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Antibacterial Activity of the Herbal Gargles

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ABSTRACT

Teeth and mouth are the supportive organs to enable us in doing all the functions of the body by consuming. So, cleanliness of the oral areas is important for the healthy and nourished body which is devoid of microorganisms. Herbs have been in the investigations for the antibacterial and antiviral properties along with the history of the medical systems. They were effective and safer relative to the available synthetic drugs. Three formulations of gargle were prepared using Extract of Neem, Aloe Gel and clove oil which were named as GNF, GAF and GCF. The physical parameters were estimated, such as pH and one formulation failed in the stability study. The prepared GNF and GAF showed better antibacterial activity against the bacteria extracted by swabbing the mouth and also the three strains like E coil, Streptomyces and staphylococcus.

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INTRODUCTION

Human skin is the largest organ in the body that is covering it, and a considerable extent of it is exposed to the various kinds of micro-organisms and allergens. These organisms utilize the secretions of the body and convert them into metabolites that result in the human body characteristic odour [1]. Teeth and mouth are the supportive organs to enable us in doing all the functions of the body by consuming. So, cleanliness of the oral areas is important for the healthy and nourished body which is devoid of microorganisms. Herbs have been in the investigations for the antibacterial and antiviral properties along with the history of the medical systems. They were effective and safer relative to the available synthetic drugs. They have rich chemistry that acts potently against most of the organisms out of which polyphenols, tannins, alkaloids and flavonols are the major classes. They exhibit antibacterial properties against varied bacterial strains [2].

Herbs like neem, tulsi, turmeric etc. which come

along in the Indian kitchen are famous for their antibacterial and antiviral properties. They had been in conjunction with other herbs like coriander, cinnamon, coleus, mint. All of these plants shoe antiviral and antifungal properties, too [3–5]. There is documented evidence for the essential oils like terpenoids that exhibit the potency against the bacteria they are Tea tree oil, clove oil, lemongrass etc. [6]. In this work, a Herbal Gargle was prepared using the extracts of Neem, Aloe vera and clove oil and some gel bases and investigated for eh anti-bacteria property.

Preparation

Herbs Material

The fresh leaves of Neem are collected and authenticated using a botanist, and the leaves were subjected to drying. The dried leaves were collected and powdered and extracted for chemical constituents using ethanol as solvent. The maceration of the powder was performed in a beaker, and then they are shaken occasionally. The macerate was collected and then filtered. The filtrate was collected and evaporated to get a thick concentrate of extract.

Aloe vera leaves were collected, and the fresh gel was extruded out and store for further use. Clove oil was collected from the local market store and used in the experiments.

The neem leave extract was mixed with ethanol to make a final concentration of 250mg/ml by accurately weighing and dissolving the neem leave extract in ethanol.

Formulation of Gargle

The accurately weighed amounts of neem extract, aloe vera gel were taken and mixed with distilled water in a sterilized beaker. It was added with a measured quantity of the clove oil as per table. The flavouring is adjusted using artificial flavours and colours too. The final volumes were made to 1000ml. All the colours and flavours used in the formulation are natural origin (Table 1).

Evaluation of Gargle solution

Evaluation of Physical properties

The gargle solution that was prepared was evaluated and examined for clarity, colour, odour and feel in the mouth.

The pH was determined using a digital pH meter; the stability testing was performed using street testing and random freeze-thawing guidelines. The prepared solutions were stored at 4^0C, 25^0 C and 45^0C for about 14 days, and then they are suddenly brought into the room temperature. The parameters like sedimentation, freezing cycle, instabilities, froth and the pH were determined in the formulations.

Antibacterial assays

Disc-diffusion method

A freshly prepared agar medium was sterilized and spread on to a Petri plate. This was let to solidify, and the cultures of E.coli, Streptomyces, Staphylococcus are inoculated into the agar plates separately. This was set aside, and a filter paper discs of approximately 1cm diameter were cut and were dipped in the gargle solution, which was compared to the marketed gargle. These discs were placed on the agar plate and were incubated for about 24 hrs in an oven. The next day, the plates were taken out, and the zone of inhibition was measured using a scale or callipers [6, 7].

Turbidity method

A healthy human volunteer who has no signs of any mucosal bleeding or an injury the mouth were chosen and are proceeded for the cotton swab method. Nutrient broth medium was prepared using the standard broth that was bought from a seller, and this was sterilized and then poured into the two test tubes in the equal amounts. Some amount of broth was set aside as a control in the UV comparison. The human volunteer was allowed to swab his teeth and oral cavity with a sterile cotton swab, and this was inoculated into the nutrient broth medium. This was then incubated for 24 hrs. Now this person is allowed to gargle his mouth for about 20 secs and then washed his mouth. Then another cotton swab was used to collect the swabbing in his mouth. This was also inoculated into another test tube and then incubated in the oven at the same temperature for about 24 hrs [8].

The solutions were taken out and made proper dilution to obtain a wavelength maximum at 600nm in UV spectrophotometry. This was then compared with the standard/ blank which was taken in the starting of the experiment. The absorbance values were noted and recorded for comparison.

RESULTS AND DISCUSSION

Three formulations were prepared by varying the herbs. The prepared formulations were named as GNF, GAF and GCF, which indicates that the formulations contained neem in GNF, aloe vera in GAF and clove oil in GCF respectively. All the three formulations were pale greenish-brown in colour and had a very pleasant taste and smell. The pH values and stability test results were tabulated inTable 2. Out of the three GCF was found unstable and so eliminated from further study.

Table 1: Formulation of the Gargle solution

S.No	Ingredients	GNF	GAF	
1	Neem Extract	10ml	–	10 ml
3	Aloe Vera Gel	–	10 g	10 g
4	Clove Oil	–	–	5ml
5	Alcohol	10 ml	10ml	10ml
6	Peppermint oil	0.5 ml	0.5 ml	0.5 ml
7	Citric acid	10 mg	10mg	10mg
8	Sugar	2g	2g	2g
9	Annatto seeds	2 ml	2ml	2ml

Table 2: pH and stability test of the prepared formulations

Formulation	Day	Temperature 0c	pH
GNF	1	40c	7.28
		250c	7.29
		450c	7.30
	7	40c	7.29
		250c	7.28
		450c	7.30
	14	40c	7.29
		250c	7.29
		450c	7.28
GAF	1	40c	7.27
		250c	7.28
		450c	7.29
	7	40c	7.27
		250c	7.29
		450c	7.30
	14	40c	7.27
		250c	7.28
		45^0c	7.30

Table 3: Zone of inhibition of the prepared Gargles

Organism	Zone of inhibition (mm)			
	GNF	GAF	Control	Marketed gargle
Escherichia coli	30 ± 0.4	24±0.32	10± 0.9	17 ± 0.6
Staphylococcus	25 ± 0.16	23±0.05	7 ± 3	12 ± 0.18
Streptococcus	17 ± 0.15	16±0.11	No inhibition	8 ± 0.13

Table 4: Absorbances of the Gargles solutions

Formulation	Absorbance*
GNF	0.152±0.04
GAF	0.163±0.05
Marketed Gargle	0.207±0.08
Control solution	0.812±0.07

In the disc diffusion method, the gargles showed a clear difference in change in the microbial flora of the mouth and oral cavity. This was compared to the market available formulation and was found significantly better than that. The zones of inhibition were calculated and compared. The prepared formulation GNF showed the highest activity compared with other formulations. This might be because of the variability in chemical constituents (Tables 3 and 4).

In the turbidity method, the formulation GNF showed a very low absorbance value compared to the other formulation and the marketed gargle too. This means lower absorbance value is the result of the higher activity. This shows that the prepared formulations have better activity.

CONCLUSIONS

Three formulations of gargle were prepared using Extract of Neem, Aloe Gel and clove oil which were named as GNF, GAF and GCF. The physical parameters were estimated, such as pH and one formulation failed in the stability study. The prepared GNF and GAF showed better antibacterial activity against the bacteria extracted by swabbing the mouth and also the three strains like E coil, Streptomyces and staphylococcus.

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Conflict of Interest

Authors declared no conflict of interest.

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