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EXTRACTION, FORMULATION AND EVALUATION OF POLYHERBAL HAND SANITIZER

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ABSTRACT

Hands are the most common mode of transmission of pathogens to patients and proper hand hygiene can prevent health care associated infections and the spread of antimicrobial resistance. The aim of present study was to prepare herbal hand sanitizer incorporating the extracts of *Azadirachtaindica* (Neem), *Ocimum sanctum Linn.* (Tulsi) and *Zingiber officinale* (Ginger) herbal combination having multidimensional activities for evaluate their respective antimicrobial efficacy and safety of hands. The objective is to have a formulation with low risk of side effects, lower cost, increase safety and compliance of individuals. The formulation was evaluated for organoleptic properties and antimicrobial properties against the specified microorganism (Bacteria- *Escherichia coli* and *Staphylococcus aureus*) by using cup plate method. The results revealed that the herbal hand sanitizer is efficient in reducing number of organisms as it gives larger inhibition zone than the marketed synthetic hand sanitizer.

1. INTRODUCTION

The commonly used ingredient in hand sanitizer is alcohol and inactive ingredients include a thickening agent, humectants etc. Alcohol based hand sanitizer are very effective in killing microorganisms than compared to soaps[1].The hands of health care workers are the primary routes of transmission of multidrug resistant pathogens and infection to patients. Hence it brings up the use of antiseptics for hand washing purposes [2].The soaps or solutions help to reduce health care associated transmission of contagious diseases more effectively but they have some shortcomings or adverse effects[3]. *Staphylococcus aureus* and some other bacterias are causative agents of skin infections[4].Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties[5]. The phytochemical analysis of *Azadirachtaindica* (Neem) extract had earlier been reported. Phytochemical screening of the stem bark extract of *Azadirachtaindica* (Neem) in the present study also revealed presence of terpenes and glycosides. Phytochemical screening of the stem bark extract of *Azadirachtaindica* (Neem) in the present study also revealed presence of terpenes and glycosides. The neem oil, also known as oil of Margosa, is believed to have medicinal properties, such as antibacterial antifungal and antidiabetic[6].

The genus *Ocimum* (Labiateae or Lamiaceae) comprises 30 species which are found in tropical and subtropical regions. Leaves and flowering tops are used for extracting essential oil. Oil of *Ocimum sanctum Linn.* (Tulsi) has revealed the presence of five fatty acids (stearic, palmitic, oleic, linoleic and linolenic acids). It is a good source of beta carotene, calcium, vitamin C and it also contains volatile substances (including estragol, linalool, eugenol, methyl chavicol and small quantities of methyl cinnamate, cineole, and other terpenes), tannins, camphor, flavonoids, triterpene: urolic acid. Various studies have been performed with Ocimum sanctum for its antibacterial, antioxidant, antiulceric, antimalarial, antidiabetic, anti-inflammatory, antilipidemic, anticancer and immunomodulatory properties [7]. Ginger has been used as a spice and medicine in India and China since ancient time. Many oils exhibit antimicrobial properties due to the presence of components such as thymol, eugenol, 1,8-cineole, α- and β- pinenes, linalool, α-terpineol etc [8]. Considering this; an attempt has been made to screen classical literature for the herbs with antimicrobial properties and found that, *Azadirachtaindica* (Neem), *Ocimum sanctum Linn.* (Tulsi) and *Zingiberofficinale* (Ginger) has those antimicrobial activities.

2.0 EXPERIMENTAL:

2.1 Drugs and chemicals: Aerial parts of *Azadirachtaindica* (Neem), *Ocimum sanctum Linn.* (Tulsi) and rhizomes of *Zingiberofficinale* (Ginger) were collected from local area of Vidarbharegion and authenticated by Department of Pharmacognosy, Priyadarshini J. L. College of Pharmacy, Nagpur,Maharashtra, India.Nutrient Agar (For bacterial cultivation), Marketed synthetic hand sanitizer (Lifebuoy) were purchased from retailer. Culture of microorganisms (overnight incubated), Alcohol were of lab grade. *Escherichia coli and Staphylococcus aureus p*athogens selected for evaluation of anti-microbial activity.

2.2 Extraction of plant material: Extraction is done by maceration method. Two grams of grinded fine powdered ofeach dry plant material (neem, tulsi and ginger) were added separately in 60 ml of methanol solution (95%v/v). It was allowed to stand for seven days in warm place with frequent shaking twice day.

2.3 Formulation of herbal hand sanitizer: Carbopol 940 was added to distilled water with constant stirring. After uniform mixing, the pH was adjusted to 7.0 by addition of triethanolamine with slow stirring to avoid formation of possible air bubbles in the product and kept aside for 24 hrs. Above extract were added to denatured alcohol along with glycerin, polysorbate 20 were mixed with aqueous phase. Finally, 0.25% each of methyl and propyl parabenwas added as preservative and 0.5% of perfume and mixed with slow stirring to obtain uniform product. Prepared product was stored in air tight containers (See Table No. 1).

Table No.1: Composition of herbal hand sanitizer

Sr.No.	Ingredients and Excipients	Quantity taken (g/mL)	Uses
1	Deionized water	9.0	Vehicle
2	Alcohol (denatured)	18.6	Antibacterial
3	Extract	0.9	Antibacterial
4	Carbopol 940	0.15	Thickening agent
5	Triethanolamine	0.21	Solubilizing agent
6	Glycerin	0.69	Emollient
7	Polysorbate 20	0.15	Emulsifier
8	Perfume	0.15	Fragrance

2.4 Antimicrobial activity: The screenings of antimicrobial activity of the herbal hand sanitizer against pathogens wereperformed using cup plate method as per standard procedure[9]. Test cultures used were *Escherichia coli and Staphylococcus aureus.* Responses of organisms to the herbal hand sanitizer formulation were measured and compared with the response of the marketed synthetic formulation.

3.0 RESULTS AND DISCUSSION:

The formulation was evaluated for organoleptic properties and for antimicrobial properties against the specified microorganism (Bacteria- *Escherichia coli and Staphylococcus aureus*) by using cup plate method. The results of organoleptic evaluation is depicted in Tablet No. 2

Table No.2: Organoleptic evaluation of hand sanitizer

Sr.No.	Parameters	Observations
1.	Appearance	Good
2.	Color	Light Green
3.	Odour	Fragrant
4.	PH	6.5
5.	Transparency	Transparent
6.	Skin irritation	No irritation

* Results are mean of five samples (n=5)

Table No.3: Antimicrobial activity of hand sanitizer

Sr.No.	Hand sanitizer Concentration (mL)	Zone of inhibition (mm)	
		E.Coli	S.aureus
1.	Extract	21	19.3
2.	HS1	15	17.6
3.	HS2	17.6	15.3
4.	HS3	14.3	17
5.	Standard	14.3	16

* Results are mean of five samples (n=5)

The two formulations HS1 and HS3 showed significant inhibitory activity. This indicates that the herbal hand Sanitizerhas antimicrobial activity particularly against *Escherichia coli and Staphylococcus aureus.* (See Figure 1).

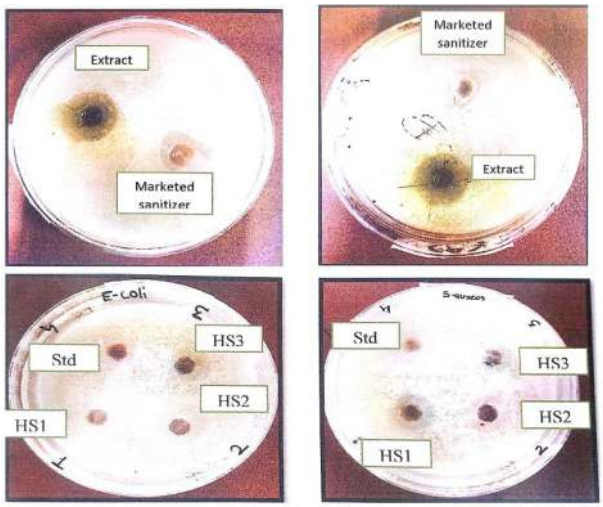

Figure 1: Study of Antimicrobial activity by cup plate method; **A)** Marketed synthetic hand sanitizer with combined methanolic extract of plant part in *E.coli* culture **B)** Marketed synthetic hand sanitizer with combined methanolic extract of plant part in *S.aureus* culture **C)** Marketed synthetic hand sanitizer with prepared herbal hand sanitizer in *E.coli* culture **D)** Marketed synthetic hand sanitizer with prepared herbal hand sanitizer in *S.aureus* culture

In the present context the plants under study are rich in these varied compounds and hence are more effective against skin pathogens. The methanol extract is efficient in extracting the phytochemical and acids which act on the pathogens. The main ideology behind combining the plan materials is to observe the additive effect of the active constituents of different plants. The combination proves to be beneficial and hence it is used in preparation of herbal hand sanitizer. The results clearly prove that the herbal hand sanitizer prepared is far more active than the marketed synthetic formulation.

4.0 CONCLUSION:

The prepared formulation showed significant results against two bacteria species *Escherichia coli and Staphylococcus aureus* compared to marketed synthetic hand sanitizer. We can conclude that the active compounds in the herbal hand sanitizer are more effective in killing and removing organism than the synthetic chemicals used in marketed formulation. Thus these compounds can be extracted and incorporated in herbal formulation with less or no side effects. This might be rational basis for use of herbs in preparation of handsanitizer and use of these compounds in making of antimicrobial hand sanitizers in place of synthetic chemicals.

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