become infected via dentinal tubules, carious lesions, or periodontal disease (Adriana Lígia de Castilho et al 2013)

Development of resistance against antibiotics and antiseptics is a growing cause of concern which have limited the preventive measures. Therefore, there is a continuing need to search for new agents (Cai and Wu1996)

In the present study, we evaluated the antibacterial activity of G. glabra against oral pathogens.

Antibacterial activity:
The microorganisms used in this study included Streptococcus Mutans (PTCC 1683) & Enterococcus Faecalis (ATCC 29212) as oral pathogens. The bacterial strains were cultured in brain heart infusion (BHI).

Materials and method:
The materials used in this study are:
1. Test materials used:
a. Licorice root
b. Aqueous water
c. Ethanol
d. CHX (0.2%&2%).
2. Microorganism:
a. Streptococcus Mutans (PTCC 1683)
b. Enterococcus Faecalis (ATCC 29212)
3. Brain heart infusion & blood agar.
4. Vernier Caliper.

Preparation of alcoholic licorice root extract
Licorice extract was prepared by mixing 50 g of dry powder with 100 ml of 70% (w/v) ethyl alcohol for a week in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filters paper and was stored at 4°C for further use. Stock solutions of crude extracts were prepared by mixing well the appropriate amount of dried extracts with an inert solvent dimethylsulfoxide to obtain the final concentrations.[9]

Preparation of aqueous licorice root extract:
After washing all, the root specimens were dried for 3 days and powdered. Thus, obtained powder is weighted up to 50 g and then it is mixed with 100 ml of sterile distilled water in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filters paper and kept in an airtight amber colored container.9

Microbial analysis
Revival of the organisms
The bacterial strains from the stock were revived by plating on blood agar medium. After overnight incubation at 37°C, isolated colonies were selected and the identities of the organisms were confirmed. Isolated colonies were transferred to sterile brain-heart infusion (BHI) broth for the bacteria and once again incubated overnight. The growth concentration was adjusted to 105 organisms/ml by using 0.5 McFarland's turbidity standard.[9]

Agar ditch plate method for testing the antibacterial properties

Agar well-diffusion assay was used to evaluate the anti-microbial potential of the extracts. Petridishes containing 18 m1 of BHI agar for S. Mutans and Enterococcus Faecalis were inoculated with approximately 100 µl of microbial strain using swab technique.

Wells of 8 mm diameter were cut into solidified agar media using a sterilized standard device. One hundred µl of each extract was poured in the respective well and the plates were incubated at 37°C for 48 h. To ensure the consistency of all findings, the experiment was performed and repeated under strict aseptic conditions. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.[9]

Same procedure was repeated for microbial analysis of chlorhexidine.

Results
At the end of 48 h, statistically significant anti-microbial activity was demonstrated by all the test specimens used in this study (P = 0.002).

The results were comparatively evaluated with Chlorhexidine using it as a standard chemotherapeutic agent. The results depict that the plant extracts have marked activity against the tested samples showing significant zones of growth inhibition.

Discussion
The current anti-microbial strategies used to treat dental caries have consisted primarily of mechanical removal of dental plaque or generalized killing of oral bacteria with anti-bacterial compounds. These remove all, kill-all approaches have shown limited efficacy, since a cleaned tooth surface provides an equal opportunity for commensal and pathogenic bacteria to re-colonize in the non-sterile environment of the oral cavity.

Cariogenic bacteria usually re-dominate the Dental plaque after the treatment and start another cycle of cariogenesis. This study proposes to develop a targeted anti-microbial therapy against S. Mutans by killing or inhibiting the cariogenic bacteria within a pathogen.

Liquorice or Muleti is a perennial herb or undershrub that is about 1 m high. Its dried peeled or unpeeled underground stems and roots constitute the drug, which is an important constituent of all cough and catarrh syrups, throat lozenges and pastilles. This has been used in medicine for more than 4000 years. However, its commercial cultivation has not yet been possible and the domestic requirement is largely met through imports. [5]

The compound glycyrrhizic acid, found in Liquorice, is now routinely used throughout Japan for the treatment and control of chronic viral hepatitis, and there is a possible transaminase- lowering effect[6].

Roots of this shrub contain a number of compounds, the most important being a glycoside, glycyrrhizin, that gives glycyrrhetinic acid on enzyme hydrolysis. Glycyrrhizin has anti-diuretic, anti-inflammatory, expectorant, anti-ulcerous, and antihistaminic properties. Liquorice may be useful in conventional and naturopathic medicine for both mouth ulcers and peptic ulcers.

In India, it is used as a tonic, diuretic, demulcent, expectorant, emenagogue laxative and laxative.

It is also used:
(1) For allaying coughs and catarrhal infections,
(2) In irritable conditions of mucous membrane of urinary organs,
(3) Sorting out other diseases like sore throat, cough, anorexia and persistent low fever.

Besides its use in medicinal field, less light has been shed for the use of licorice in Dentistry. Hence, this study was aimed to assess the anti-microbial effect of licorice root extracts on caries causative microorganisms.

CHX was considered as positive control in this study. The bisbiguanide CHX, which has been studied extensively for over 25 years, is currently the most potent anti-microbial agent against mutans streptococci and dental caries. Its method of action has been comprehensively reviewed by Hugo, whose classical studies demonstrated that CHX w concentrations is a potent membrane active against both gram-positive and -negative bacteria, including the release of K+,

At higher bactericidal concentrations, CHX induces precipitation of cytoplasmic protein and nucleic acids. It abolishes the activity of the phosphoenolpyruvate-phosphotransferase sugar transport system, and thereby markedly inhibits acid production, in oral streptococci cariogenic bacteria in subjects with a high risk of developing caries.7
Another study conducted by Toors and Herczog aimed to measure acid production from an experimental, non-sugar licorice, its separate constituents and some other foodstuffs in bacterial suspensions. It was found that the experimental licorice proved to be relatively well fermentable by both S. mutans and the plaque-saliva mixture.8

Sunil Lingaraj Ajagannanavar et al in 2014 the inhibitory effect shown by alcoholic licorice root extract against S. mutans and L. acidophilus was superior when compared with that of aqueous form and CHX.[9]

However study by R. Segal et al. showed that neither licorice promoted growth or induced plaque formation. In the presence of sucrose, glycyrrhizin inhibited bacterial growth, but the adherence (plaque formation) was markedly inhibited and at 0.5-1% concentration inhibition of plaque formation was almost complete [10]

Another study by Ramachandran Sudarshan et al in 2012 stated that it has well known properties such as antiviral, glucocorticoid, anti-inflammatory, antioxidant, antiulcerative, anticarcinogenic and many more. Its indications in dentistry are also well noted such as in oral lichen planus, apthous stomatitis. It was tried in the vesicullobullous disorder which is not well recognized. It also portrays the properties, medicinal values such as antiviral, glucocorticoid,

Hypocholesterolaemic, antioxidant, toxicities, anticancer, hepatitis, gastric ulcer, hepatocellular carcinoma and dosage of this precious herb. [11]

Fereshteh Sedighinia1 et al, the antibacterial activity of Glycyrrhiza glabra against oral pathogens by diffusion methods and determined the minimum inhibitory concentration (MIC) by both broth and Agar dilution methods and minimum bactericidal concentration (MBC) by broth dilution methods.[12]

In this study by Gupta VKet al, Glabridin, the active component of Glycyrrhiza glabra, exhibited antimicrobial activity against both Gram-positive and Gramnegative bacteria as well as mycobacteria.The antimycobacterial activity of Glycyrrhiza glabra was found at 500 microg/mL concentration. The results indicated that licorice can be used as potential antitubercular agent.13

Patil R. C. et al 2007used aqueous, alcohol and hydroalcohol extracts of Glycyrrhiza glabra to evaluate the antimicrobial effect on multiple drug resistant (MDR) strains of Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae,Serratia marcescens, Proteus mirabilis and Salmonella typhi from clinical isolates in Mumbai, Maharashtra, India. The result demonstrated that Glycyrrhiza glabra extracts had more antibacterial activity than the Tinospora cordifolia extracts. Aqueous Glycyrrhiza glabra extract showed maximum antimicrobial activity against all the screened MDR strains followed by alcohol and hydroalcohol.[14]

Similarly,

Aparajita Gupta et al in 2013 stated that both methanolic /acetonic extracts of G. glabra had potential antibacterial activity against all the studied gram-positive and gram-negative bacterial strains.

Eesha Jain et al 2013study leads to the conclusion that Liquorice extracts used in child patients showed antimicrobial efficacy and led to a rise in the pH of saliva. The present study confirms the antimicrobial and cariostatic effi cacy of liquorice extracts and recommends that liquorice can be used as a preventive regimen in pediatric practice.15

Conclusion:

This study showed that Glycarrhiza glabra have antimicrobial property which are comparable with gold standard. It can also be used specially in pediatric population as it can reduce side effects of chlorhexidine such as staining & alteration of taste. Above study results reveal that the seasonal plants have lots to offer.

Whenever we talk about Indian medicinal plant the western world and Indian Laurent seems to be focusing on wellknown plants like Tulsi, Neem, Turmeric, Aloe Vera etc.But the Indian folk medicine & Western Ghats have much more to offer.

Even the World Heritage Organization has acknowledged the Western Ghats regarding its bio-diversity.

Due to cariostatic properties of licorice, it can be involved into caries preventive regimen by selectively inhibiting cariogenic bacteria basically targeted for use in pediatric population.This study results can be considered an a tip of an ice berg in the sea of the medicinal plants which can be helpful for further research of undiscovered /hidden properties of this medicinal plant which can be useful for easier application in pediatric population.

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