Research Article

Investigation on antibacterial effect of *Eulophia herbacea* against *Streptococcus mutans*

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Abstract

Objective: The study was aimed to evaluate *in vitro* antibacterial effect of various extract of *Eulophia herbacea* against *Streptococcus mutans*, and *Lactobacillus*. **Materials and methods:** Plant leaves material was collected, washed, dried, coarsely grinded and defatted with petroleum ether and extracted by using ethanol and water. The both extracts were taken and performed the preliminary phytochemical tests, powder character and antimicrobial activity on *Staphylococcus mutans* and *Lactobacillus*. Pure strains of test organisms were obtained from Hi media (Mumbai). Using Agar well diffusion method, zone of inhibition of various extract of *Eulophia herbacea* against test organism were performed. All tests were performed in triplicates manner. **Results:** Phytochemical studies of different extracts of *Eulophia herbacea* revealed the presence of carbohydrates, proteins, amino acids, tannins, saponins, flavonoids and alkaloids. The various extracts of *Eulophia herbacea* were showed significant antibacterial efficacy against the oral test microbes. Zone of inhibition showed good efficacy against *S. mutans* and moderate efficacy against *Lactobacillus*. Overall non toxic and equally efficacious herbal product can be an interesting alternative to synthetic drug. **Conclusion:** The study reveals that the various extracts of *Eulophia herbacea* can be used as anticariogenic or antiplaque agent.

Keywords: *Eulophia herbacea*, agar well diffusion, *Streptococcus mutans*, dental caries, antimicrobial

Introduction

In developing countries as well as in India, dental problems due to microbial infections are a very common (Rajalaxami and Lakshami, 2017). In the development of dental caries and periodontal disease bacteria existing in the dental plaque or biofilm play an important role (Marsh, 2006). The biofilm is the main factor that causes dental caries by encourages the aggregation of bacteria on the tooth surface. Dental caries, also known as tooth decay (Saini et al., 2003). *Streptococcus mutans* is one of the most cariogenic microorganisms that are involved in the development of dental caries and dental plaque in humans. The major source of dental plaque or biofilm is *S. mutans* which can produce acid and synthesizes water insoluble glucan by the action of glucosyltransferase (GTFase)

(Dos Santos et al., 2002; Wiater et al., 1999). Oral cavity pathogens other than *Streptococcus mutans* include *Lactobacilli*, *Streptococcus salivarius*, *Halobacterium* sp., *Veillonella* sp. etc. These bacteria grow and attack the tissues causing gingivitis, characterized by inflamed gums that bleed easily (Ghada et al., 2013). Approximately 60-65% Indian population suffers from dental caries (Shouri, 1941; Ramchandran et al., 1973). Pathogenic bacteria had developed or increased the resistance to currently used antibiotics and chemotherapeutics. There are many products for the oral cavity such as toothpastes, gums, or mouthwashes that can reduce the risk of tooth decay. Numbers of commercially chemical agents are available, such as chlorhexidine, triclosan, or sodium fluoride which is used as antibacterial and antiplaque agents in the oral cavity. Unfortunately, these agents cause oral mucosa irritation and have undesirable side effects such as vomiting, diarrhea, and tooth staining (Park et al., 2003). Hence, there is a need to develop some alternative products against dental caries. Various traditional plants and natural

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products for the treatment of oral diseases have been reported (Cowan, 1999; Kalembe et al., 2003). Various antimicrobial agents have been tested against these microorganisms. The goal of this study was to assess the efficacy of *Eulophia herbacea* extract and chlorhexidine mouth rinse on *S. mutans* and *Lactobacillus*.

Materials and methods

Collection of *Eulophia herbacea*

The fresh rhizomes of *Eulophia herbacea* were collected from the Toranmal hilly region.

Collection of the test organisms

The test organisms used in this research consisted of *Streptococcus mutans* and *Lactobacillus* bacteria and purchased from Hi media (Mumbai). The medium used for the study is Tryptic Soy broth (TSB). The medium was also purchased from Hi media, Mumbai.

Preparation of aqueous extract of *Eulophia herbacea*

Twenty grammes of fine grounded powder of rhizome of *Eulophia herbacea* was weighed on an electronic weighing balance. This was dispensed into two beakers, each containing 80ml of distilled water. These were soaked for 72 hours after which the solution was carefully filtered with muslin cloth into a sterilized conical flask of 100 ml and the filtrates obtained were stored in the refrigerator at a temperature of 4°C until required (Chigozie and Samuel, 2017).

Preparation of ethanol extract

Twenty grammes of fine grounded powder of *Eulophia herbacea* was dispensed into a beaker containing 80ml of 95% ethanol. They were soaked for 72 hours while the resulting supernatant was decanted into a conical flask of 100 ml and kept in the refrigerator for further study. The extraction of the plants was carried out according to the method of Chigozie and Samuel, (2017).

Qualitative phytochemical screening of *Eulophia herbacea*

Simple standard chemical tests were carried out for the qualitative phytochemical screening of *Eulophia herbacea*. These tests were used to detect the presence of bioactive agents such as the alkaloids, tannins, saponins, amino acid, carbohydrates, cardiac glycosides and proteins. Preliminary Phytochemical investigations aqueous extracts was tested for various chemical constituents by different chemical tests (Evans et al., 2005; Khandelwal et al., 2006).

Alkaloid test

Two grams of aqueous extracts of *Eulophia herbacea* and 2 ml honey were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath at 60°C for 5 minutes. The sample was filtered with

a 3 layered muslin cloth. One ml of the filtrate was treated with few drops of Dragendoff's reagent and Hager's reagent. Reddish brown precipitate and yellow precipitate respectively indicates presence of alkaloids.

Cardiac glycosides

Five grams of the extract and 5 mL of honey were dissolved separately in 2 mL glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml concentrated H₂SO₄. A brown ring at the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form which just gradually spreads throughout the layer.

Flavonoids test

Five millilitres of diluted ammonia solution was added to aqueous filtrate of the test sample followed by the addition of 1 mL concentrated H₂SO₄. A yellow colouration indicates the presence of flavonoids

Amino acids test

Two grams of aqueous extracts of *Eulophia herbacea* and 2 ml honey were stirred with 2ml of Millions reagent white precipitate indicates presence of amino acids.

Carbohydrates test

Two grams of aqueous extracts of *Eulophia herbacea* and 2 ml honey were stirred with few drops of alcoholic - naphthol, then few drops of concentrated sulphuric acid through sides of test tube were added. Purple to violet color ring appears at the junction which indicates presences of carbohydrates.

Saponin test

Two grams of aqueous extracts of *Eulophia herbacea* and 2 ml of honey were shaken separately with distilled water in a test tube. Stable froth (foam) is formed was taken as preliminary evidence of the presence of the saponins.

Tannins test

Two grams of aqueous extracts of *Eulophia herbacea* and 2 ml of honey were stirred separately with 100 ml distilled water and filtered. 1 ml of ferric chloride reagent was added to the filtrate, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Proteins

Two grams of aqueous extracts of *Eulophia herbacea* and 2 ml Biuret reagent was added, violet color indicates presence of proteins.

Antibacterial activity by diffusion method

The pure strains of *Streptococcus mutans* and *Lactobacillus* test organism was grown in TSB medium for 24 h at 37°C and concentration was adjusted to 0.5 McFarland standards (Jorgenson and John, 2015). About 20ml of sterile media was aseptically poured into each dish. The dishes were gently rocked for proper mixture and the nutrient agar was allowed to solidify. Afterwards, well was dug in each plate with the aid of a sterilized cork borer of 6mm diameter. The various concentrations of the test drugs (ethanolic and aqueous extract of *Eulophia herbacea*) were impregnated into well. The concentration was 10mg and 20mg of test drug. The standard drug serves as positive control (0.2% chlorhexidine) was separately impregnated into well. They were allowed to stand for one hour for proper diffusion and then incubated at 37°C for 24 – 48 hours. The sensitivity of the test organisms to ethanol and water extract of *Eulophia herbacea* was indicated by zone of inhibition around the wells. The diameter of the zone of inhibition was measured in millimeter using a transparent ruler.

Statistical Analysis

Data were expressed as Mean $\pm$ S.D. and were evaluated by student's 't' test. Values of $p < 0.005$ were considered statistically significant.

Results

Preliminary phytochemical tests

Preliminary phytochemical investigation of aqueous and ethanolic extracts of *Eulophia herbacea* revealed the presence of carbohydrates, proteins, amino acids, tannins, saponins, flavonoids and alkaloids given in table 1.

Determination of zone of inhibition by *Eulophia herbacea* extract against test organisms

For the preliminary study of inhibition of bacterial growth, as an antibacterial activity of the aqueous and ethanolic extract of *Eulophia herbacea* against *S. mutans* and *Lactobacillus* were investigated by the agar well diffusion technique. *S. mutans* and *Lactobacillus* were exposed to 10 and 20 mg/ml aqueous and ethanolic extract of *Eulophia herbacea*. The results showed that the antibacterial activity against *S. mutans* and *Lactobacillus* was dose-dependent and increased with increasing concentrations of aqueous and ethanolic extract of *Eulophia herbacea*. The zone of inhibition for aqueous extract against *S. mutans* was found to be 9.50 ± 0.50 mm and 12.75 ± 0.5 mm. Also the zone of inhibition for aqueous extract against *Lactobacillus* was found to be 8.25 ± 1.0 mm and 11.50 ± 1.0 mm. The zone of inhibition for ethanolic extract against *S. mutans* was found

Table 1. The results of different phytochemical test performed for *Eulophia herbacea* extracts

S. No.	Test	Aqueous extract	Ethanolic extract
1	Alkaloids	+	+
2	Cardiac glycosides	-	-
3	Flavonoids	-	+
4	Amino acid	+	+
5	Carbohydrates	+	+
6	Saponins	+	-
7	Tannins	+	+
8	Proteins	+	+

Table 2. Zone of inhibition of aqueous and ethanolic extract against test organisms

Test drug	Concentration of test drug	Zone of inhibition (mm) test organism <i>S. mutans</i>	Zone of inhibition (mm) test organism <i>Lactobacillus</i>
Ethanolic extract of EH	10mg	16.00 ± 0.5	14.50 ± 0.5
	20mg	18.75 ± 0.25	17.25 ± 1.0
Aqueous extract of EH	10mg	9.50 ± 0.5	8.25 ± 1.0
	20mg	12.75 ± 0.5	11.50 ± 1.0
Chlorhexidine	0.40 mg	22.00 ± 1.0	20.00 ± 0.5

to be 16.0 ± 0.50 mm and 18.75 ± 0.25 mm. The zone of inhibition for ethanolic extract against lactobacillus was found to be 14.50 ± 0.50 mm and 17.25 ± 1.0 mm. Chlorhexidine, which has the most powerful antibacterial activity against *S. mutans* and was used as the positive control in this study. The zone of inhibition of 0.2 % chlorhexidine against *S. mutans* and lactobacillus was found to be 22 ± 1.0 mm and 20 ± 0.5 mm, respectively.

Discussion

Among all cariogenic microorganisms *S. mutans* is considered as the main culprit for dental caries. The pathogenesis of dental caries determines that initially there is adhesion mediated attachment of *S. mutans*, which adhere by hydrophobic bonds to the enamel surface and ferment sucrose (Jeon et al., 2011). Due to further metabolism of sucrose firm adherence and aggregation of the bacteria to the tooth surface is observed and results in acid production and cavity formation (Palombo et al., 2011). The use of an antimicrobial agent in the oral cavity might inhibit the growth of microorganisms, thus preventing the development of dental caries. Chemical agents such as fluoride and chlorhexidine, were used to prevent dental caries for several decades, but associated with some side effects such as staining of teeth and fluorosis. Thus, there is no perfect antimicrobial agent to prevent dental caries until now (Gold, 2008). But it is believed that for thousands of years in folk medicine, natural products have been the new source of antimicrobial agents (Xavier and Vijyalakshmi, 2007). Secondary metabolites such as alkaloids, flavonoids, polyphenols and lectins present in the herbs are prone to show the antimicrobial activity (Fatima et al., 2000). Due to high incidence of oral diseases and increased bacterial resistance to antibiotics, there is a continuous need for alternative therapies from natural herbs which can effectively tackle against the bacterial infection which is affordable and not toxic to the society (Rishton, 2008). In tribal parts of India peoples used routinely tubers of Amarkand as food and therapeutic entity for their better health (Aarti et al., 2016). Moreover, different solvent extracts of *E. ochreata* Lindl were found to have potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* (Devkar et al., 2009). Hence in the present study we assessed the efficacy of ethanolic and aqueous extract of *Eulophia herbacea* against the oral cavity pathogens. The results of zone of inhibition against test organism shows the almost equal efficacy to that of chemical agent (chlorhexidine) used as positive control.

Conclusion

It is suggested that the significant antibacterial efficacy of isolated extract of *Eulophia herbacea* was found in this study and could be attributed as extracts used for the study are rich in

alkaloids, flavonoids, terpenoids and tannins. The inhibitory effect shown by the isolated extract against *S. mutans* and lactobacillus were significant and thereby such herbs products can be used as an alternative to chemical products for treatment of dental caries.

Conflict of interest

None

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