PREPARATION AND EVALUATION OF HERBAL TOOTH POWDER COMPOSED OF HERBAL DRUGS WITH ANTIMICROBIAL SCREENING.

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Summary

The medicinal plant clove is famous for its dental analgeic potential in the herbal world is concerned. Apart from this, the ginger, asafoetida, amla, ajowan, pepper, neem bark, acacia bark, alum, mentha leaf and mustard oil were used and the formulation of herbal tooth powder was carried out. The extracts prepared using cold maceration techniques were subjected to qualitative chemical analysis. The evaluation of herbal tooth powder was done and results found to be within the limits. The extracts were screened for its antimicrobial activity by Agar well diffusion method against *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans.* The alcoholic extract showed antimicrobial activity with the zone of 8 mm. This proves that the extract can be useful to treat the dental caries and dental plague with the scientific documentation.

Key words: Herbal tooth powder, *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans.*

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Introduction

Tooth powders are more popular in suburban and rural areas. The constituents of tooth powder and tooth pastes are same except that tooth powders do not contain humectant, water and binding agents[1]. The primary function of tooth powder is the cleaning of the accesible surfaces of the teeth[2].

Herbal tooth powder is a tooth-cleaning agent that is almost entirely made from all-natural ingredients. Its purpose is to freshen breath, help heal gums, rid teeth of bacteria, and reduce the amount of inflammation in the mouth. Herbal tooth powder has been around for centuries and many believe it to be an essential part of any teeth-cleaning regimen[3].

Neem is a traditional tooth cleaner. From various forms and extracts of tender twings of Neem was prepared[4]. Antimicrobial activity of various herbal tooth powder and paste was evaluated against *Candida albicans, Staphylococcus aureous* and *Escherchia coli*[5,6,7]. In vitro evaluation of antibacterial activity of an herbal dentifrice was carried against *Strptococcus mutans* and *Lactobacillus acidophilus*[8]. In vitro antimicrobial activity of Endodontic pastes with propolis extracts and calcium hydroxide was studied[9]. Herbal tooth powder was also evaluated for its piperine contents[10]. The antimicrobial potential of 14 natural herbal dentifrices was studied by diffusion method[11].

A literature survey and screening of scientific data revealed that a large number of Herbal and non herbal tooth powders have already been investigated. The several tooth powder formulations are available in market. Many tooth powders having cleansing property or other special therapeutic activity have been formulated. Therefore in the present work, following aspects of Herbal tooth powders were planned for the formulation, standardization of herbal tooth powder and antimicrobial screening of the extracts of herbal tooth powder.

Plant /Drug profile

The following drugs are used in formulation of Herbal Tooth powder.

1. Clove
2. Ginger
3. Asafoetida
4. Amla
5. Ajowan
6. Pepper
7. Neem bark[12]
8. Acacia bark[13]
9. Alum[14]
10. Mentha leaf [15]
11. Mustard oil

Materials and Methods

Formula setup:

Formula 1 (10 %)

Ingredient	Quantity
Clove	10 gm
Ginger	7 gm
Asafoetida	5 gm
Amla	20 gm
Ajowan	10 gm
Pepper	7 gm
Neem bark	12 gm
Acacia bark	20 gm
Alum	5 gm
Mentha leaf	5 gm
Mustard oil	q. s.

Formula 2 (15 %)

Ingredient	Quantity
Clove	15 gm
Ginger	10 gm
Asafoetida	5 gm
Amla	15 gm
Ajowan	15 gm
Pepper	10 gm
Neem bark	10 gm
Acacia bark	10 gm
Alum	7 gm
Mentha leaf	7 gm
Mustard oil	q. s.

Collection, processing and authentification

All drugs are collected from the market except Neem bark, Acacia bark and Mentha leaf. The Neem bark, Acacia bark and Mentha leaf are collected from the region of Dhulgaon, Yeola, Nashik, Maharashtra, India. in August- September 2010. The authentification of drugs was done by the experts. The material was dried under shade. Powdered it and used for the formulation of Herbal tooth powder.

Extractive values

Ethanol soluble and Water soluble extractive values

Formulation 1, formulation 2 and standard drug were extracted with ethanol and water by standared method. Vicco vajradanti was used as standard formulation[16].

Qualitative Chemical Investigation

Methanol and aqueous extract obtained by maceration method[16,17]. Both the extracts were subjected to proximate chemical analysis.

Standardizations / Evaluation parameters

Volatile matters and moisture: A specific amount of the product required to be taken in a dish and drying was done till constant weight. Loss of weight will indicate percentage of moisture and volatile matters[2,16,18].

Foaming character: This test was specially required for foam forming tooth powders. Specific amount of product can be mixed with specific amount of water to be shaken. The foam thus formed was studied for its nature, stability, washability[1,2].

Flow property: Flow property is determined by Angle of repose in Funnel method. (θ =Tan-1 h/r)[19].

Bulk density: Bulk density was determined by Tapped and untapped volume of the powder [2, 16, 18].

Pharmacological screening

Test Samples and standards

The saturated ethanolic extract and aqueous extracts were the test samples. The extracts of Vicco vajradanti tooth powder was the standard samples.

Statistical Analysis

All values shown as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test $P<0.05$ was considered statistically significant

Antimicrobial activity

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine[20]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases[21].

The following bacterial strains obtained were used in the bioassay: *Esciahericha coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (NCIM3471).

Agar well diffusion method

The percent inhibition of microbial growth at fixed dose (200µg/ml) of extract was investigated using gram-positive and gram-negative bacteria and fungal strains using broth dilution method[22]. Muller-Hinton and Potato dextrose agar were used for bacteria and fungi respectively. The alcoholic extracts were dissolved in alcohol and aqueous extracts were dissolved in distilled water to obtain 1000 µg/ml stock solution. The Muller-Hinton and Potato dextrose agar was inoculated with 200 µl of the inoculum (1×10^8 cfu/ml) and poured into the petri plate, allowed to dry, a well was prepared in the plates with the help of a borer (6 mm), and 100 µl of test compound was introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition[23].

Results

Extractive values

The yield of extracts was 7.24% w/w, 5.6% w/w, 8.06% w/w, 6.5% w/w for water and ethanol extracts of formulation 1 and formulation 2 respectively.
The yield of extracts was 10.22% w/w 6.82% w/w for water and ethanol extracts respectively of vicco vajradanti (Table no.1).

Qualitative determination of extracts

The all extracts were subjected to preliminary phytochemical screening; which showed the presence of tannins, flavonoids, alkaloids and glycosides (Table no.2).

Evaluation of herbal tooth powder

Moisture content

The Percentage of Volatile matter is 12.67 ± 0.441%w/w, 14.5 ± 0.2887%w/w,39.83±4.055 of formulation 1, 2 and standard formulation respectively.

Foaming character and Bulk density

The foaming character and bulk density of 1% and 2% solution of formulation 1 and formulation 2 were shown in Table no.3 and 4.

Flow property

Flow properties determined by angle of repose were found to be 47.2 ± 0.2292, 47.37 ± 0.2719 of formulation 1 and formulation 2 respectively (Table no.5).

Pharmacological Screening

The results of anti-microbial activity of all extracts against *Esciahericha coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (NCIM3471) as shown in Table no.6

Discussion

Dental caries are the most common oral infectious disease among children and old age. The prevention strategy against dental caries includes the elimination of cariogenic microorganisms from the oral cavity, inhibition of their plaque formation and the enhancement of tooth resistance to demineralization. In the former strategies, phytochemicals have been widely studied for their antimicrobial activity. A variety of plants with potent activity are known to be traditionally used for dental hygiene world-wide. Antibiotics and other antimicrobial agents are effective in the prevention and treatment of dental caries, but they also cause undesirable side effects such as Furthermore, viridians group streptococci including *S, mitis, S.mutans*, *C.albicans* most representative human cariogenic bacteria are moderately resistant to antibiotics Therefore, search for the herbal dental care formulation could offer an effective alternative to antibiotic strategies for oral infection disease like dental caries.

Conclusion

Among the two formulations of herbal tooth powder, alcoholic extracts of formulation-2 (15 %) is effective against the Micro organisms like caries produce bacteria and fungi. Developing countries like India having the percentage of poor people more, to meet with the demand of the poor public, this herbal formulation may serve the purpose once the evaluation and detailed studies may over.

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Table No.1. Percentage of dry extracts in terms of air dried powder of formulation 1, 2 and standard formulation

Extracts	% yield
10% aqueous of formulation 1	7.24% w/w
10% ethanolic of formulation 1	5.6% w/w
15% aqueous of formulation 2	8.06% w/w
15% ethanolic of formulation 2	6.5% w/w
Standard aqueous	10.22% w/w
Standard ethanolic	6.82% w/w

Table No.2. Qualitative determination of extracts of formulation 1, 2 and standard formulation

Extracts	Tannins	Glycosides	Alkaloids	Flavonoids
10% aqueous	+	+	-	+
10% ethanolic	+	+	+	+
15% aqueous	+	+	-	+
15% ethanolic	+	+	-	+
Standard aqueous	+	+	-	+
Standard ethanolic	+	+	+	+

+ = presence of compound,

−= absence of compound.

Table No.3. Foaming character of formulation 1, 2 and Standard formulation

Sr. No	Percentage of Solution	Volume of foam (ml)
Formulation 1	1 %	0.5 ± 0.05774
	2 %	0.3333 ± 0.08819
Formulation 2	1 %	0.5 ± 0.05774
	2 %	0.2667 ± 0.08819
Standard formulation	1%	4.6667 ± 0.3333
	2%	9 ± 1.528

Each value is presented as Mean ± SEM ($P<0.05$) one way ANOVA followed by Dunnett's test

Table No.4. Bulk density of formulation 1, 2 and Standard formulation

Type		Bulk density (gm/ml)
Formulation 1	Untapped density	0.528 ± 0.017
	Tapped density	0.391 ± 0.011
Formulation 2	Untapped density	0.514 ± 0.029
	Tapped density	0.47 ± 0.07
Standard formulation	Untapped density	0.478 ± 0.022
	Tapped density	0.613 ± 0.033

Each value is presented as Mean ± SEM ($P<0.05$) one way ANOVA followed by Dunnett's test

Table No.5. Flow property of formulation 1, 2 and Standard formulation

Type	Radius (cm)	Height (cm)	h/r	θ =Tan-1 h/r
Formulation 1	3.24 ± 0.0251	3.46 ± 0.0333	1.077 ± 0.0064	47.2 ± 0.2292
Formulation 2	3.2 ± 0.0325	3.46 ± 0.0333	1.08 ± 0.0057	47.37 ± 0.2719
Standard formulation	3.5 ± 0.0288	3.76 ± 0.0666	1.07 ± 0.0286	47.09 ± 0.716

Each value is presented as Mean ± SEM (P<0.05) one way ANOVA followed by Dunnett's test

Table No.6. Zone of Inhibition of aqueous, ethanolic extracts of formulation 1, 2 and standard formulation

Sample	*E. coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923	*Candida albicans* (NCIM3471).
10 % aqueous	No zone	No zone	No zone	No zone
10 % ethanolic	No zone	No zone	No zone	No zone
15 % aqueous	No zone	No zone	No zone	No zone
15 % ethanolic	No zone	No zone	**8 mm**	**8 mm**
Standard aqueous	No zone	No zone	No zone	No zone
Standard ethanolic	No zone	No zone	No zone	No zone
Ofloxacin	**23 mm**	**20 mm**	**20 mm**	-
Fluconazole	-	-	-	**32 mm**

Each value is represent n=3.

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