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Antimicrobial activity of a poly-herbal extract against dental micro flora

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ABSTRACT

The extracts of six herbs (cf. medicinal plants) namely *Azadirachta indica* A.Juss., *Emblica officinales* Gaertn., *Terminalia belerica* Linn., *Terminalia chebula* Retz., *Terminalia arjuna* (Roxb.ex DC) Wight and Arn., and *Mangifera indica* L, as individual and as combination were tested for animal toxicity, anti-microbial activity *in vitro* and for Total Viable Counts of dental plaque *in vivo* studies. The formulation was then prepared and its anti-microbial activity was compared with the marketed chlorhexidine mouthwash. The results indicate that this poly-herbal mouthwash holds promise in improving the oral hygiene in healthy individuals and help in preventing dental caries and gingivitis through plaque control.

Keywords: Antimicrobial activity, poly-herbal extract, dental plaque, mouthwash

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INTRODUCTION

Dental plaque formed on the gum margin and adjacent tooth surface causes inflammation of gums. The bacteria in the plaque release toxins which cause swelling, redness and bleeding of gums. Periodontitis is a more severe and destructive gum disease which may progress irreversibly in breaking down supporting periodontal structures. [1] As it is impossible to eliminate oral bacteria causing dental plaque, it is important to achieve plaque control by limiting growth of harmful bacteria. Bacterial flora in plaque is variable according to the site such as – sub gingival/supra gingival, and tooth/gingiva associated.[2] Complex oral flora makes it difficult for the antimicrobial agent to be targeted at a particular organism in the plaque. Thus a non specific plaque removal or inhibition has been accepted as the practical approach to control dental plaque formation.

Various products such as toothpastes, gels, pastes for application, mouthwash, lozenges, etc. have been available for years. However, in the recent years, use of mouthwash has been on the increase as it is relatively easy to use for maintaining oral hygiene. Commercially available mouthwashes containing synthetic and semi synthetic active agents have several disadvantages like staining on the teeth, irritation during use, high degree of alcohol content etc. To overcome the above disadvantages naturally occurring antimicrobial herbs can be used individually or in combination. The herbal medicines are normally considered safer than the non-herbal medicines because the natural active ingredients present in herbal medicines are in combination with other components.

In the present study the herbs used for the preparation of extract are *Azadirachta indica* [3], *Terminalia chebula* [4], *Terminalia belerica*[5], *Emblica officinales*[6] *Terminalia Arjuna[*7], and *Mangifera indica*[8]. These herbs are known for their medicinal properties and are traditionally used in India.

MATERIALS AND METHODS

The following herbs were authenticated and then parts of the herbs were used for the extraction-

Azadirachta indica-leaves, *Terminalia chebula*-whole fruit, *Terminalia belerica*- whole fruit, *Emblica officinales* - Dried pulp of fruit, *Terminalia arjuna*- bark, and *Mangifera indica*-leaves.

Other materials used for the extraction and *in vitro* and *in vivo* evaluation are 95% v/v Ethanol, Distilled water, Nutrient Agar (Hi media), Sodium chloride(AR), peptone, meat extract, chlorhexidine (IPCA Labs.)

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Dental micro flora (OD at 530 nm = 0.1) was collected from the Department of Dentistry - Deenanath Mangeshkar Hospital and Research Center, Pune.

EXPERIMENTAL

Extraction of individual herbs

Extraction process

20 g of powdered herb was macerated in 100 ml of solvent system (ethanol and water in proportion of 70:30) by cold maceration method and then filtered. The filtrate was concentrated to yield 20 ml of a semisolid extract. This extract was then used for antimicrobial activity evaluation.

Evaluation of antimicrobial activity of individual extract

Antimicrobial activity was evaluated by using Agar Well Diffusion Assay method against dental micro flora. 0.1 ml Dental micro flora was spread by Spread Plate Technique on sterile pre solidified Nutrient Agar Medium. The standard procedure was adopted and the zone diameters were measured from six different angles for each well and the average Zone diameter was recorded.

Table 1 : Zone of inhibition shown by the individual herb extract

Sr. No.	Hydro alcoholic Extract (30: 70)	Zone of Inhibition (mm)
1	Azadirachta indica	18.00
2	Terminalia chebula	17.0
3	Terminalia belerica	18.0
4	Emblica officinales	18.0
5	Terminalia arjuna	18.0
6	Mangifera indica	17.0
	Average zone of inhibition in mm	18.0

Results are mean of five experiments (n = 5)

The results showed that all the herbs used have antimicrobial activity. [Table 1]

Extraction of Poly-herbal herbs (Poly-herbal Extract)

Extraction process

Total 20g of Poly-herbal powder of selected parts of herbs in the proportion (*Azadirachta indica*, *Emblica officinales*, *Terminalia belerica*, *Terminalia chebula*, *Terminalia arjuna*, and *Mangifera indica* 1 : 0.25 : 0.25 : 1.5 : 1 : 1 respectively) was macerated using 100 ml mixture of ethanol and water (in proportion of 70:30). By cold maceration method and then filtered. The filtrate was concentrated to yield 20 ml of a semisolid extract.

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Evaluation of antimicrobial activity of poly-herbal extract

Table 2: Zone of inhibition shown by Poly-herbal extract of powders of herbs

Sample used	Average Diameter of the Zone of inhibition (mm)
Poly-herbal extract of powders under study	18

Results are mean of five experiments (n =5)

Antimicrobial activity was evaluated by using Agar Well Diffusion Assay against dental micro flora as explained earlier. [Table 2]

Animal toxicity study of Poly-herbal extract

The Poly-herbal extract was evaluated for animal toxicity as per the standard procedure. The median lethal dose (LD_{50}) after oral administration as a single dose in Sprague Dawley rats both male and female was estimated to be more than 5000 mg per kilogram of the body weight.

***In vivo* study of the Poly-herbal extract**

Preparation of patients and collection of samples

In vivo antimicrobial activity was conducted at Deenanath Mangeshkar Hospital and Research Center, Pune after approval by Institutional Ethics Committee *(ICH-GCP guidelines)*. Initially seven participants were subjected to dental procedure. At the start of the procedure, oral prophylaxis (Scaling and Polishing) was done. After the prophylaxis step, participants were asked to gargle for 30 seconds using plain water. Plaque samples were collected from buccal surface of maxillary right first permanent molar and adjacent buccal mucosa (control). The extract under study was then applied with sterile cotton bud on the buccal surface of maxillary left first permanent molar and adjacent buccal mucosa (test) and left for a period of 5 minutes. The plaque samples were collected from the test site, after one hour. [9,10]

Total Viable Count (TVC) estimation

Table- 3 : Percentage of Subjects with Reduction in Total Viable Count (TVC)

Total No. Of Participants	Number of participants in which reduction in total viable count took place	Percentage of Positive results
7	6	85.75

The (TVC) of both samples (test and control) were estimated by standard Spread Plate Technique on nutrient agar incubated at 37^{o}C. The count was taken at anywhere between 24 to 48 hrs. [Table 3]

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Preparation of Formulation

Preparation of mouthwash

The extract of Poly-herbal powders was formulated by using pharmaceutical excipients like Soothing and Cooling agent (menthol and thymol), Thickening agents (glycerin, propylene glycol), Flavoring agent (Raspberry oil), into a mouthwash. The formulation was prepared using 10% w/v of poly-herbal extract, in any single dose of the formulation, to provide optimal antimicrobial activity and palatability. [11, 12]

Evaluation of antimicrobial activity of mouthwash

Table 4 : Zone of inhibition shown by the formulation (mouthwash) prepared from the Poly-herbal extract

Sample used	Average Diameter of the Zone of inhibition (mm)
Formulation under study	18
Chlorhexidine (0.2 %w/v)	19

Results are mean of five experiments (n = 5)

The study was carried out by using Agar Well Diffusion Assay method with undiluted marketed formulation of chlorhexidine (0.2 % w/v) as Positive Control and Sterile distilled water as Negative Control. The antimicrobial activity of the formulation was found to be comparable with antimicrobial activity of marketed Chlorhexidine formulation. [Table 4]

Toxicity studies of formulation

The Mouthwash was tested for oral toxicity on Sprague Dawley rats in compliance with the regulatory guidelines. The median lethal dose (LD_{50}) of formulation after oral administration as a single dose in Sprague Dawley rats both male and female was estimated to be more than 5000 mg per kilogram of the body weight.

RESULTS AND DISCUSSION

The herbs used in the formulation *Azadirachta indica*, was reported to be used widely in oral care formulations. *Terminalia chebula, Terminalia belerica, Emblica officinales* appear to be synergistic to the antimicrobial activity of *Azadirachta indica* in maintenance of oral hygiene. *Terminalia arjuna*, and *Mangifera indica* were selected because of their astringent and antioxidant properties in addition to antimicrobial activity. Extraction was done for individual herbs and was evaluated for antimicrobial activity against dental micro flora. The results showed that all the plants used in the study have antimicrobial activity.

Instead of preparing extracts of individual herbs and combining them to get a Poly-herbal extract, a novel approach was used. The powders of the selected part of herbs were

mixed in a set proportion and the Poly-herbal powder was extracted to obtain a Poly-herbal extract. The results of antimicrobial study showed that the extract prepared by using Poly-herbal powders is equally effective as antimicrobial and has advantage of consuming less solvent system and thus is also cost effective.

This extract of poly-herbal powders was studied for the antimicrobial activity in the range of concentration from 0.05% w/v to 80%w/v of extract. It was found that the antimicrobial activity at 10 % w/v of the extract was found to be substantial and at the same time is tolerable as regards the palatability due to bitterness. So the formulation was prepared by using 10% w/v of extract which provides optimal antimicrobial activity and palatability to the formulation. A sample of the poly-herbal extract was tested for oral toxicity and was found to be safe. *In vivo* antimicrobial activity test of the poly-herbal extract include *in-vivo* plaque collection and total viable count estimation. The total viable count reduction was observed in 85% of participants out of seven participants, confirming the anti-microbial activity of the Poly-herbal extract.

The formulation of 10%w/v of extract in the form of mouthwash showed significant effect in terms of antimicrobial activity, comparable with the marketed synthetic drug like chlorhexidine. The mouthwash was also evaluated for the animal toxicity and was found to be very safe.

CONCLUSION

Thus, the poly-herbal extract was found to be effective in its activity in the formulation containing 10% w/v concentration of extract. The Poly-herbal extract and formulation containing poly-herbal extract was found to be safe in animal toxicity studies. This extract in suitable formulation therefore can be an effective yet safe and non toxic alternative in the treatment of dental plaque and associated disorders. Thus the formulation can also be routinely used for improving oral hygiene of healthy children and adults as well as in patients with dental caries and gingivitis.

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