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FORMULATION, DEVELOPMENT AND EVALUATION OF NOVEL POLYHERBAL HANDWASH CONTAINING MEDICINAL PLANT: (MIMOSA PUDICA, AZADIRACHTA INDICA, OCIMUM SANCTUM AND GLYCYRRHIZA GLABRA)

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ABSTRACT

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. The present work deals with the formulations and development of novel polyherbal handwash from the methanolic extracts of leaves of Azadirachta *indica*, *Mimosa pudica* and *Glycyrrhiza glabra*. Hand-washing is very important process in day-to-day life. Hands are major source through which microbial infections may occur thus proper hand was must be required using appropriate hand wash formulation. After preparation of a formulations and development of polyherbal hand wash evaluation parameter were checked such as physical properties like appearance, pH, spread ability, grittiness, viscosity , foam stability and cleaning action, The antimicrobial activity of prepared formulations of novel polyherbal hand wash was checked by agar diffusion method against skin pathogens like *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtills*. The results revealed that prepared herbal hand wash formulations showed significant zone of inhibition and its efficacy was checked and compared with the standard commercial marketed hand wash formulations. So, these plant materials can be used in the preparation of novel polyherbal hand wash on commercially scale.

KEYWORDS: Novel Polyherbal handwash, *Azadirachta indica Mimosa Pudica, Glycyrrhiza glabra*, antimicrobial activity.

INTRODUCTION

Plant extracts and their products have been used for long time like a traditional medicine, functional food, natural dyes, cosmetics, as a detergent as well as in the treatment of diseases.[1,2,3] The skin being the most exposed part of our body requires protection from skin pathogens. The hands of Health Care workers (HCWs) are the primary routes of transmission of multidrug resistant pathogens and infection to the patients. Hence, it brings up the use of antiseptic for hand wash purpose. Many of the chemical antiseptics are now available in market as alcohol-based sanitizers, chlorhexidine products etc. These soaps or solutions help to reduce health care associated transmission of contagious diseases more effectively but they have some adverse effects. Their frequent use can lead to skin irritation and resistant among pathogens. Organisms such as *Bacillus subtills, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* are some of the skin pathogens.[4] The main advantage of using natural source is that they are easily available, cheap and harmless compared to chemical products. Therefore, research has been increased tremendously towards making natural products with improved quality yet less expensive and no side effect over chemical products. Hand washing is an important way to help fight the spread of disease. Hand washing removes visible dirt from hands and reduce the number of harmful microorganisms. harmful bacteria and viruses such as, *E. coli* and *Salmonella* can be carried by people, animals or equipment and transmitted to food.

Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi. When use plants as source of medicine has been inherited which play important component of the health care system in India.[5] The emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antimicrobial agents. In present study we formulate herbal hand wash using different plant extracts with potential antibacterial activity and thereby establishing them as a potent antimicrobial agent in the formulation of herbal hand wash. Especially hands are needed to protect from bacterial pathogens as they are the most exposed part of the body. Proper hand hygiene is the single most important, simplest, and least expensive means of preventing health care associated infections. Most of the hand wash available in market are made from the emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antimicrobial agents. In present study we formulate herbal hand wash using

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Skin, especially hands are needed to protect from bacterial pathogens as they are the most exposed part of the body. Proper hand hygiene is the single most important, simplest, and least expensive means of preventing health care associated infections. Most of the hand wash available in market are made from synthetic chemical and have more side effect such as rashes, dryness of skin etc.[6,7]

Tulsi (Ocimum sanctum) -This is traditionally used in many diseases. Indian mythology attaches a great significance to Basil by recognizing it as a holy herb. Perhaps, such significance comes from the actual health applications of the herb. Its use is recommended as a first aid in the treatment of respiratory, digestive, and skin diseases. Apart from these common ailments, Ayurveda also recognizes its use for the diseases ranging up to tumorous growths. Experimental studies identify it to be a highly promising immunomodulator, cytoprotective and anticancer agent. Following is the benefits & uses of Tulsi plant or Holy Basil/Ocimum Sanctum. Holy basil contains vitamin C and antioxidants such as eugenol, which protects the heart from the harmful effects of free radicals. Eugenol also proves useful in reducing cholesterol levels in the blood. Vitamin C and A, phytonutrients, in Holy Basil are great antioxidants and protect the skin from almost all the damages caused by free radicals. Tulsi acts a mild diuretic & detoxifying agent which helps in lowering the uric acid levels in the body. Acetic acid present in holy basil helps in the breakdown of the stones. Tulsi is a natural headache reliever which can also relieve migraine pain. Holy basil helps kill bacteria and infections. The primary active compound of holy basil oil is eugenol which helps fight skin related disorders. Ocimum Sanctum helps treat skin infections both internally and externalities' is an age-old ingredient for treating fever. It is one of the prime ingredients in

the formulation of various ayurvedic medicines & home remedies. Tulsi's anti-inflammatory properties help promote eye health by preventing viral, bacterial and fungal infections. It also soothes eye inflammation and reduces stress. Tulsi is a natural mouth freshener and an oral disinfectant. Ocimum Sanctum can also cure mouth ulcers. Holy basil destroys the bacteria that are responsible for dental cavities, plaque, tartar, and bad breath, while also protecting the teeth. Due to the presence of compounds like camphene, eugenol, and cineole, tulsi cures viral, bacterial, and fungal infections of the respiratory system. It can cure various respiratory disorders like bronchitis & tuberculosis.[8,9]

Fig:- TULSI.

Neem (Azadirachta indica)

This tree is also known as Margosa or Indian lilac. Various parts of the Neem tree have been used as traditional Ayurvedic medicine in India. Neem oil, the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Neem leaves possesses a wide spectrum of antibacterial action against gram-negative and gram-positive microorganisms' Antibacterial activity Recent research shows the isolation and identification of the antibacterial active compound from petroleum ether extract of neem oil. The study of Zhong *et al*. showed an antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. Elavarasu *et al.* studied *in vitro* anti-plaque microbial activity of neem oil. Antiviral Galhardi *et al*. studied the *in vitro* antiviral property of *Azadirachta indica* polysaccharides for poliovirus. The study of Saha *et al.* showed water extracted polysaccharides from *A. indica* leaves with anti-bovine herpes virus type 1 (BoHV-1) activity. The research of Xu *et al*. showed the *in vitro* antiviral activity of neem seed kernel extracts against duck plague virus. Sexually transmitted disease Few researchers have focused

on neem efficacy in treating sexually transmitted diseases. The reports that have been completed are overwhelmingly positive. Recent research of Shokeen *et al.* showed the evaluation of the activity of 16 medicinal plants against *Neisseria gonorrhoeae* Neem and the immune system Neem trees can reach 15–30 meters (49–98 feet) in height and have attractive rounded crowns and thick furrowed bark. The compound leaves have toothed leaflets and are typically evergreen but do drop during periods of extreme drought. The small fragrant white flowers are bisexual or staminate (male) and are borne in clusters in the axils of the leaves. The fruit is a smooth yellow-green drupe and has a sweet-flavored pulp. Neem is usually grown from seed but can be propagated from cuttings or root suckers.[10,11]

Fig. Neem.

Lajwanti (Mimosa pudica**)** –This is also known as touch me not, live and die, shame plant and humble plant is a prostrate or semi-erect subshrub of tropical America and Australia, also found in India heavily armed with recurved thorns and having sensitive soft grey green leaflets that fold and droop at night or when touched and cooled. It is often grown for its curiosity value: the compound leaves fold inward and droop when touched or shaken, defending themselves from harm, and re-open a few minutes later.

The species is native to the Caribbean and South and Central America, but is now a pantropical weed, and can now be found in the Southern United States, South Asia, East Asia, Micronesia, Australia, South Africa, and West Africa as well. It is not shade- tolerant and is primarily found on soils with low nutrient concentrations.

It majorly possesses antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac, and various other pharmacological activities. The herb has been used traditionally for ages, in the treatment of urogenital disorders, piles, dysentery, sinus, and also applied on wounds The infusion of the twigs with leaves possess antipyretic, soothing and sudorifie properties. Apart from the toxicity from mimosine, the plant has shown a

significant hypotensive, sedative, and anti-inflammatory effect. An alcoholic extract has shown to lower blood glucose concentrations in diabetic rats. The alkaloidal extracts of the roots is antagonistic to both acetylcholine and histamine and histamine. A crude ethanol extract of the leaves was found to have insecticidal activity against one Trifolium species.[12]

Fig. Mimosa pudica.

Mulathi (Glycyrrhiza glabra) - This is a native of south-east Europe and south-west Asia, including Iran. Antimicrobial activities of roots and rhizomes have been studied in previous researches, but there are a few reports about the effect of licorice leaves against microorganisms.[13]

Lemon oil which is obtained from fruits of Citrus limon L. belonging to the family Rutaceae is traditionally used for the purpose of cleaning due to its disinfectant properties. Lemon juice is also used as a short-term preservative in some food preparations. Lemon juice is used in Indian medicinal systems because of the anti-microbial properties of lemon. It is also used to add taste to many food preparations.

Fig. Mulathi.

MATERIALS AND METHODS

Collection of plant materials and chemical

Glycerin, Sodium lauryl salphate, methyl parabens, carbopols 940 and lemon oil are received

from our pharmaceutics lab from L.N. Pharmacy college Campus Baitalpur Deoria, India as for research work under the supervision of our head of department and do our research work successfully.

The plants *Mimosa pudica* L and *Azadirachta indica* were collected from the herbal garden of L.N. Pharmacy college Campus Baitalpur Deoria up., India and send to authentication purpose in Pharmacognosy department.

Preparation of herbal leaf extracts

The collected plants *Mimosa pudica* L, *Azadirachta indica and Ocimum sanctum* leaves are taken and make coarsely powdered. After that 10 grams of coarsely powdered leaves of both plants were soaked in 200 ml of methanol and kept for maceration for about 3-4 days. After maceration the extract is filtered and the filtrate was collected and used for making hand wash.

Method of extraction of *Glycyrrhiza glabra*

10 grams powered of *Glycyrrhiza glabra* were extracted with 100 ml of ethanolic solution (9 parts of ethanol and 1 part of distilled water by means of extraction). The mixture was heated in water both at 60^0C for 1 hour. The content was filtered thought Whatman filter paper. The filtrate was collected and stored at room temperature.[14]

Method of preparation of polyherbal handwash

Azadirachta indica, Glycyrrhiza glabra and *Mimosa pudica* with lemon juice were measured accurately and dissolved by gentle heating. After heating keep the solution aside for some time. The required Sodium lauryl Sulphate dissolved in 10ml distilled water along with Glycerin were mixed in above aqueous phase with continuous stirring. Herbal hand wash was prepared by using Carbopol 940 as a gelling agent in 0.5%w/v concentration with distilled water over night then the swelled polymer was stirred using mechanical stirrer to ensure the uniform dispersion of polymer the pH was adjust to 7.

The methyl paraben was dissolved in remaining quantity of purified water and dispersed into the extract. The swelled polymer (Carbopol 940) was stirred using a mechanical stirrer to ensure the uniform dispersion of polymer and finally added into the above mixture to form a homogenous Gel and then the required quantity of neem oil was added for fragrance. And the formulation was stored in well closed container and labelled suitably for further analysis.

Table 1: (Formulation of polyherbal handwash).

Sr. No	Composition	Formulation (A)	Formulation (B)	Formulation (C)	Formulation (D)
1	Methanolic extract of *Azadirachta indica*	20ml	-	-	10ml
2	Methanolic extract of *Mimosa pudica*	-	-	20ml	5ml
3	Methanolic extract of *Glycyrrhiza glabra*	-	20ml	-	5ml
4	Methanolic extract of *Ocimum sanctum*	15ml	-	-	10ml
5	Glycerin	10ml	10ml	10ml	10ml
6	Lemon oil	2ml	2ml	2ml	2ml
7	Carbopol 940	15ml	15ml	15ml	15ml
8	Sodium lauryl sulphate	6gms	6gms	6gms	6gms
9	Methyl barabean	0.1gm	0.1gm	0.1gm	0.1gm
10	Distilled water	q. s	q. s	q. s	q. s

Evaluation of Polyherbal Handwash

1-Organoleptic properties

The poly herbal hand wash was subjected to Physical evaluation visually. The test parameters of were organoleptic properties like colour, odor, and texture was carried out. colour and texture were evaluated by visual and touch sensation respectively.[15]

2- pH

1 gm of sample of Herbal Based hand wash was taken and dissolved it into 100 ml distilled water. The pH of solution was taken in previously standardized digital pH meter.[16]

3- Homogeneity and Appearance

Homogeneity and appearance were evaluated by visual inspection,[17]

4-Viscosity

The viscosity of poly herbal hand wash Based hand wash was determined by using digital Brookfield viscometer.

5-Grittiness

1ml hand wash was taken on fingertip and rubbed between two fingertips, then formulation was evaluated.[18]

6-Antimicrobial activity

Agar well diffusion method was performed for the determination of antimicrobial activity. After sterilization of the nutrient medium and petri dishes transfer into laminar air flow unit for aseptic transfer. 0.25um of each bacterial inoculums were added to 25 ml of nutrient medium and pour into Petri dishes. After solidification cups were made by using borer (5mm). 50um of test formulation A, B, C, & D and marketed herbal hand wash.[19]

7-Spreadability

0.5 gm of sample of polyherbal hand wash was taken and passed between two slides and left for 5minute where no more spreading was expected diameter of spreader circle was measured in cm and was taken as comparative value for spread ability.[20]

8-Foam height

1 gm of sample of polyherbal handwash was taken and dispersed in 50 ml distilled water dispersion was transferred into measuring cylinder. Volume was made up to 100 ml with water. This solution was taken in 10 test tubes in the series of successive portion of 1, 2, 3….10ml and remaining volume is made up with water to 10 ml. Then the test tube was shaken for 15 sec. Then the test tube allowed to stand for 15 min. and the height of foam was measured.[21]

RESULTS AND DISCUSSION

The Anti-microbial efficacy of the formulated Herbal hand wash was tested on *S. mutans* and *Staphylococcus aureus*, by Disc-diffusion method. The results of showed that the hand wash prepared from ethanolic extract of *Glycyrrhiza glabra* root extract plant materials had significant activity as compare the commercially available hand wash. The activity of herbal hand wash formulation showed significant inhibition of bacterial growth in the culture plates All the prepared formulations were evaluated for parameters such as colour, odor, texture, appearance, homogeneity, grittiness, spread ability, foam height and pH.

The observation reveals that the polyherbal handwash were translucent in appearance, Smooth in texture, and the pH of all prepared formulations was found to be in range of 6.8 – 7.9. All the formulations showed good spread ability. Also, from the above data it was observed that increase the concentration of plant extract increases the spread ability. All the prepared formulations showed good homogeneity with absence of lumps. The developed preparations were much translucent.

Table 2: Combined evaluation table of herbal hand wash formulation.

	Formulation	(A)	(B)	(c)	(D)	Formulation available in market
1	Colour	Green	Orange	Light green	Dark brown	Light orange
2	Odour	Lemon like	Lemon like	Lemon like	Lemon like	Pleasant
3	pH	7.5	7.3	6.8	7.9	7.8
4	Homogeneity and Appearance	Translucent	Translucent	Translucent	Translucent	Translucent
5	Grittiness	Non-gritty	Non-gritty	Non-gritty	Non-gritty	Non-gritty
6	Spread ability	3.1	3.0	3.4	3.5 cm	3.8 cm
7	Foam height	10ml	9ml	11ml	13 ml	17ml

Table 3: (Anti-microbial activity/Zone of inhibition table a of polyherbal handwash).

Sr. No	Microorganism (Antibacterial activity)	(A)	(B)	(c)	(D)	Formulation available in market
1	Escherichia coli	14mm	18mm	18mm	25mm	17mm
2	Staphylococcus aureus	13mm	14mm	16mm	24mm	15mm
3	Pseudomonas aeruginosa	10mm	16mm	18mm	23mm	18mm
4	Bacillus subtills	12mm	15mm	17mm	25mm	17mm

CONCLUSION

Most of the people do not even bother to use hand washes in our daily life. Nobody knows the significance of liquid hand washes even these are much better than plain soaps due to their ingredients and effectiveness on our skin of hands and as well as suitable for all type of skin might be that was most sensitive. Mostly poly herbal hand washes protect us from many daily encounter bacteria. Nowadays there is a lot of demand for herbal formulations in the market due to their cost effectivity and absence of any side effects. From the above experimental data, it is clear that a polyherbal handwash formulation with herbal ingredients such as *Glycyrrhiza glabra*, *Mimosa pudica* and *Azadirachta indica* and Ocimum sanctum has good characteristics and also possesses a good antimicrobial activity against *Bacillus subtills, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtills,* (standard) were added to each cup. Then the plates were incubated at 37^0C for 24hrs in the incubator. After incubation, the diameter of clear zones of inhibition produced around the well and it was measured in mm compared to the standard (Marketed formulation). standard was added to each cup. Then the plates were incubated at 37^0C for 24hrs in the incubator. After incubation, the diameter of clear zones of inhibition produced around the

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