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DESIGN AND DEVELOPMENT OF SANITIZER SOLUTION INCORPORATING HERBS

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ABSTRACT

The human body consists of skin organ which is covered externally and acts as thermo regulation. Skin secrets sensible substances and various microbial agents harbors by matrix. Skin secrets sensible substances undergoesmetabolization and form skin order compounds. Acquired infections are avoided by hygienic hands. The present study is conducted on poly herbal hand sanitizer consists of bioactive components like Turmeric, Aloe vera and Neem undergoes formulation and evaluate the variety of methods produce effectivity of antibacterial properties. The herbal hand sanitizer prepared was checked for its efficacy using disc diffusion and turbidimetric method and the results clearly proved that the herbal sanitizer thus prepared is far more active than the commercial hand sanitizer.

Keywords :-Hand hygiene, Anti bacterial activity, Herbal antimicrobials, Standardization, Disc diffusion method, Turbidimetric method.

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INTRODUCTION

The human body consists of skin organ which is covered externally and acts as thermo regulation. Skin secrets sensible substances and various microbial agents harbours by matrix. Skin secrets sensible substances undergoes metabolization and form skin order compounds. Acquired infections are avoided by hygienic hands. In the present years, the accelerated dietary supplements are extracted from the plants. In invivo, the plants contains 2° metabolites like alkaloids, terpinoids, flavanoids and tannins which are having anti microbial components. The traditional healers treat the infections by using plants. The anti infectious agents are produced by the plants. The hand wash may contains anti microbial components of herbs such as coleus vettiveroids , coriandrumsativum,citrus lemon juices, vetieriazizanioide oil, azadirachtaindica contains constituenys like nimbinin, nimbin and nimbidin. The tea tree oils, myrrh and clove contains antimicrobial property are extracted from the plants. The essential oils and plant extract of rosemary, peppermint, bay, basil, tea tree oil, celery seed and bottle brush shows antimicrobial properties in different publications. The coleus vettiveroides protects from bacteria and produce deodorant and cooling components, while the skin is protected by citrus juice lemon from oxidant activity. The present study is conducted on poly herbal hand sanitizer consists of bioactive components like Turmeric, Aloe vera and Neem undergoes formulation and evaluate the variety of methods produce effectivity of antibacterial properties.

FORMULATION DESIGN

Collection and preparation of extracts

The plant materials used in the formulation were collected from the whole sale supplier of herbal crude

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drugs. All the chemicals used in the experiments were procured from SD Fine Chem LTD., Mumbai, India. Commercial available herbal hand sanitizer was of good brand and procured from local retail store. The dried leaves of *A.indica* and *Aloe vera* and rhizomes of *Curcuma longa* were finely ground separately. 500 g of each powder was extracted with 95% ethanol and filtered. The filtrate is dried and 50 g of each extract was dissolved in 120 ml of ethanol. This was concentrated to a final volume of 135 ml (300 mg/ml).

Preparation of Sanitizer

Weighed quantities of *Aloe vera gel* and glycerin were taken with alcohol in a beaker. With constant stirring solutions of extracts (neem and coriander) were added and sodium dodecyl sulphate was added and mixed. Finally volume is made upto 100ml with distilled water. Then a little quantity of rose oil is added as a flavouring agent and annatto seed extract is added as a colour. All the formulations were made according to table 1. The formulations are named as ARC1 and ARC2.

Characterization of the Extracts

The prepared poly herbal hand washes were evaluated for their physical appearance like colour, odour and texture. The pH of the prepared formulations was determined by using digital pH meter (New Delhi, India), stability studies had been performed by freeze thaw cycling method. The formulations were stored at temperatures 4, 25 and 45^0c for 14days and they were exposed to ambient room temperature. Then the pH, sedimentation and any change in physical appearance.

Anti-Bacterial activity

Disc diffusion method

Nutrient agar was prepared and sterilized. It was aseptically spread on three sets of Petri plates; each set containing three plates and was marked as test, control and standard. Test cultures used were *E.coli*, *S.aureus* and *P. aeruginosa*. The plates were inoculated with test cultures and incubated at 37°C for 24 h. Next day two filter paper discs were loaded with herbal hand sanitizer and a Commercial hand wash and each disc was placed in the respectively marked plate and an empty disc was placed in the plate marked as control. It was taken care that the sterile discs completely absorb the hand sanitizer. Disc of SLS was maintained as control. After 24 h, the test determines the efficacy of the product in terms of zone of inhibition of the organism. Higher the zone of inhibition, the more effective is the test product[9].

b. Turbidimetric method

Healthy volunteer without any clinical signs of dermal abrasion, trauma and infection were included in the study. Sterile cotton swab sticks were used to take swabs from both hands. Sterile test tubes were serially numbered and were divided into three parts and one was marked as before application (Bf) and the other as after application (Aft). Nutrient broth was prepared and equal volume of it was transferred into the sterile test tubes. One set of the test tubes was taken as control. 5 ml of sterile nutrient broth was kept aside to be used as reference. Approximately 0.5 ml of formulated Herbal Hand Sanitizer was squeezed out on the palms of the subjects and they were asked to rub the gel thoroughly on the palms, back of the hands, fingernails until the hands became dry. After 15 seconds of application the swabs were taken by the same method and inoculation was done in the respective tube, in the part marked Aft. The same procedure was followed with a Commercial hand wash, selected as standard. They were incubated for 24 h at 37°C in an incubator[10]. The absorbance of the medium from all the test tubes was determined in an UV-Vis Spectrophotometer at 600 nm. The method was repeated for seven consecutive days and the absorbance readings were noted. Also the smear prepared from the culture was stained by Gram's stain and were examined for bacterial presence.

Statistical analysis

Statistical analysis was carried out by using STATS software and results were expressed as mean S.D. All the parameters were statistically analyzed at 95% confidence level in the column. Statistical result of psychometric evaluation was further tested by ANOVA [One way analysis].

RESULTS

Both the prepared formulations were light green-emerald green in color and had a pleasant agreeable odor. The results of the pH and stability tests were tabulated in table 2. The results of disc diffusion method (table 3) showed that the hand sanitizer prepared from ethanol extract of the combined plant materials had greater activity than the activity of the commercially available hand sanitizer. Disc of SLS was maintained as control. The zone size obtained with disc of SLS showed that significant antibacterial activity of the formulated herbal hand sanitizer is not solely due to addition of 30% SLS but is the result of the combined activity of the phytoconstituents.

The results of Turbidimetric method (table 4) shows the high absorbance values of the control followed by the commercial handwash, with the Herbal Hand sanitizers showing least absorbance value. The higher the absorbance values the lower the antibacterial efficacy. This again infers that the formulated Herbal hand sanitizer is comparatively effective against various bacterial strains.

Table 1. Formulation composition

S.No	Ingredients	Formulation ARC1	Formulation ARC2
1	Neem Extract	10 ml	10 ml
2	Curcumin Extract	10 ml	---
3	Aloe Vera Gel	10 g	10 g
5	Cetyl alcohol	20 ml	20 ml
6	Glycerin	30 ml	30 ml
7	SLS (30%)	10 ml	10 ml
8	Lavender oil	3 ml	3 ml
9	Preservatives	2 ml	2 ml

Table 2.pH and stability test of the prepared formulations

Formulation	Day	Temperature ^{0}c	pH
ARC1	1	4	7.29
		25	7.30
		45	7.30
	7	4	7.28
		25	7.29
		45	7.30
	14	4	7.28
		25	7.29
		45	7.39
ARC2	1	4	7.27
		25	7.27
		45	7.28
	7	4	7.28
		25	7.27
		45	7.28
	14	4	7.28
		25	7.27
		45	7.29

Table 3. Anti-microbial sensitivity of the formulation in disc diffusion method

Organisms	Zone of inhibition (mm)			
	ARC1	ARC2	Commercial hand wash	Control
Escherichia coli	29 ±0.4	24±0.35	17 ± 0.8	9± 0.7
Staphylococcus aureus	24± 0.23	23±0.06	11± 0.37	7± 2
P. aeruginosa	16 ± 0.19	15±0.9	8 ± 0.25	No inhibition

All of the values are represented as Mean ± SD (n=3)

Table 4.Anti-microbial sensitivity of the formulation in turbidimetric method

Formulation	Absorbance*
ARC1	0.2654±0.04
ARC2	0.2871±0.06
Commercial Hand wash	0.2989±0.05
Control	0.793±0.07

*Mean of triplicate readings

DISCUSSION AND CONCLUSION

In the present context the plants under study are rich in these varied compounds and hence are more effective against skin pathogens. The leaves of *A.indica* are widely used for medicinal purposes[7]. The ethanol extract is efficient extracting the phytochemicals, which act on pathogens. The main ideology behind combining the plant materials is to observe the additive effect of the

active constituents from different plants. The combination proves to be beneficial and hence it is used in preparation of a herbal hand sanitizer. The herbal hand sanitizer prepared was checked for its efficacy using disc diffusion and turbidimetric method and the results clearly proved that the herbal sanitizer thus prepared is far more active than the commercial hand sanitizer. Nimbin, Nimbolide and Nimbidin from Neem may be responsible for the anti-bacterial activity of the formulation[5]. Thus, these compounds can be extracted and incorporated in bases, in order to prepare superior anti-microbial sanitizer with less or no side effects. Hence a new way can be found to combat antibiotic resistance of pathogenic organism and provide safe and healthy living through germ free hand, although the removal is not 100% but a major number can be reduced.

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